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Complementary and Alternative Medicine: Immunization/Vaccinology

Editor

Mughees Aizaz Alvi
Majeeda Rashid
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Shizray Imtiaz Toor

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PREFACE

Immunization is an essential aspect of veterinary medicine, pivotal for preventing disease and safeguarding the health of livestock, poultry, aquatic species, and companion animals. *Complementary and Alternative Medicine: Immunization/Vaccinology* explores both the foundations and frontiers of veterinary immunization, encompassing conventional vaccine strategies and emerging innovations like CRISPR, mRNA technology, and nano-vaccines. This book serves as an in-depth guide to understanding immunization's role in modern animal health and offers insights into future advancements. The book begins with an overview of vaccine history, physiology, and evolution, charting milestones in immunization and the development of diverse vaccine types. Building on this foundation, it delves into the biological mechanisms of immunity, highlighting processes like antigen processing, cytokine responses, and T cell activation. Readers will explore how vaccines trigger immune responses, including in dairy cows and poultry, to provide protection against specific pathogens, demonstrating the science behind successful immunization. As disease prevention becomes increasingly essential in livestock and aquaculture, advanced topics like precision vaccinology and nano-vaccine applications are examined for their potential to offer targeted protection with minimal side effects. Chapters cover a range of disease-specific strategies, such as vaccines for controlling parasitic infections like bovine babesiosis and malaria, as well as infectious diseases in aquaculture, which impact fish health and sustainability. Additionally, the role of vaccines in preventing zoonotic diseases, including COVID-19 and Hepatitis B, underscores the relevance of animal immunization in global health. Beyond science and technology, this book addresses critical social and economic considerations in vaccine development and implementation, including vaccine accessibility, hesitancy, and equity. These perspectives highlight the importance of building trust and improving the availability of vaccines, particularly in areas with high disease prevalence or limited resources. By understanding the social factors that impact immunization efforts, the book provides a well-rounded view of the challenges and opportunities in veterinary immunization. In exploring the One Health approach, this book illustrates how immunization contributes to animal health and public health, recognizing the interconnectedness of human and animal populations in disease prevention. From traditional vaccines to cutting-edge immune boosters, it discusses how immunization strategies help prevent zoonotic transmission, control disease spread, and enhance the welfare of animal populations, benefiting human health in turn. This book is designed for veterinary students, researchers, and professionals committed to advancing animal health through immunization. With its combination of historical context, scientific insights, and innovative perspectives, *Complementary and Alternative Medicine: Immunization/Vaccinology* aims to inform and inspire future developments in veterinary immunization, helping readers envision new solutions to the complex challenges of animal disease control in a rapidly changing world.

Editor

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Chapter 01

Usage of CRISPR Technology in Development of Vaccines and Immunization against Various Diseases in Poultry

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ABSTRACT

CRISPR, a modern approach of genome editing, has paved a new way to the development of vaccines and immunization along with several of its other uses in different fields of biology. The CRISPR-Cas9 system was initially discovered in prokaryotes. Constituents of CRISPR-Cas 9 system include a Cas operon, an AT rich ladder and repeat sequences separated by unique spacer sequences. It has been employed in genetics researches, in domains such as biomedical modeling and diagnostics and other medical researches. The genome editing technology has also been used in development of vaccines against various diseases of poultry and this usage has also been discussed in detail in the chapter. CRISPR technology has many advantages over conventional live and attenuated vaccine production. We can modify our vaccine production strategies to immunize the poultry by keeping in view the overcoming and drawbacks of the vaccination and immunization techniques in poultry. CRISPR technology can be a future of vaccination and immunization of the birds against viral and bacterial diseases.

KEYWORDS
CRISPR technology, CRISPR-Cas 9 system, Genome editing technology, Biomedical modeling, Diagnostics, Vaccine production

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INTRODUCTION

Genome editing is the precise and effective DNA modification within a cell by modifying its genetic coding. Every method for altering a gene in a cell is predicated on the particular site-specific cutting of double-stranded DNA by DNA endonucleases. Researchers were able to edit nearly any genomic sequence thanks to programmable nucleases, which also made it possible to study human diseases using animal models and cell lines and opened up new avenues for gene therapy treatment (Munawar et al., 2021).

The CRISPR, or clustered regularly interspaced short palindromic repeats system, is now the newest approach to editing of genome, offering remarkable specificity, effectiveness, and variety in genetic manipulation. CRISPR developed as a prokaryotic system (immune system) that provides acquired immunity and resistance to invading genetic elements like bacteriophages. Recently, it has been developed into a tool for the precise targeting of nucleotide sequences inside eukaryotic complex genomes for genetic manipulation. The power of CRISPR lies in its straightforward nature and simplicity of use, the ability to be targeted to any particular nucleotide sequence by the use of an easily synthesized guide RNA, and its facile capacity to undergo further scientific breakthroughs (White et al., 2017)

CRISPR Technology

The CRISPR-Cas system is a well-studied defense mechanism that protects archaea and bacteria from bacteriophages and plasmids. It is currently being utilized to modify the genomes of various creatures. CRISPR-Cas system is a significant

genome editing mechanism that is straightforward, cost-effective, precise, and user-friendly (Singh, 2020).

CRISPR, or Clustered Regularly Interspaced Short Palindromic Repeats, originates from bacteria that have evolved to defend against invading genetic elements such as plasmids or viral DNA. This system represents a form of adaptive immunity in bacteria infected by bacteriophages. Bacteria store a record of the invading DNA, and when the same DNA enters again, they mount an acquired immune response to destroy it. CRISPR includes numerous direct and short DNA sequence repeats, with each repeat flanked by approximately 30 bases of spacer DNA (Lino et al., 2018).

These spacers are small DNA segments taken from plasmids or bacteriophages. When the host encounters the same plasmid or bacteriophage again, it recognizes the foreign DNA by matching it with CRISPR RNA (crRNA). Once the crRNA pairs with the complementary foreign DNA, the Cas9 protein (a nuclease) degrades and eliminates the invading DNA or RNA. In this process, single-stranded guide RNA (sgRNA) binds with Cas9, and this complex directs the endonuclease activity to the region near the protospacer adjacent motif (PAM). When the sgRNA identifies a specific DNA sequence, the attached Cas9 cuts three nucleotides upstream of the PAM (NGG) on both the positive and negative DNA strands, creating a double-stranded break with a blunt end (Cencic et al., 2014).

CRISPR technology has been effectively used in genetic editing, diagnosis, and treatment for years since it is getting more stable and mature (Chen, 2023).

CRISPR Technology in Research

Versatile CRISPR-enabled genome editing has been used in research in a number of ways, including chromosome imaging, genome-wide screening, transcription regulation, and epigenome modification. CRISPR systems are now being utilized in the clinic to treat blood and ocular diseases in humans, and they will probably soon be used to treat genetic abnormalities in animals as well. China and the US have approved two CRISPR-Cas9-based clinical trials for targeted cancer medicines. Beyond biomedical uses, these instruments are currently being employed to create novel antibiotics, speed up the breeding of crops and cattle, and employ gene drives to suppress disease-carrying insects (Barrangou and Doudna, 2016).

At the moment, the CRISPR/Cas mechanisms utilized in bacteria confront problems, such as the high off-target rate of Cas9, weak cleavage activity of Cas12a, and inadequate growth of endogenous systems (Liu et al., 2020).

CRISPR Applications

The prokaryote-derived CRISPR-Cas genome editing tools have revolutionized our potential to modify, identify, photograph, and interpret specific DNA and RNA sequences in living cells of various species. The ease of application and stability of this technology have altered genome editing for research ranging from basic science to translational medicine (Li et al., 2020).

Aside from DNA, CRISPR-Cas-based RNA-targeting devices are being created for use in research, medical care, and diagnostics. Cas proteins that are nuclease-inactive and target RNA have been combined to a variety of effector proteins to control gene expression, epigenetic changes, and chromatin interactions. Overall, the new advancements significantly improve our comprehension of biological processes and move CRISPR-Cas-based techniques into clinical usage in cell and gene therapies (Pickar-Oliver and Gersbach, 2019).

It is quickly becoming a popular tool in the biological sciences, with applications ranging from genome editing to the treatment of genome-derived disorders. The CRISPR/Cas technology allows the biological DNA sequence to be mend, cut, changed, or inserted. It has the ability to successfully modify human stem cells and is likely to produce therapeutic benefits. CRISPR is a more accurate, effective and convenient genome editing technology than ZFN and TALEN (Wang et al., 2019).

CRISPR Applications in Biomedical Modeling

Replicating mutations or variations unique to a patient is not the sole challenge in creating authentic disease models, but using appropriate controls is also a significant obstacle. In this regard, the redesign of both phenotypes (disease-related models or gene correction alternatives) has been made easier by the CRISPR/Cas9 system. The CRISPR/Cas9 system has been utilized to quickly create a large number of disease-based models for major human pathologies, such as cancer, cardiovascular disease, neurological diseases, and other Mendelian or complex genetic illnesses. These models have made it possible to study the molecular mechanisms underlying pathogenesis of these diseases. These novel models are also great aims for conducting a high quality research in gene therapy and drug screening (Martinez-Lage et al., 2017).

CRISPR Applications in Diagnostics

The CRISPR/Cas system has grown in prominence as a diagnostic mechanism for diseases that are caused by microbes or by non-microbes. Pardee et al. (2016) created the first CRISPR-based diagnostic system to identify the Zika virus. Subsequently, SHERLOCK was designed as a Cas13-based diagnostic drive for nucleic acid identification. Several other researchers also build CRISPR-based nucleic acid detection approach, the most current use of which is in the identification of COVID-19. In addition to being accurate, DETECTR, a CRISPR-based DNA detection technology, also requires shorter turnaround time. The off-target effect, which can provide false positive results, and the requirement for a high nucleic acid level (viral load), which raises the possibility of false negative results, are the main drawbacks of CRISPR-based diagnostics

(Jamal et al., 2016).

In addition to recognizing viral, bacterial, and fungal infections, CRISPR may also detect cancer cells. Nonetheless, CRISPR-based diagnosis appears to be a promising technique for straightforward, quick, and cost-effective diagnosis of infectious and non-infectious disorders (Prasad et al., 2021).

CRISPR-Cas Systems as Infectious Disease Diagnostics

There have been several reports of CRISPR-based methods for identifying etiologic pathogens' nucleic acids. The primary focus of early research was on type II systems' Cas9. CRISPR-Cas system-based traits were quickly established once the collateral pursuit of type V (Cas12 and Cas14 effector) and VI (Cas13 effector) techniques was utilized (Li et al., 2021).

CRISPR/Cas9 GENE Editing in Avian Vaccines Development

Viral vector has been edited using a variety of techniques in an effort to create recombinant vaccination candidates that are resistant to poultry viruses. The present techniques for altering virally vectored vaccines are labor-intensive, time-consuming, and inefficient, particularly when it comes to purifying steps. Thus, the development of viral vectored vaccines urgently requires a more potent and trustworthy genome editing approach. The CRISPR/Cas9 genome editing strategy is the greatest option since, in addition to offering a simpler and more alternative method than old procedures, it also presents a chance to create multivalent recombinant vaccines that offer concurrent shelter against important avian diseases (Vilela et al., 2020).

CRISPR Technology in Poultry

In the field of genome editing and avian transgenic, chicken is given the status of a model organism for research (Chojnacka-Puchta and Sawicka, 2020).

To counter the foreign invading agents like viruses and plasmids, the natural heritable adaptive immunity is provided to prokaryotes by CRISPR/Ca9 (Barrangou and Marraffini, 2014). This naturally present system of CRISPR is made up of the constituents such as

- Cas genes also known as Cas operon
- An AT sequence rich ladder
- Repeat sequences separated by a spacer sequence, which is unique (Bayat et al., 2018)

The interest in producing the disease resistant chicken having the ability to give more production is increasing with the increase in demand, with genetic engineering considered as the ultimate way to achieve the goal (Sid and Schusser, 2018). It is doubtless that in poultry sector the vaccines are the most reliable disease control strategy.

With the advancement in recombinant DNA technology and the biology of pathogens, the problems related to conventional vaccines have been overcome to a greater extent (Khan et al., 2016; Nadeem et al., 2020).

Mutagenesis (by the use of UV and chemicals), nuclease mediated and recombinase mediated were genome editing methods in animals before endonuclease CRISPR/Cas9 guided by the RNA was discovered (Rocha-Martins et al., 2015).

In CRISPR- Cas system the DNA sequence is cleaved by the Cas nuclease guided by the RNA guide , three bases up streamed of the PAM i.e. protospacer adjacent motif. CRISPR-Cas system has been identified and classified into two classes and six types. The most widely used CRISPR system in biotechnology, translational research purposes and bioengineering is CRISPR-Cas system type II, which have single effector protein. The example of CRISPR-Cas system type II is from *Streptococcus pyogenes* (SpCas9) (Marraffini et al., 2016).

The double strand break is induced in the target sequence with precision by the single guide RNA (sgRNA) with the other components of CRISPR-Cas9 system i.e. Cas9 protein, CRISPR RNA (crRNA), a Cas9 nuclease-recruiting sequence transactivation crRNA (tracrRNA). A homology directed pair (HDR), in the presence of a homologous sequence, or non-homologous end joining (NHEJ) trigger the repair of double strand break (DSB) in the host cell. Some other variants and orthologues of Cas9 have also been described (e.g., StCas9, SaCas9, Cas9n, and Cas12a) .Distinct cleavage actions are performed by these variants and recognize different PAM sequence**.** The recombinant vaccine study has used CRISPR system, using its editing capability, to target the viruses (Soppe et al., 2017), yeasts (Mitsui et al., 2019), bacteria (Zhang et al., 2020) and plant cells (Cao et al., 2020).

In the recent advancement in the use of CRISPR-Cas9 technology in developing the vector and recombinant vaccines, there is a major focus on recombinant viral vaccines. In many biological research programs of animals, humans and plants CRISPR associated systems (Abdi-Hachesoo et al., 2014) and interspaced short palindromic repeats (Teng et al., 2018) are used as the new generation technology of gene-editing.

Editing the viral genomes with a huge potential of research has been made simpler and more efficient using the powerful and straightforward approach of CRISPR/Cas9 system. Presently, the viral genome editing by CRISPR-Cas9 mainly is effectively used for virus gene editing of DNA viruses. There are a lot of technical hindrances that encounter the researchers in developing RNA virus vaccines, as the application in the RNA virus mutagenesis is more difficult to achieve (Pushko et al., 2016).

An endogenous RNA from bacteria have been targeted by a newly discovered Cas9 nuclease of *Francisella novicida* (FnCas9) , which is a Gram-negative bacteria, a eukaryotic cell +ssRNA virus is targeted by the reconstructed gRNA (Vilela et al. 2020), which in turn leads to the inhibition of synthesis of viral proteins in the animal cell. This has

provided a possibility for treating RNA virus infection, thus paved a way to treat the viral infections usingCRISPR-Cas9 system (Teng et al., 2021).

To induce a targeted double-stranded break in foreign DNA, an RNA duplex employed by the complex the endonuclease Cas9 known as crRNA:tracrRNA. This complex can be engineered and adapted into single guide RNA (sgRNA), maintaining its essential characteristics including: a nucleotide sequence for DNA target recognition (crRNA) and a duplex RNA structure capable of binding to Cas9 (tracrRNA) (Doudna and Charpentier, 2014).

CRISPR usage in Vaccine Development against Viruses of Family Herpesviridae

Marek's disease (MD) is caused by the virus of family herpesviridae named Marek's disease virus (MDV), is a most important neoplastic ailment in domestic birds, primarily managed through mass vaccination (Boodhoo 2016). Viral based delivery involves the transfer of the DNA of plasmid to the host cells with the aid of virus as vectors. To one's surprise this method is giving a great advantage in numerous experiments using the CRISPR technology, its application in vaccine development using viral vectors may encounter challenges due to the insufficiently understood mechanism of antagonistic interactions among multiple viruses within host cells. Lipofectamine, a widely utilized chemical transfection technique, facilitates the delivery of CRISPR components. The role of delivery system that is by making the plasmid DNA encapsulated is fulfilled by this chemical vector. Following delivery, cells typically incubate for a period ranging from 12 to 24 hours (Tang et al., 2018).

Scientists in genomics have also achieved success in creating a stable vaccine vector through deleting the virulence factor simultaneously and inserting the antigens into another virus i. e. Infectious Laryngotracheitis of family Herpesviridae by the use of NHEJ-CRISPR/Cas9 and Cre-Lox System (Atasoy et al., 2019).

CRISPR Usage in Vaccine Development against other Viruses

The utilization of this fascinating technology of CRISPR/Cas9 has transformed the process of constructing the vaccines of HVT, making them to be utilized as a potent tool for making vaccines such as; multivalent vaccines. In a study, the vaccinologist have achieved successful generation of a triple gene inserts containing HVT, that is a recombinant HVT., thereby creating a promising candidate for recombinant viral vaccine capable of strongly eliciting protection to combat against three highly virulent avian viral diseases: Avian Influenza Virus (AIV); an avian virus of family Orthomyxoviridae of viruses, Infectious Bursal Disease Virus (IBDV); an avian virus of family Birnaviridae of viruses, Infectious Laryngotracheitis Virus (ILTV); an avian virus of family Herpesviridae of viruses as well as Marek's Disease Virus (MDV); which is also a virus of family Herpesviridae of viruses (Tang et al., 2020).

Likewise, the vaccinologists have also. employed the techniques of HDR-CRISPR/Cas9 for creating a bivalent recombinant HVT candidate for vaccine that expresses the surface antigens of influenza A virus i. e. hemagglutinin (HA) antigen (Chang et al., 2019).

The HDR approach of CRISPR/Cas9, devoid of errors, has been harnessed for the generation of a bivalent HVT-AIV vaccine. Chang et al. (2019) introduced a novel method for selecting HVT-HA recombinant by developing one of the most precise, simple, and rapid adsorption assay for erythrocyte, diverging from conventional methods. This process resulted in an approximate accuracy of ~6% for the insertion of HA of H7N9, suggesting that the NHEJ predominantly plays an important role in silencing of the GFP-negative HVT viruses. CRISPR-associated Cas9 facilitates site-specific genome engineering by inducing a double-strand break (DSB) at a chromosomal location specified by the guide RNA (Cong et al., 2013).

Utilizing the error-free homology-directed repair (HDR) mechanism of CRISPR/Cas9, researchers have developed a bivalent HVT-AIV vaccine. In a study by Chang et al. (2019), the selection of recombinant HVT having HA (hemagglutinin antigen) was facilitated through the developing a very precise and straightforward method with an additional property of giving the assay of rapid erythrocyte adsorption which is a departure from the conventional methods. This innovative approach achieved an approximation of an accuracy of 6% for the insertion of HA of H7N9, this indicates that the NHEJ has predominantly silenced the majority of HVT viruses negative for GFP (Vilela et al., 2020).

CRISPR Usage in Vaccine Development against Retro Viruses

HTLV-1, a human retrovirus with tumorigenic properties, contributes to the development of the carcinogenic conditions like leukemia/lymphoma (ATL) and a disorder of nervous system (TSP/ HAM). It attains immortality and persistence in ($CD4^+$ T) lymphocytes throughout the lifespan of the host. Key contributors to HTLV-1-induced proliferation and transformation are the viral genes hbz and tax. The later i. e. Tax, is being transcribed by the plus-sense strand, which in- turn is vital for both initial infection and cellular immortalization. Hb is transcribed from the minus-strand that helps in the proliferation and the survival of the infected cells by the means of mRNA forms and its protein. Disruption in the function or the expression of hbz and/or tax via editing in genome and the break repair of mutagenic double-strand that could be a reason of hindering of the growth and potential damage to the immortality of HTLV-1-infected cells, potentially preventing immune modulation and associated diseases. Additionally, the HTLV-1 viral genome displays high conservation and sequence homogeneity, allowing for more precise guide RNA targeting. Furthermore, numerous models of animals that are well-established exist for investigating the in vivo infection of HTLV-1 and in vitro immortalization of the cell. As a consequence, research on HTLV-1 might offer valuable insights into advancing genome editing approaches to combat and

boost immunity against retroviral infections when came into utilization in vitro as well as in vivo (Vilela et al., 2020).

Future of CRSPR

There has been a rapid increase seen in the number of technologies that can perform gene editing from several years in the past. Both DNA and RNA have been edited with a great precision and a very high frequency. For a wide range of disorders, these technologies hold a great potential to be used as therapeutics because they can correct the diseases that cause mutation via its repair mechanism. These mutations are seen in most types of cells, which makes the uses of these technologies more versatile. It should also be considered though that overlapping capabilities are seen in many of these platforms. A critical assessment of the immunogenicity will also be extensively required for an effective implementation of next-generation CRISPR technologies. Theoretically it is believed that DNA editing technologies specifically have the capability to control their own expression by self-inactivation. DNA technologies do not need to be continuously monitored to assure their sustained effect. On the other hand RNA technologies need a continuous expression for a sustained effect when used in therapeutics, which could lead to the risk of generating an immune response in the host. The study of the adverse effects of use of next- generation technologies of CRISPR or the continuous expression of Cas13 can be conducted by studying and using them as models in researches. Summarizing this all , it can be said that next-generation DNA and RNA genome editing technologies possess a magnificent capability to be used in an advanced level of gene and cell therapies (Gaj, 2021).

Prospects of CRISPR Technology

Until recently, most researches have been conducted on viruses and CRISPR/Cas9 gene editing mechanism and these researches have focused on three primary aspects:

(1) Employing the CRISPR/Cas9 technique to remove viral pathogenic genes shows promise for gene therapy applications against various diseases. However, extensive research and practice are necessary before it can be clinically applied.

(2) Modifying certain functional viral genes is a quick and direct genetic engineering method that can be utilized to study the mechanisms of virus-induced pathogenesis and tumorigenesis.

(3) Creating modifications in the genome of virus through deletion of gene, substitution of gene, and insertion of gene to create strains of vaccine possible with diversity in recombinant DNA technology, resulting in a more innovative and effective approach to recombinant vaccine researches and productions (Teng et al., 2021).

However, long time would be taken to determine the gene editing site specifically and stability of passages of the reconstituted viruses. Creating and developing any creative approach is an important step in the advancement of science and technology. Without a doubt, gene editing technology based on CRISPR/Cas9- offers limitless opportunities for advanced researches in science and developing the vaccines in future to address key unresolved difficulties in biology (Teng et al., 2021).

Conclusion

The gateway to various scientific opportunities in the molecular interrogation of viral genetic determinants of the interaction of the viruses with the hosts, their pathogenicity , their gene deletion and/or recombinant vaccines, have been opened by the recent advancements in the development of this technology that is completely accordant with CRISPR/Cas9. Thanks to its unique advantages of simple design, high efficiency, low cost, and broad application; however, some shortcomings, particularly the possibility of "off-target" effects, are causing some concern.

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Chapter 02

Introduction to Livestock Vaccines and Immunity

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ABSTRACT

The ultimate goal of livestock vaccination is to protect the healthier animals from pathogen invasion, capable of causing diseases. It's a convenient, steadfast, and secure means to protect the animals and mitigate further infection. Vaccine hinders pathogenicity by, elevating the immune system and meddling the pathogen cascade of infection. This section covers several aspects of livestock vaccination such as historical background, purpose of vaccination, methods of administration, expostulation in vaccination, and immune feedback. A vaccine is an attenuated biological agent administered to healthy people or maybe to animals with a paramount goal of protection from pathogens. Nevertheless, Vaccination is important for dairy farms as an outbreak if struck might cause economic losses. Livestock vaccinations on the other hand have numerous challenges from, the supply and demand sides, along with technical, logistical, and economic issues, and socioeconomic and psychological impairments. A multifaceted task such as trust-building, mass communication, and awareness regarding vaccination is required to cope with these challenges and boost up immunization and the health status of the herd. This chapter covers several sections pertaining to livestock vaccination and host immunity that will assist the researcher and veterinarian to protect the animal's health and improve the well-being and productivity of livestock herds.

KEYWORDS
Vaccine, Livestock, Pathogen, Health, Socioeconomics

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INTRODUCTION

The process of vaccine intrusion into a host so that to protect an organism from a disease or infection is called vaccination. It is a convenient, steadfast, and secure means to protect the animals and mitigate further infection. Vaccine hinders the pathogenicity by, elevating the host immune system and meddling the pathogen cascade of infection. As a result of vaccination the host immune system synthesizes antibodies similar to the way it does in actual pathogen infection. Vaccines, however, do not cause the disease because they only contain weaker or killed forms of bacteria or viruses (World Health Organization [WHO], 2021). Across low- and middle-income nations, livestock is vital to smallholder households' general well-being. Due to zoonotic illnesses and the detrimental social and economic effects that animal diseases have on those whose livelihoods depend on livestock, there is an unbreakable link between the health of animals and humans. Vaccines serve as a vital link between the pastoralist livestock industry and intensive industrial animal systems, maintaining the health of cattle. They are essential in helping people who have a hard time gettingstronger and bouncing back from tough situations. The development of livestock vaccines is progressing in a way that is consistent with the Sustainable Development Goals (SDGs). The SDG Goal 2 is specifically focused on eradicating hunger, guaranteeing food security, improving nutrition, and promoting sustainable agricultural practices (United Nations, 2021). Vaccination is the most used method in veterinary medicine and the most economical intervention for the prevention and control of illnesses in animals (Roeder and Rich, 2009).

Vaccine

A vaccine is a biological agent designed to be given to healthy people or animals in order to protect them from pathogens that might cause illness, such as bacteria, viruses, or other microbes. These bacteria are either eliminated from their capacity to cause disease or their toxic effects are countered (Altuğ et al., 2013). The disease agents'

antigenic structures, toxins released from their cells, or inactivated (dead) or attenuated (weakened) forms can all be utilised as vaccines (Shams, 2005). Most vaccinations used in modern cattle procedures across the world are either attenuated or inactivated. Additionally, vaccines are essential to the ongoing worldwide effort to eradicate peste des petits ruminants (PPR), a disease that has serious economic ramifications in many regions of the world (Baron et al., 2017). In veterinary medicine, where this technology has been embraced far more quickly than in human medicine, viral vectored vaccinations, particularly those employing vectors like adenovirus, herpesvirus, and poxvirus, are commonly used (Draper and Heeney, 2009).

Smallpox Vaccination: A Historical Illustration of the Connections between Human Vaccination and Animal Vaccine

The best example of the close relationship between animal and human immunization is the history of smallpox vaccines, which is also arguably the most documented. In light of the 1979 declaration of the disease's eradication and the "long and arduous hunting down of the disease," as French historian Pierre Darmon put it, it seems fitting to reminisce a little about this intriguing tale that illustrates the connections between animal and human vaccination.

History of Livestock Vaccines

In order to prevent chicken cholera in 1879 and sheep and cattle anthrax in 1881, a vaccine was created by French scientist Louis Pasteur. To conduct research and development in the fields of livestock and veterinary sciences, the United States government established the Bureau of Animal Industry under the Department of Agriculture in 1884. Prevalent vaccination types included live attenuated, inactivated, subunit, recombinant, polysaccharide, conjugate, and toxoid vaccines before the introduction of mRNA vaccines (Plotkin et al., 2013). According to Patel and Heldens (2009), the use of live-attenuated or killed whole organism-based vaccinations proved to be highly effective in controlling the transmission of many infectious illnesses, such as rinderpest, equine infectious anemia, and classic swine fever. The mRNA vaccines were created to cure cancer; infectious diseases were not frequently linked to them (Pardi et al., 2018).

The Need for Livestock Vaccination

In developing nations, the need for dairy, eggs, and meat is increasing due to the expanding human population as well as rising living standards (Henchion et al., 2021). The livestock species have seen changes in recent decades, with larger and denser populations. Infectious diseases that impair the well-being and productivity of livestock herds result in economic losses and unstable food supply. Moreover, public health is seriously threatened by the zoonotic spread of pathogenic organisms from food producing animals to people. A variety of measures are employed to manage infectious diseases affecting livestock species, such as maintaining facility hygiene, isolating or eliminating contaminated animals, selecting genetic stock that is resistant to the disease, administering antibiotics, and vaccinating animals (Kraham, 2017; Kahrs, 2008).

Purpose of Vaccination/Importance of Veterinary Vaccines

The main goal of livestock vaccinations is protecting the herd from dangerous diseases. Immunizations are crucial for sustaining the health of livestock by preventing infections that could have serious adverse effects on an animal's health. Vaccines minimize the chance of disease by boosting the immune system and training the body to identify and fight particular pathogens. Additionally, vaccination is vital for dairy farms because disease outbreaks can result in enormous financial losses. Vaccination reduces the need for culling, veterinary expenses, and production losses by lowering the risk of spreading diseases within the herd (Hairgrove and Hammack, 2023; Lombard et al., 2017). Moreover, vaccination improves herd health generally, which lowers stress and enhances living conditions for animals. Another important advantage of vaccination is group protection. The vaccines work effectively when given to a whole population of livestock, building a collective resistance against common diseases (Sander et al., 2020). Additionally, vaccination contributes to the herd's development of immunological memory. The immune system is prepared to identify specific germs and build a quick defense if exposed to each vaccination and booster, thereby enhancing the protective effects of immunization (Roth, 2011). The veterinary vaccinations are used in livestock and poultry to keep animals healthy and increase overall productivity. More efficient animal production and improved availability of high-quality protein are critical for feeding the rising population (FAO, 2009). The veterinary vaccination in livestock is intended to prevent and manage animal illnesses, as well as to prevent disease in food animals in order to avoid zoonosis or infection in human consumers and to increase the efficiency of food animal production (Roth JA. 2011). Veterinary vaccinations are critical to animal health, welfare, food production, and public health. This is an inexpensive way to prevent animal illness, improve food production efficiency, and minimize or prevent the spread of zoonotic and foodborne pathogens to humans. The safe and effective animal vaccinations are necessary for contemporary.

Vaccines are made to mimic the existence of a pathogen (a bacteria or virus), without truly causing the disease (Rappuoli and Vozza, 2022). Vaccines work by introducing inactive components of the pathogen, such as genetic material or proteins, which successfully trigger the immune system. After vaccination, the immune system considers these foreign substances invaders. Specified cells, like antigen-presenting cells, ingest and process the components of vaccines (Storni et al., 2005). This encounter trains the immune system that becomes adapted to the pathogen. The memory cells (T and B cells) are programmed to identify the particular pathogen. If that individual later comes into contact with the real

pathogen; For example, through a natural infection this priming enables a quick and effective response. The body directly reacts, as it already possesses a memory of the pathogen from the vaccine. Pathogens are neutralized by the antibodies, and the infected cells are destroyed by the killer T lymphocytes (Siegrist, 2008; Aickelin et al., 2004).

Challenges Facing Livestock Immunization Systems

The research on the fairness and efficacy of vaccination distribution networks has underlined many of these restrictions, yet a gendered analysis and deeper knowledge of the vaccine system remain lacking (Acosta et al., 2019). The livestock vaccination systems have challenges from both supply and demand sides, including technical, logistical, and financial concerns, as well as socioeconomic and psychological impediments (Alders and Spradbrow 2009). The livestock keepers in impoverished nations are shown to have low demand for vaccines due to cost and distance, as well as a lack of faith in the system and safety concerns (Abakar, 2018). The desire to pay for vaccinations and the willingness to vaccinate also rely on the level of awareness about vaccination programs and the market orientation of cattle.

Application of Genomics to Improve Livestock

Nearly 25 years have gone since much of the early molecular genetics research in cattle began. Since then, initiatives in the livestock community have included gene identification, gene mapping, quantitative trait loci (QTL) discovery, genome-wide association studies, and, most recently, the completion of the first sequencing for all major livestock species. Many individual genes have been found and used in livestock selection methods, and whole genome analysis and genomic selection have lately gained traction (Rothschild and Plastow, 2014). The use of gene identification, mapping, and QTL discoveries in cattle resulted in the development of a variety of DNA markers that were used in selection programs across various species. These include but are not limited to gene markers DGAT1, GHR, MC1R, MSUD, ASS, and CAST in cattle.

Types of Vaccines

Vaccines come in various forms, all aimed at training the immune system of the host to defend it against pathogens and the life-threatening diseases they cause.

Inactivated Vaccines

Inactivated vaccines, sometimes known as killed vaccines, are made up of bacteria, viruses, or other infectious pathogens that have been cultured and then destroyed to make them incapable of causing sicknesses. These immunizations stimulate an immune response in animals, resulting in the formation of memory cells and antibodies. For instance, a vaccine to treat swine pyrexia, influenza in birds, FMDs (Foot and Mouth Disease), and also brucellosis comes under the category of dormant or inactivated vaccines. The vaccines are credible to be used for pregnant animals and those having fragile immune systems. Such vaccines enhance and sustain the health status of the animals which had a counter impact in the form of long-lasting food yield and economic stability (Fanelli et al., 2022; Tizard, 2019).

Live-attenuated Vaccines

This class of vaccine can be tagged often modified live virus vaccines also play a pivotal role in the health status of the cattle. During such vaccination, the real existing virus has been genetically altered and is then intruded into the host, such vaccines own the potential to trigger and provoke the immune system with the peculiar goal of disease containment. Nevertheless, these modified viruses still possess ample ability to infect but are introduced to mitigate the disease. The immune responses due to these vaccines may be of variable nature as may be in the form of cellular response or response via antibodies (humoral response). Several vaccines as a precedent include those used to treat swine pyrexia, bird influenza, and foot-and-mouth disease (Mebatsion, 2021; Yadav et al., 2020; Lauring et al., 2010; Domenech et al., 2010).

Messenger RNA (mRNA) Vaccines

The use of messenger ribonucleic acid (mRNA) vaccines is a unique approach to safeguarding animal health. These vaccines operate by stimulating an immune response in cattle via messenger RNA. When administered, the vaccine prepares the animal's immune system to recognize and eradicate certain infections. It is worth noting that the mRNA remains within the targeted cells and is never released into the food supply. Contrary to popular belief, there are no mRNA vaccines in the food we consume. Producers make educated immunization selections after conferring with herd veterinarians. Correcting misunderstandings and supporting appropriate vaccine usage will unlock the potential of mRNA vaccines in protecting animals while ensuring food safety (Rzymski et al., 2023; Villa et al., 2021).

Subunit, Recombinant, Polysaccharide, and Conjugate Vaccines

Subunit Vaccines

These vaccines are not manufactured from the entire organism, but rather from specific sections of pathogens. They concentrate on the immune system's reaction to dangerous bacteria coated in sugar. These vaccines function by targeting the bacterial surface, allowing the body to develop immunizations and combat the infection. Subunit vaccines are safe for a range of cattle, including those with impaired immune systems, because they do not contain live pathogens (Josefsberg and Buckland, 2012).

Recombinant Vaccines

Recombinant vaccines use genetic engineering methods to generate particular pathogen proteins. These proteins being vaccine ingredients have the potential to generate immunological responses in the form of a cascade of reactions. Recombinant vaccines are potent enough to guard cattle from a variety of infections (Hansson, 2000).

Polysaccharide Vaccines

These kinds of vaccines focus on the capsulated or sugar-coated bacteria and generate immune reactions against them. The mode of response in such a case is targeted and focuses on the bacterial surface in order to completely destroy the bacterium (Bashiri, et al., 2020).

Conjugate Vaccines

Such vaccines are blend or mixture of the pathogen ingredient (usually a polysaccharide) and other molecules. It consequently elevates the immune feedback. This type of immunization is useful while mitigating and abolishing infections caused by sugar-coated bacteria which finally improves the health of cattle to a greater extent (Berti and Adamo, 2013).

Toxoid Vaccines

Toxoid vaccines are given when an infection is caused by toxins of a bacterium. These vaccines are comprised of toxoids, which are inactivated forms of bacterial toxins. Toxoid immunizations can protect animals from illnesses like tetanus and botulism by strengthening their immune systems against these toxins (Zaragoza et al., 2019).

Viral Vector Vaccines

Vaccines with viral vectors, which employ viruses as vaccine agents, are a new veterinary medicinal therapy. To make these genetically engineered vaccinations, DNA encoding key antigens is introduced into a viral vector. The viral vector expresses these antigens upon injection, triggering cellular and humoral immune responses. The benefits include increased safety, lower manufacturing costs, dual immunological responses, and the capacity to discriminate between vaccinated and sick animals. These immunizations prevent illness in wildlife, food animals, and companion animals (McCann et al., 2022; Aida et al., 2021).

Vaccine Administration

In cattle, numerous vaccine delivery routes provide effective immunization. For example, vaccines are delivered intramuscularly (IM) by injecting them into a muscle, usually the thigh or neck. Intramuscular injections are commonly used to maintain systemic immunity (Cserep, 2008). Then the subcutaneous injections are administered in the cervical (neck) and near the ear. Such vaccinations are convenient and effective as well for a wide range of species (Turner et al., 2011; Morton et al., 2001). Another route for vaccine administration includes the oral route which is considered beneficial for large-scale management. The final method is administration through the nasal route which is widely used in pigs and poultry and has a positive impact and generates both systemic and local immunological feedback (Davis, 2001; Bowersock and Martin., 1999).

For managing and preventing infectious diseases, boosting immunity, and maintaining the overall health and production of herds, vaccination is the better option and an important activity. Herd immunity is a situation in which a significant part of a herd develops resistance to a particular disease as a result of earlier exposure or immunization (Balakrishnan and Rekha, 2018). Even animals who have not received vaccinations remain safe when a significant portion of the herd possesses immunity, which significantly reduces the infectious pathogen's ability to spread. In order to keep the herd healthy and prevent disease outbreaks, this phenomenon is essential. By obtaining herd immunity through extensive immunization, we can protect livestock populations and enhance the well-being of all animals (Reiss, 2015; Meeusen et al., 2007).

Livestock vaccination’s acceptability and effectiveness are influenced by a number of issues and debates. The most common challenges to vaccinations include the unwillingness of livestock owners to receive vaccinations, concerns about side effects and safety, misinformation, economic considerations, and adherence to regulations. To overcome these difficulties and enhance immunization and overall herd health, trust-building, open communication, and education are required (Hubach and Tonne, 2022 Lewis and Roth, 2021).

The Immune Response to Vaccination

Distinguishing factors for acquired immunity are memory and specificity of the pathogen. Such an immune system is triggered upon exposure to pathogens or may to antigens. Humoral immunity and cell-mediated immunity (CMI) both together constitute acquired immunity. Within humoral immunity, plasma cells a kind of specialist B lymphocytes synthesize the primary feline classes of immunoglobulin namely IgG, IgM, IgA, and IgE (Margie et al., 2013). The CMI consists of T lymphocytes, which include verities of T cells as, helper, regulatory, and T cytotoxic cells which all together assist in vaccine immunity (Saalmuller, 2006). Soon after vaccination B and T cells get activated, and these cells have the potential of epitope recognition located on the foreign invader or pathogen such as virus, bacteria, and parasites. Based on life span effector cells linger for a very little while (days to a week) whereas, B and T cells last for the long term and may

turn or develop into effector cells upon the repeated invasion by similar pathogen. Memory cells do not linger around just because of the repeated exposure of the pathogen but sometimes may be due to the activation of the non-specific agents for instance commensal bacteria or may be due to environmental irritants, which cause low-level cellular development. B and T Memory cells collaborate to protect the vaccinated animal against infection later in life. The immunological memory provides the foundation for protective vaccinations (Zinkernagel, 2003).

If vaccination stops future infection, the concerned animal is considered to have sterilizing immunity, and the immunological responses are not at peak since the first day of pathogen intrusion. This type of immunity can arise after being immunized against feline panleukopenia virus and rabies virus (Schultz, 2006). When vaccination fails to prevent infection (e.g., feline herpesvirus-1 and feline calicivirus), systemic and local CMI, as well as humoral immunity, particularly local IgA antibodies, provide protective but non-sterilizing immunity, lowering disease severity (Gaskell et al., 2002).

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Chapter 03

Introduction, History, Types of Vaccines, and Physiology of Immunization

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ABSTRACT

This book chapter provides a comprehensive overview of the mechanisms through which vaccines protect against diseases and contribute to public health. It begins by describing the natural defense of the body against pathogens, including skin and mucous membranes like physical barriers, and the immune response, highlighting the role of antigens and antibodies. The immune system goes on to produce precise antibodies against the pathogen; this can either be a bacterium, virus, parasite, or fungus. These antibodies precisely target the pathogen's antigen. Vaccines work by priming the immune system for it to later recognize and destroy infectious microbes, in short, by injecting a weakened, inactivated, or partial form of the pathogen into the body. In response, our immune system produces antibodies as "warriors," which will destroy that specific infection, while producing at the same time the memory cells that will stay in our body for a very long period after the first exposure. In the case of re-exposure to the same pathogen, these cells are capable of producing the antibody at a much quicker rate, hence the reaction in order to prevent illness is quicker and stronger. Vaccines also evoke herd immunity. If a large portion of people have become immune to an infection, there is little chance of one getting infected by another person. This gives even them some level of protection to people who cannot be vaccinated—such as those with some allergic conditions, diseases, or other diseases resulting from other health conditions—because the disease has less of a chance to establish itself in the population. The bottom line: in general, vaccines are critically important for the prevention of disease. They have helped control or eliminate many killer infections and have resulted in improved health and longevity around the globe.

KEYWORDS
Immunization, Smallpox, Influenza, Herpes simplex, Malaria, Gonorrhea, HIV, Vector, Recombinant vaccines

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INTRODUCTION

A vaccine is a synthetic or naturally occurring substance that protects against a specific illness or infection by inducing an immune response. In order to defend organisms against real attacks or diseases, the pathogen must first be attenuated or destroyed before it can be introduced into them. This process then activates the body's immune system to manufacture antibodies. In most cases, vaccination will include an agent that mimics a disease-causing bacterium; this agent may be a deceased or weakened version of the microorganism, its toxins, or even a surface protein. An immune response is triggered when an agent is introduced into the body. The immune system then goes on to eliminate the agent and any associated bacteria. (Immunization: The Basics, CDC, 2022).

The administering of vaccine is termed vaccination. Vaccination or immunization is the most effective approach to preventing the infectious diseases (United States CDC, 2011.) Indeed, mostly due to the immunity of the people created by vaccination, smallpox has been eradicated worldwide. From much of the planet, diseases such as tetanus, measles, and polio are now brought down chiefly by the immunity of the people created by vaccination. The World Health Organization (WHO) reports that twenty-five specific preventable diseases are now licensed for immunizations (WHO, Global Vaccine Action Plan 2011-20.).

History

Intentional variolation with the smallpox virus may prevent smallpox before vaccines made from cowpox were used. Around the ninth century, the use of variolation as a treatment for smallpox was first noticed in China (Needham et al., 2000). Variolation was initially used by the Chinese in the fourteenth century, which is the first recorded use of the term. They began using a method called "nasal insufflation" wherein powdered smallpox material, typically scabs, was blown into the nose. Several blowing methods were documented in China in the 1600s and 1700s (Williams, 2010). In 1700, the Royal Society in London received two papers about the practice of inoculation in China: one from Clopton Havers and the other

from Martin Lister, who had acquired information from a Chinese representative of the East India Company (Silverstein, 2009). In his French work "Voltaire, 1742%," the author claims that the Chinese were engaged in inoculation with virus almost a century ago.

After seeing variolation in Turkey, Mary Wortley Montagu brought her four-year-old daughter back to England in 1721 and had her variolated in front of Royal Court physicians (Williams, 2010). Six inmates at London's Newgate Prison were subjected to experimental variolation by Charles Maitland later that year (Fenner et al., 1988). After the successful experiment, the royal family began to acknowledge variolation and play a role in its widespread adoption. However, in 1783, a few weeks afterprince Octavius of the United Kingdom was sick andexpired (Baxby, 1984). In 1796, medical professional Edward Jenner collected fluid from wound off the hand of a milking man suffering the cowpox invaded into the elbow of a kid who was approximately 8 years old, James Phipps and a half months later variolated his child with smallpox, subsequently finding that he did not get smallpox (Stern, 2005: Dunn, 1996). Jenner intensified his experiments and, in 1798, claimed that his vaccine proved harmless in both kids as well as elders, and might transfer immunity together, which removed dependency on unstable supply from infected cows (Baxby, 1984). In 1804, the Spanish Balmis smallpox vaccine moved to Spain's colonies Mexico, and Philippines using the arm-to-arm distribution strategy to get around the reality the vaccine lasted for only 12 days *in-vitro*. They exploited cowpox (Burgen, 2021). Since cowpox vaccination was substantially safer than other variants vaccine, the latter, although still routinely used in England, was forbidden in 1840 (Van Sant, 2008: Didgeon, 1963).

Following the work of Jenner, the second generation of vaccinations was stimulated by Louis Pasteur in the 1880s. Pasteur invented vaccination for anthrax and chicken cholera. From the last decades of the 19th century, prideful nationalistic perspective was taken toward vaccines (Pasteur, 1881). It was formed national immunization strategies, and required vaccination statutes were adopted (Stern and Markel, 2005). In the year 1932, Alice Miles Woodruff and Ernest Goodpasture found that fowl pox virus could easily be propagated in the embryonated chicken egg. Replication of the fowl pox virus has also occurred in embryos and replicated in other embryonated bird eggs. Eggs were also exploited for viral proliferation in the production of a vaccine used to prevent yellow fever in 1935 and a vaccine for influenza in 1945. The medium for growth and cell culture technology overtook eggs as the main technology of viral replication for vaccines in 1959 (Louten, 2016).

Thus, vaccinology moved very successfully through the twentieth century - if at all - in the sense that during this century, a whole set of successful vaccines was discovered against various diseases: diphtheria, rubella, measles, and mumps. Other major successes comprised the advancement of a polio vaccine in the mid-fifties and the elimination of smallpox in the sixties and seventies. Maurice Hilleman stood out among the vaccine developers in the 20th century. With their popularity, many began to take it for granted; however, vaccinesfor some of the most important infections, e.g., gonorrhea, herpes simplex, HIV, and malaria, still remain elusive (Stern and Markel, 2005: Baarda and Sikora, 2015).

Types of Vaccines

Vaccines usually hold live weakened, killed, or inactivated bacteria or viruses or their pure derivatives. There are different types of immunization in use ('Vaccine kinds,' 2012). These demonstrate various approaches that are used to decrease the hazard of disease while maintaining the ability to raise a good immunological response.

Attenuated Vaccine

Few immunizations include live weakened microbes. Some of these are virus strains cultured during circumstances that weaken their infection causing capabilities or use closely associated but less risky species to elicit a heightened immune response. While many vaccines are virus-based, some are bacteriological. Examples of the former cases are yellow fever, rubella, measles, and mumps, while that of the latter case is typhoid. The Calmette and Guérin live vaccine developed for M. *tuberculosis* (TB) contains a non-infectious strain of the live bacterium. It has a virulently altered strain, which has been named "BCG," designed to induce an immune response from the body upon vaccination with it. For the vaccination against plague, a live attenuated vaccine using strain Y. pestis will be produced. Weakened vaccines have so many pros and cons. Live vaccines or live attenuated vaccines have the disadvantage that they tend to provoke an immune response that is more prolonged than that of non-replicating or killed vaccines. However, the pathogenic forms of the fungi may not be acceptable for use in immunodeficient persons and may develop very rarely from the nonpathogenic forms (Sinha and Bhattacharya, 2014).

Inactivated Vaccine

Some vaccines contain deactivated, former, and harmful microorganisms that have been killed by heat, chemical agents, or radiation—ghosts, along with an intact but blank bacteriological cell membrane ('Vaccine Types,' 2022). They are said to be in between attenuated and inactivated vaccines. For instance, rabies vaccines; most influenza vaccines; IPV, which is inactivated polio vaccine; and hepatitis A vaccine ('Different Types of vaccinations: History of Vaccines,' 2019).

Toxoid Vaccine

These vaccines are those developed from deactivated dangerous chemicals that are source of the disease, except the germs. For example, one of the vaccines based on toxoids is for tetanus and diphtheria. In no way do toxoids apply just to

bacteria; for example, the use of Toxoid Crotalus Atrox is evident for dog vaccination against bites from rattlesnakes ('Different Types of Vaccines: History of Vaccines,' 2019).

Subunit Vaccine

Instead of the whole-agent vaccination that exposes the immune system of an organism to an entire agent or to microorganisms that are attenuated but would compromise it, a subunit vaccination involves only the use of a fragment. Some of the examples include vaccine of Hepatitis B composed of the surface antigen of the virus (originally derived from blood serum of persistently infected persons, but now it is produced by recombination of viral DNA into yeast) ('A Look at Each Vaccine: Hepatitis B Vaccine,' 2014). For instance, take the edible algal vaccines. One of such vaccines, derived from the main capsid protein of the virus and represents a virus-like particle vaccine, is against the human papillomavirus (HPV) ("HPV Vaccine," 2019). Furthermore, proteins named hemagglutinin and neuraminidase of the influenza virus represent yet one more example ("History of Vaccines," 2019). Subunit vaccines are being used in plague immunization (Williamson et al., 1995).

Conjugate Vaccine

Some bacteria bear a polysaccharide in their outermost coat, which is weakly immunogenic. Attachment of such polysaccharides to a protein (like a toxin) may force the immune system of the host to recognize the polysaccharides in a manner similar to a protein antigen. This is applied to an approach in an outer membrane vesicle of influenza type B ('Polysaccharide Protein Conjugate Vaccines,'2019).

Outer Membrane Vesicle Vaccine

This has in turn proved membrane vesicles to be innately immunogenic and hence the potential to manipulate them into yielding strong inoculations. The most recognized outer membrane vaccines are those created for serotype B meningococcal disease (Pollard and Bijker, 2020: Pol et al., 2015).

Heterotypic Vaccine

Heterologous vaccinations also referred to as "Jennerian vaccines," are those that contain pathogens of another species that also don't cause sickness or else the organism being treated only generates a mild disease. The classic instance is the use of cowpox for protection against smallpox by Jenner. The use of BCG vaccine is a current example, derived from the bacteria Mycobacterium bovis, for prevention of T (Scott, 2004).

Gene-based Vaccine

Genetic vaccines act on the nucleic acid principle of adsorption into the host cells; then, a protein is formed in accordance with the nucleic acid design. Subunit vaccines, on the other hand, tend to be less effective in the induction of response to the immune system; they, in general, consist of single antigenic proteins combined with small molecules of adjuvants. In general, the surface protein is normally the immunodominant-antigen from the infectious pathogen that enables stimulation for the development of neutralizing antibodies.

Viral Vector

Viral vector vaccines work by introducing harmless viruses into the body to deliver pathogenic genes that mostly code for surface proteins—a specific type of antigen that recruits the immune system to fight off infection ('Vaccine Types,' 2019: 'Understanding and Explaining Viral Vector COVID-19 Vaccines,' 2021).

RNA

The vaccine having mRNA is a new type of vaccine made of mRNA as part of the central part capsule in a vector with lipid particles (Garde and Feuerstein, 2020). Among these are some COVID-19 vaccines, including various RNA vaccines intended for the COVID-19 pandemic; a number of them have been officially permitted or have received reserve use authorization in different countries. Studies highlight that Moderna and Pfizer-BioNTech mRNA vaccines have been approved and recommended by the FDA for both adult and child administration. ('Covid-19 and Your Health,' 2021: Banks, 2020: Branswell, 2020).

DNA

A DNA plasmid is used by a DNA vaccine (pDNA) that encrypts for a protein based on antigen derived from the disease against which the vaccination was aimed. Since pDNA is relatively low-cost and stable, it therefore becomes very safe to use in the delivery of vaccines (Cuffari, 2021). The potential advantages of this approach over prior ones include B- and T-cell activation, increased vaccine durability, no involvement of an infectious agent in administration, and mass production being easier ('DNA Vaccines,' 2024).

Experimental Vaccine

Many new vaccinations are also under research and usage.

- Dendritic cell vaccines merge dendritic cells with antigens to transport them to the WBCs, so eliciting an

immunological response. These have demonstrated some positive early outcomes for the treatment of brain tumors and are also explored in the treatment of malignant melanoma (Kim and Liau, 2010: Anguille et al., 2014).

- Recombinant vector — by combining the DNA of one microorganism with another, immunity may be produced to fight with the ailment that has intricate contamination processes. RVSV-ZEBOV vaccine is a prime example that is licensed to literature that has been in use (2018) to fight Ebola in Congo (McKenzie, 2018).
- There is a type of vaccine that is under development for several disorders employing infected individuals of stomatitis, Valley Fever, and atopic dermatitis and known as T-cell receptor peptide vaccines. These peptides have been identified to impact cytokine production and increase cell-mediated immunity.
- Targeting identified bacterial proteins that are associated with complement suppression would nullify the key bacterial virulence pathway (Meri et al., 2008).
- Plasmid usage was established in preclinical experiments to say it a defensive vaccine method for tumors and transferrable illnesses. However, in medical research, this method has failed to provide substantial improvement clinically. The general efficiency of plasmid DNA vaccination relies on enhancing the plasmid's immunogenicity while correcting for parameters that are involved in the specific immune effector cell activation (Lowe, 2008).
- Bacterial vector – These are similar in principle to viral vector vaccines, but utilizing bacteria alternatively (Pollard and Bijker, 2021).
- Antigen-presenting cell (Pollard and Bijker, 2021).

Whereas many vaccines are developed from deactivated or weakened agents from microorganisms, synthetic vaccines are made up almost entirely or entirely of synthetic peptides, polysaccharides, or antigens.

Valence

Vaccines come either in univalent or polyvalent. A univalent vaccine is an immunization given by injection into the human body to elicit an immune response or protection against one antigen or single disease only. Vaccines that are polyvalent are that which aim at immunizing against two or more genotypes of a single pathogen or two or more additional pathogens. ('Polyvalent vaccination,' 2012). Multivalent vaccine valency may be expressed with the use of a Greek or Latin prefix (e.g., bivalent, trivalent, tetravalent/quadrivalent). In this view, a monovalent vaccine may have utility in that it may rapidly engage an intense immunological response. These two vaccines can interact when two or more immunizations are mixed in the same composition ('Question and Answers on Monovalent Oral Polio Vaccination Type 1,' 2005).

Interactions

When two vaccines can interact two or more immunizations are mixed in the same composition, this maximum commonly occurs with live weakened immunizations, when one of the vaccine mechanisms proves to be more robust than the others and limits the development and immunological reactivity to other parts of the vaccine (Gizurarson, 1998).

Such effects were first noticed with the trivalent Sabin polio vaccine, which can first be observed when the quantity of Serotype 2 virus in the immunization has to be decreased to keep it from affecting the "take" of the Serotype 1 and 3 viruses in the vaccine (Sutter et al., 1999). It was further noted that the vaccines for dengue predominated with the DEN-3 serotype, thus decreasing the immune response to the DEN-1, -2, and -4 serotypes in the year 2001 (Kanesa-thasan et al., 2001).

Other Contents

A dose of vaccine contains a lot of other elements (inactivating agents, residual cultured cell materials, residual antibiotics, preservatives, etc.), with the active immunogen making up a small proportion of the total mass of liquid. One dosage may have hardly a few nanograms of viral particles or micrograms of bacterial polysaccharides. The vaccine is put into the bloodstream through different routes i.e. oral drops or even a nasal spray that is mostly water, mixed with additional compounds to enhance the immunity response and ensure safety or help in storage, and a very tiny quantity of leftover components are in it from the manufacturing process. In particular, at times, these substances could source a sensitive response in individuals who are especially subtle to them.

Adjuvants

The adjuvants added to vaccines are usually meant to improve immune response. For example, high adsorption on the alum adsorbent onto tetanus toxoid is seen. This, in essence, spreads the antigenic material in a way so as to elicit a response that is much greater than that caused by plain aqueous tetanus toxoid. People who show an unfavorable adsorbed tetanus toxoid reaction could serve to provide the basic immunization when the time for booster came (Engler et al., 2006).

The whole-cellular pertussis vaccine had been used to some extent in advance of the Persian Gulf War in 1991 as an adjuvant for anthrax inoculation. This will produce a faster immunity response compared to providing just the anthrax vaccine (Sox et al., 2000).

Preservatives

This occurs maximum and frequently with live attenuated immunizations when one of the vaccine components is proven to be stronger than the others, limiting the development and immunological reactivity to other parts of the

vaccine. The vaccines could also contain preservatives to avoid microbial contamination. Thiomersal, as a preservative agent (thimerosal in the US and Japan), was until recently present in the vaccines that were subjected to many vaccinations and did not contain live viruses. Until 2005, US-recommended influenza vaccine contained thiomersal in more than trace amounts, a flu vaccine recommended for children. It should only be given to children with specified risk factors ("Thimerosal Table," n.d.). No single-dose influenza vaccines that do not contain thiomersal are available in the UK. The preservatives are consumed at different phases in the manufacturing of vaccines, and the residues of these compounds can be detected in the final product by the most modern methods of measurements, just as these residues can be detected in the environment and the population at large ('Measurements of Non-gaseous Air Pollutants,' 2023).

Most immunizations require preservatives; among them are those for preventing sudden fatal effects, such as death from staphylococcus infection in 12 of 21 newborns treated with a diphtheria vaccine lacking a preservation agent in 1928. Some of the preservatives in the market today include thiomersal, phenoxyethanol, and formaldehyde. Thiomersal is more operative in fighting bacteria, allows shelf life for a longer time, and increases the stability and security of the vaccine. It is, therefore, no longer in use as a preservative in children's vaccines in the U.S., the European Union, and a few other advanced countries, for safety against its mercury content (Bigham and Copes, 2005). However, such accusations and controversies, whether thiomersal is the causative agent of autism or not, have no credible scientific grounds at all (Paul and Offit, 2007). Another study with the observation of children for 10-11 years, which included 657,462 children, found that the incidence of autism among vaccinated children with MMR does not increase; on the contrary, it was noted that children who had not been vaccinated increased the incidence of autism by 7 percent (Rapaport, L., 2019: Hoffman, 2019).

Excipients

Along with active ingredient in the vaccine, the following excipients and residual processing chemicals are existing or may be existing in vaccination formulations (CDC, 2022):

- Adjuvants are aluminum salts or gels used to enable a smaller vaccine dose by eliciting an earlier, stronger response that is also more persistent with immunization.
- Antibiotics are supplementary to some immunizations to suppress the formation of bacteria during production and preservation of the vaccine.
- The flu and yellow fever vaccines do contain protein from eggs, among others, that are actually prepared using chicken eggs.
- Formaldehyde is applied to deactivate the toxoid vaccines used in disabling the bacterial products. Formaldehyde is, therefore, applied in the process to deactivate undesirable viruses and eliminative bacteria that might contaminate the vaccine during its production.
- MonMSG, 2-phenoxyethanol: a stabilizer added to some vaccines in order to protect against heat, light, acidity, or moisture inactivation.
- Thiomersal is a mercury-antibiotic preservative added to multidose vials of vaccines to avoid contamination and subsequent multiplication of possible pathogens. This has resulted in the removal of thiomersal from maximum vaccines, other than multi-use influenza, where the level has been reduced to an extent such that one dose would contain less than a microgram of mercury, a similar level to consume 10 grams of canned tuna ("Mercury Levels in Commercial Fish and Shellfish (1990-2012)," 2022).

Physiology of Immunization

The germs are present all around us. They are present in the environment and in our body. In a feeble person, the attack of a harmful organism can cause a person to have a disease or even die. The body employs several ways of helping repel pathogens (disease-causing germs). The three physical barriers that help the body keep the pathogen out of the entire system are the skin, mucus, and cilia. When a virus does get inside the body, the immune defenses in our body are alerted, and then the illness is fought and gotten rid of or demolished. (WHO, Vaccines Explained series, 2020).

Natural Response of the Body

A pathogen is any type of bacterium, virus, parasite, or fungus that, in and of itself, can be a reason for sickness within the body. Each type of microorganism has a lot of sub-parts, often specific to that exact pathogen and the disease that it causes. The antigen, therefore, is the constituent of a pathogen that activates the production of antibodies. Antibodies elaborated to antigens of a pathogen remain an important part of the immune system. If you will, look at the antibodies as the soldiers of the body's defense system. Every one of these antibodies inside our system is trained or made to recognize just one single kind of antigen. There may be hundreds of different kinds of these antibodies in the body. It takes some time for the immune reaction to respond by forming specific antibodies in reaction to the presented antigen when the human body is in contact with an antigen for the first time. In the meantime, he is susceptible to getting sick. These antigen-specific antibodies, when formed, act along with the rest of the immune system toward the clearance of the infection and termination of the disease.

Antibodies to single pathogen usually don't provide immunity against another disease until when two diseases are substantially related to one another, like cousins. After the body has produced the first antibodies in reaction to an antigen, it also makes memory cells, which produce antibodies. These last a long time after the disease is destroyed by the

antibodies. But if the body should come into contact with the same disease again, then the antibody reaction will be extremely rapid and much stronger than it was the very first time around because memory cells have already prepared to send out the antibodies to respond against that antigen. This means that whenever a person is again exposed to the harmful virus in future, his/her body's immune system will be able to respond quickly in order to protect it against the disease (WHO, Vaccines Explained series, 2020).

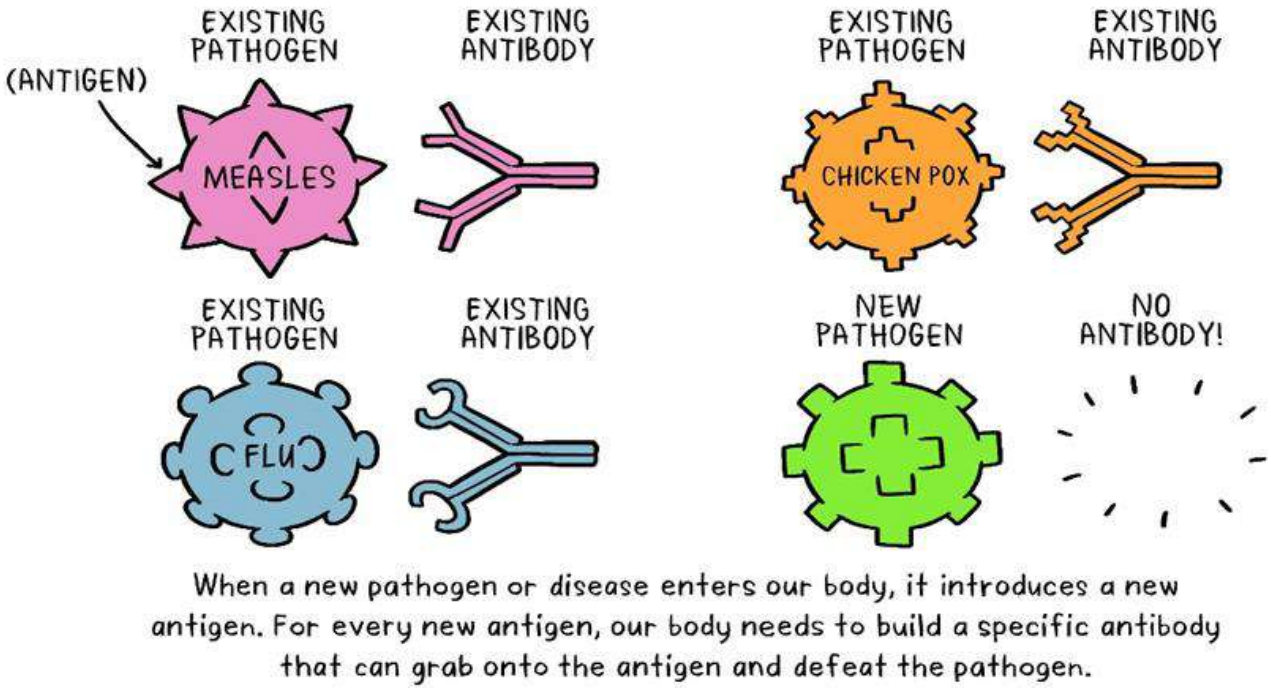

Fig. 1: Vaccines Antibody illustration (WHO, Vaccines Explained series, 2020).

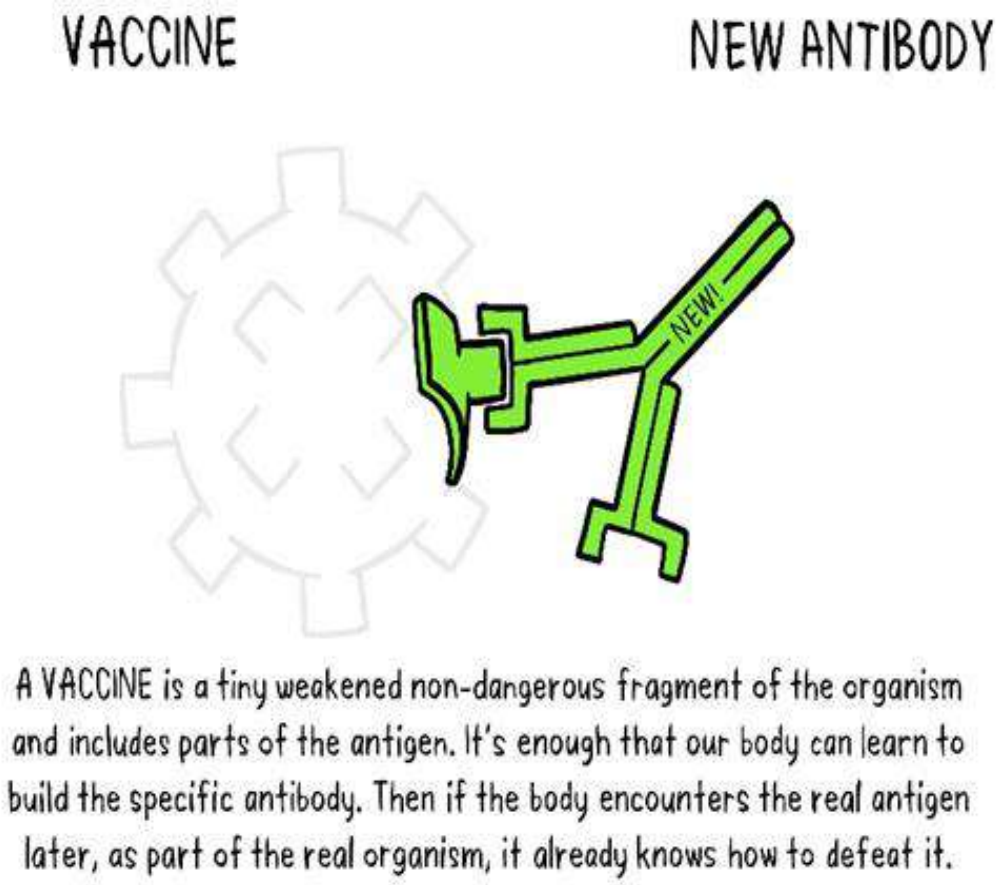

Fig. 2: Vaccines Antibody illustration (WHO, Vaccines Explained series, 2020).

How Vaccines Help

Vaccines contain live, weakened, or dead organisms (antigens) that stimulate a response from the immune system within the body. Some of the latest vaccines contain instructions for the human body to make a synthetic antigen, not the antigen itself. Whether the vaccine consists of the antigen itself or its antigenic portion/component so that it bodies the immune response, this will not be able to cause the illness in the person getting the vaccine, but it will do so in a way that allows the individual's immune system to react as fully as it would have done had it been exposed to the live pathogen for the first-time pathogen.

Some vaccines may need to be given in repeated doses with time intervals after some time as boosters. This is required in order to afford the assembly of long-lived antibodies and proliferation of memory cells. In this strategy, the body is trained to fight off the particular pathogen that causes the illness, building up the memory of the infection so as to be able to promptly fight it off should it ever be challenged in the future (WHO, Vaccines explained series, 2020)

Collective Immunity

When one person gets vaccine, he is very likely to be safe from the particular disease. However not every person can be vaccinated against infection. Some vaccinations may not be given to immunize persons who have certain underlying medical conditions that may interfere with their capability to fend off infection, such as cancer or HIV, or to people with major sensitivities to a given constituent of the vaccine. These people may still be immunized if they live in and among those who are immunized. The illness may have difficulty spreading from person to person because most of the people that it meets are immune. So, the more other people get vaccines, lesser would be the chance for such individuals who were not able to get immunized ever to expose themselves to the detrimental germs. This is called herd or collective immunity.

This is particularly important when people who can't get vaccinated and who could be even more at risk for the ailments that we're vaccinating against. No one vaccine gives 100% protection; even group immunity does not give total defense to the ones who cannot be safely vaccinated. But with this type of immunity, these people will be provided significant protection courtesy of their surroundings being vaccinated.

This will protect not just oneself but allows them to protect the other members of society who might not be able to get immunization. Man has succeeded with time by being able to manufacture immunizations against a wide range of life-threatening infections, including meningitis, tetanus, measles, and wild poliovirus. In the 19th century, at the turn of the century, an extremely common disease affected hundreds of thousands of people annually. It was in the year 1950 that two successful immunizations against it were discovered. But during this time, immunization in some countries of the world was still not at the level to prevent the spread of polio, mainly in Africa. And during 1980, a well-organized multinational movement to remove polio from the planet commenced. For many years and many decades now, polio vaccination has often been done during the frequent immunization visits, which take place on all continents, as well as the massive vaccination programs. Millions of children, majorly, had been vaccinated, and in August 2020, it was confirmed that the continent of Africa was free of wild poliovirus, so there were no longer any wild polioviruses on all other continents of the universe except Pakistan and Afghanistan, where the polio virus has not been wiped out (WHO, Vaccines explained series, 2020).

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Chapter 04

Historical Evolution of Vaccines: Milestones in Immunization

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ABSTRACT

Vaccines have played a crucial role in preventing infectious illnesses over the last two centuries and are regarded as significant achievements in the field of medicine and public health. Conventional vaccination methods have been used for several bacterial and viral infections; yet, there are certain instances when their efficacy has been lacking. However, it is expected that the increased use of newly developed pneumococcal conjugate and rotavirus vaccinations would lead to a subsequent reduction in childhood mortality. Nevertheless, several diseases that are significant for public health are intricate and/or undergoing fast changes, presenting distinct challenges to the creation of vaccines. Some of the problems include a limited understanding of the process of immunity formation, genetic diversity across hosts and pathogens and a growing concern within society about the safety of vaccines. Novel vaccine technologies have the capacity to enhance the advancement of vaccinations that specifically target several categories of bacteria that are resistant to multiple drugs. Enhanced comprehension of the mechanisms behind microbial transmission throughout populations is facilitating the adoption of more logical immunization strategies on a worldwide level, perhaps resulting in the elimination of several infections. Considerable focus is now being directed towards enhancing the accessibility of vaccinations by means of the advancement of combination vaccines and the use of less intrusive inoculation methodologies.

KEYWORDS
Vaccine, Vaccination techniques, Future prospects, Infectious diseases

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INTRODUCTION

A vaccine is a biological preparation that offers protection against a specific infectious or cancerous illness through active acquired immunity (Kavanaugh et al., 2023). A vaccine is usually composed of weakened or destroyed versions of the pathogen, its toxins, or one of its surface proteins. It usually comprises an agent that mimics a disease-causing bacterium. The agent triggers the immune system to identify the agent as a threat, eliminate it, and then identify and eliminate any associated bacteria that the body may come into contact with in the future. The process of administering vaccines is called vaccination. Vaccination is the most effective way to prevent infectious diseases and is thought to prevent 2-3 million lives yearly. An additional 1.5 million fatalities may be prevented if vaccination rates were raised globally (Frieden et al., 2011; WHO, 2020). Aside from the local eradication of certain diseases like measles, vaccinations have led to the global eradication of two important infections, rinderpest and smallpox, and can offer long-term, cost-effective protection (Greenwood, 2014; McKee et al., 2018). Vaccines are widely recognized for their safety and efficacy, often considered safer than many therapeutic medications. Their immense public health impact places them second only to safe drinking water in terms of overall health benefits.

Edward Jenner and Smallpox Vaccine

Edward Jenner has gained international recognition for his pioneering contributions to the field of vaccination and the effective eradication of smallpox (Lakhani, 1992). For an extended period, he had been informed that dairymaids who had previously been infected with cowpox had inherent immunity to smallpox. Upon careful consideration, Jenner arrived at the conclusion that cowpox had the potential to be transmitted between individuals as a deliberate defensive strategy, in addition to its role in safeguarding against smallpox. In May 1796, Edward Jenner encountered a young dairymaid named Sarah Nelms, who displayed fresh cowpox lesions on her hands and arms. On May 14, 1796, utilizing material from Nelms' lesions, Jenner inoculated an 8-year-old boy named James Phipps. Following the procedure, the boy experienced mild

fever and discomfort in the armpits. Nine days later, he exhibited symptoms of feeling cold and a loss of appetite, but by the following day, his condition had significantly improved. Jenner vaccinated the boy again in July 1796, using material obtained from a recently healed smallpox lesion. He reached the conclusion that protection was complete and no illness manifested (Willis, 1997). A small booklet named "An Inquiry into the Causes and Effects of the Variolae Vaccinae," which Jenner privately published, described the ailment known as "Cow Pox" that was seen in several western counties of England, particularly Gloucestershire (Winkelstein, 1992; Willis, 1997). Jenner chose to name this new treatment vaccination since the Latin words for cow and cowpox are vacca and vaccinia, respectively. Fig. 1 illustrates the discovery of different vaccines throughout the historic time period.

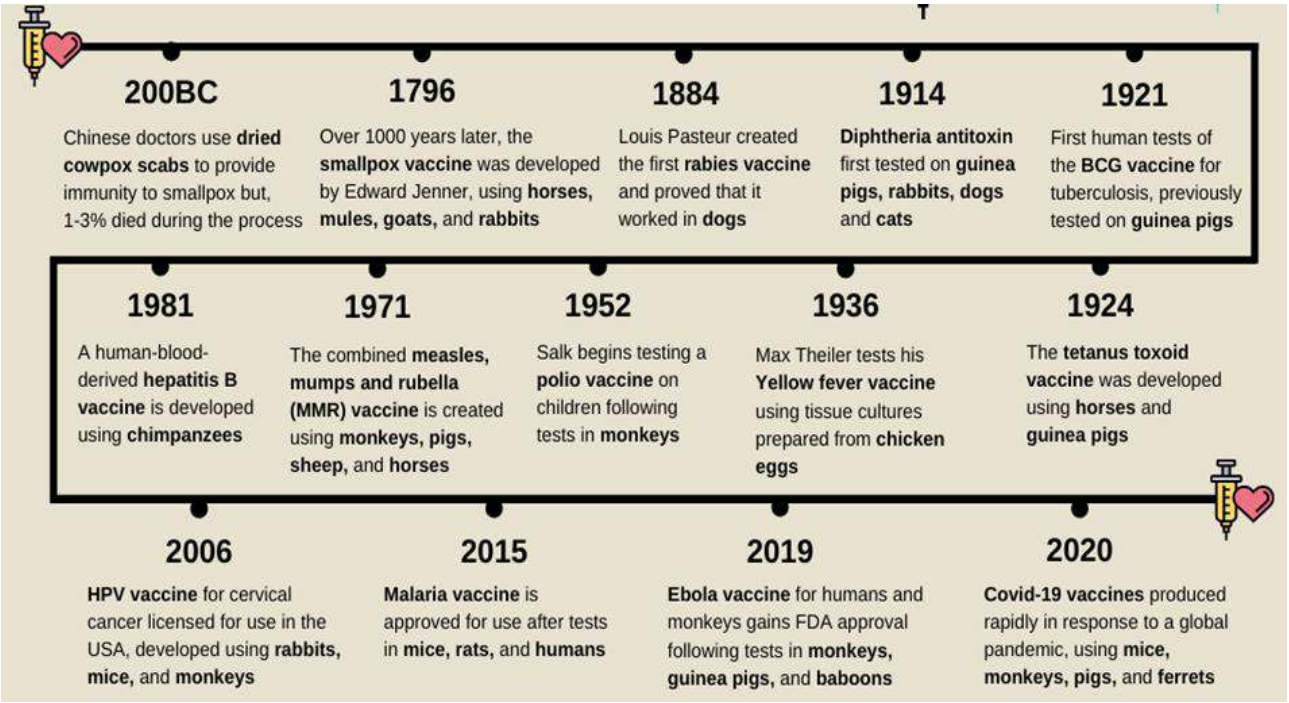

Fig. 1: Diagram showing the brief history of vaccines

Jenner's Success in Vaccination

Through the intentional use of vaccination, Jenner's achievement was the first scientific effort to manage an infectious disease. With time, the numerous examples that Jenner and others who adopted his technique documented proved that cowpox effectively protected the smallpox for the majority (though not all) of people who were exposed to it. By counting and contrasting the number of instances of smallpox among those who received the cowpox vaccination and those who did not, they employed the scientific method to study smallpox epidemics. The individuals who were immunized against smallpox with cowpox had a lower percentage of smallpox cases than the general population, indicating the efficiency of the immunization. In order to demonstrate that the cowpox vaccination was effective, Jenner and his supporters were essentially gathering and analyzing epidemiological data, even though the field of epidemiology was not founded until the middle to late 19th century.

Development of Vaccination Techniques

The development process of a vaccine may span many months or even years, often ranging from ten to fifteen years, starting with the first identification of the disease's causative agent until the vaccine is accessible to the public. The development of vaccines is a significant and ongoing problem, particularly for high-endemic areas or populations at high risk of infection.

Live Attenuated Vaccines

A live attenuated vaccine is one that maintains the pathogen's viability or liveness while decreasing its pathogenic potential (Badgett et al., 2002). An infectious agent undergoes attenuation, which changes it to make it less virulent or harmless (Pulendran and Ahmed, 2011). Attenuated vaccinations result in a rapid onset of immunity and a more robust and long-lasting immune response (Tretyakova et al., 2013; Gil et al., 2020) The way attenuated vaccines work is by stimulating the body to produce memory immune cells and antibodies in response to the particular infection that they are meant to protect against. Diverse properties of vaccines are shown in Fig 2. Vaccinations against mumps, measles, yellow fever, rubella and several types of influenza are frequently attenuated live vaccinations (Zou et al., 2018).

Inactivation of Pathogens for Vaccine Development

The development of vaccines demands extremely precise requirements for virus inactivation. To effectively elicit a host immune response, the viral proteins' structural integrity must be maintained. In particular, the capacity to trigger the production of neutralizing antibodies is frequently crucial. (Raviprakash et al., 2013; Pinto et al., 2013). Up to 2020, there were six approved viral vaccinations that were inactivated using either formaldehyde or beta-propiolactone (BPL), which have been the most widely utilized inactivating techniques for vaccine deployment since the 1920s (Wilton et al., 2014; Schneider et al., 2015). More recently, whole-virus vaccines against SARS-CoV-2 have been produced using BPL, which is also used to inactivate influenza and rabies vaccines (Heinz and Stiasny, 2021). However, the significance of these inactivation methods has been increasingly challenged as safer and more advanced alternatives have become accessible. For example, studies employing psoralens as an inactivation technique to produce a whole-virus SARS-CoV-2 vaccine have demonstrated encouraging outcomes (Sundaram et al., 2021). Compared to live-attenuated vaccinations, inactivated vaccines often have a greater safety profile, and reversion into active illness is extremely rare. In addition to being less reactogenic, they have a reduced immunogenicity and need multiple doses to produce a protective effect (Sanders et al., 2015).

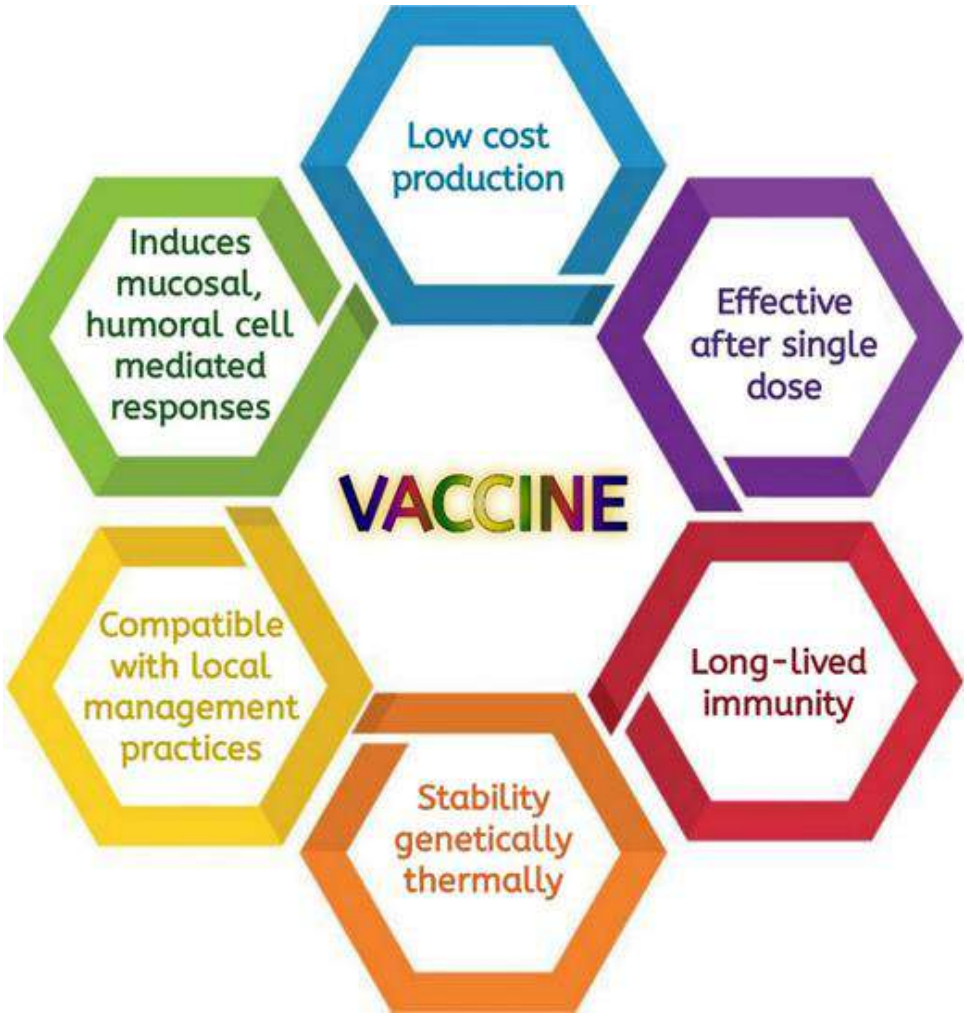

Fig. 2: Various properties of vaccines

Advancements in Vaccine Production and Administration

One of the best methods for preventing and controlling serious, and occasionally fatal, infectious diseases is vaccination, also known as vaccine administration (Strassburg, 1982). The last century of vaccine development has been characterized by conventional manufacturing technologies, which provide effective protection against illnesses such as smallpox, polio, measles, as well as others with high rates of disability and mortality. These technologies have well-established infrastructure and resources, and their development costs already spread out over time. These technologies, while well-understood and efficacious, are constrained by their slow, empirical, and costly development process, as well as their transient protection against several infections. Genetic engineering and better cell-culture techniques are examples of technological advancements that could help lower costs, increase production output, add knowledge, and improve our ability to adapt more quickly to new threats as we move to the production platforms of the next generation of vaccines (Ghattas et al., 2021).

Next-generation technologies for vaccine development such as mRNA and DNA derived vaccines, present a fascinating and promising pathway because of their low cost, high potency, safety, and quick mass deployment. These

platforms are especially important in the case of immune-evading complex infections. Furthermore, these platforms may provide effective treatments for non-infectious disorders, including cancer, in contrast to vaccines derived conventionally (Ghattas et al., 2021).

The Golden Age of Vaccines

The golden age marked a significant and constructive turning point in the history of public health worldwide. The optimism of the "golden age" promoted entry and large investments in the research, development, production, sales as well as marketing of vaccines.

Vaccines for Diphtheria, Tetanus and Pertussis

The prevalence of infectious illnesses including diphtheria, tetanus, and pertussis (DTP) has dramatically decreased over time because of global infant immunization campaigns. Nonetheless, there is mounting data that suggests that a substantial portion of the population still lacks immunity to infection. The recent diphtheria epidemic that killed over 4,000 people in the 1990s in the newly independent states of the former Soviet Union served as evidence of this (Vitek, 1998). The resurgence of epidemics like this has prompted a reassessment of disease control strategies, underscoring the significance of attaining and sustaining high vaccination coverage rates among infants and addressing the immunity gap in adults (Dittman, 1997). Due to the combined diphtheria, tetanus, and pertussis vaccination initiatives, natural exposure to these diseases during childhood is now uncommon in many regions worldwide, with immunity acquired almost exclusively through immunization. Research indicates that immunity diminishes over time, as serological data suggest a decline in serum antibody concentrations to diphtheria and tetanus with age in the general population (Matzkin et al., 1985; Gasparini et al., 1997; Matheï et al., 1997; Kjeldsen et al., 1998; Von et al., 2000).

Jonas Salk's Inactivated Polio Vaccine

Salk and his colleagues killed the poliovirus with formaldehyde while preserving its antigenic qualities. They gave the vaccine to a large number of volunteers, including him, his wife, and their kids, after proving its efficacy and safety. In 1954, Salk conducted a countrywide research study with over one million pediatric volunteers. The following year, on April 12, 1955, he unveiled the results, confirming the efficacy and safety of the immunization. According to later estimates, there were about 29,000 cases of poliomyelitis in the United States in 1955. The newly designed vaccine was mass produced, and two years later the infection rate fell to less than 6,000. The Salk vaccination was quickly embraced across the country and, by 1959, it had been distributed to nearly 90 nations (Tan and Ponstein, 2019).

Expansion of Vaccination Programs

In order to boost the global acceptance of routine pediatric vaccinations, The World Health Organization (WHO) established the Expanded Programme on Vaccination (EPI) in 1974. This campaign has achieved remarkable success, as seen by the rapid increase in coverage rates of EPI immunizations from less than 5 to over 80% in several low- and low-middle-income countries (Harris et al., 2014).

Global Eradication Efforts for Smallpox

Only one of the seven attempts to date to completely eradicate infectious diseases in humans has been successful: the smallpox epidemic (Hopkins, 1998). When Edward Jenner discovered in 1796 that those who had cowpox, a relatively minor illness, become immune to smallpox, it marked a significant advancement toward developing a preventative method against smallpox (Jenner, 2023). An alternative approach to eliminate smallpox, which did not depend on widespread vaccination, was ultimately implemented. The approach was used by smallpox workers in West Africa and known as surveillance and containment (Foege et al., 1971). The surveillance process included the use of systematic searches, enhanced reporting systems, and active source tracking to identify and investigate cases. Several nations provided financial incentives to individuals who provided information that resulted in the identification of a smallpox case. Containment measures included the practice of isolating patients and administering vaccinations to all individuals in known or suspected contact. The primary aim of this technique was to effectively contain epidemics within designated geographic regions, hence mitigating the spread of the disease into unaffected areas.

Vaccines for Measles, Mumps and Rubella

The MMR vaccine is designed to elicit an immunological response that confers protection against measles, mumps, and rubella. The vaccination in question is classified as live attenuated, indicating that it is a benign and less potent form of the infectious pathogens it aims to protect against. Due to its live attenuated nature, the MMR vaccination exhibits exceptional efficiency, albeit it needs multiple doses to attain immunity. The projected effectiveness of the MMR vaccination in preventing measles with a second dose is 99%, while its efficiency in preventing mumps is above 95%, and its efficacy in preventing rubella after a single dose is 90%.

Progress in Vaccine Development through Technological Advancements

While conventional vaccinations have demonstrated significant effectiveness, numerous infectious diseases remain

unaddressed due to the absence of effective vaccines. The progress made in creating vaccines for several human infections, including tuberculosis (TB), human immunodeficiency virus (HIV), cytomegalovirus (CMV), respiratory syncytial virus (RSV), Epstein-Barr virus (EBV) and herpes simplex virus (HSV) has been lacking (Pandey and Galvani, 2019). The need to overcome limitations in traditional vaccine platforms has led to the development and progress of novel vaccination technologies. The aforementioned methodologies encompass viral vector vaccines (Barouch and Picker, 2014) and nucleic acid vaccines. The aforementioned developing technologies have the potential to tackle unaddressed medical requirements, which include the production of vaccinations containing antigens that pose difficulties or the rapid creation of vaccines for novel diseases. The mRNA exhibits characteristics of non-infectiousness, non-integration, and rapid degradation through normal cellular processes following injection. This characteristic serves to mitigate the potential for toxicity and long-term adverse consequences. mRNA vaccines have garnered significant interest in recent years due to their promise to accelerate vaccine development, enhance safety and effectiveness, and address diseases that have proven resistant to alternative methods.

Conjugate Vaccines

One variant of bacterial vaccination involves the chemical conjugation of a protein molecule with a minute quantity of the polysaccharide constituent of the bacterium's cell covering. The administration of the vaccination enhances the immunological response. Illustrative instances encompass Haemophilus influenzae type b (Hib), meningococcal and pneumococcal conjugate vaccines. Conjugate vaccines have demonstrated remarkable success since their introduction over two decades ago. The conjugate vaccine targeting Haemophilus influenzae Type B has effectively mitigated the prevalence of invasive Haemophilus influenzae Type B disease throughout significant regions globally. The primary factors contributing to its efficacy lie in its capacity to elicit immunologic memory and mitigate asymptomatic carriage, hence facilitating the dissemination of infection. The initial pneumococcal conjugate vaccination was granted licensure in the year 2000 and has since garnered significant popularity in the United States. In 1999, the UK started a statewide effort to introduce the initial meningococcal conjugate vaccine (Mäkelä and Käyhty, 2002). Notwithstanding the extensive body of research conducted on conjugate vaccines, the specific molecular mechanisms underlying the processing of polysaccharides that are conjugated to protein carriers as T-dependent antigens remain elusive, necessitating further investigation. The efficacy of these vaccines in practical applications is noteworthy, despite our limited comprehension of the underlying mechanism. Hence, it is expected that the *N. meningitidis* serogroup C and multivalent *S. pneumoniae* conjugate vaccines would be promptly approved for licensure (Goldblatt, 2000).

Advancements in Adjuvants and Vaccine Delivery Systems

A substance that augments the immune response against a vaccination antigen is commonly referred to as an adjuvant. Oftentimes, the antigen itself exhibits low immunogenicity, necessitating the use of an adjuvant to enhance the immune response. The introduction of adjuvants in vaccines can serve to direct the specific immune response that is elicited. The consideration of this aspect holds particular significance in the context of vaccine development for human immunodeficiency virus (HIV), cancer or the mucosal immune system. An adjuvant is incorporated into a vaccination to stimulate a qualitative modification of the immune response. Adjuvants are being increasingly employed in the creation of vaccines to enhance specific types of immunity that are not efficiently produced by the non-adjuvanted antigens. Some of the benefits encompassed in this context are the acceleration of robust immune responses, extension of their duration, stimulation of local mucosal immune responses, generation of antibodies with enhanced affinity and neutralization capacity, augmentation of immune responses in individuals with compromised immune systems, enhancement of response rate in individuals with low responsiveness, and reduction of the necessary quantity of antigen. As a result, the incorporation of these adjuvants has the potential to result in decreased expenses within vaccination initiatives.

The primary objective of incorporating adjuvants into vaccine delivery methods is to selectively target their effects primarily on Antigen Presenting Cells, while minimizing their impact on non-immune cells. Prior to developing vaccine delivery methods capable of eliciting robust immune responses in both the target population and the pathogen, some prerequisites must be met. The utilization of adjuvant system (AS) technology has facilitated the advancement of research in the field of combined delivery of antigens with one or more adjuvants. The interface between the innate immune response and the subsequent impact on the adaptive response can be further controlled in the context of the AS strategy.

New Frontiers in Vaccinology

Vaccines against Emerging Infectious Diseases

Vaccines are the most reliable way to reduce the danger of a pandemic and epidemic, serving as the fundamental basis for managing outbreaks of infectious diseases. Each type of vaccine is specifically functional for specific targeting, as shown in Fig. 3. Using mRNA technology, two COVID-19 vaccines (Pfizer–BioNTech and Moderna) were created (Polack et al., 2020; Baden et al., 2021), both of which shown excellent effectiveness and safety. The vaccines for human papillomavirus and hepatitis B consist of virus-like particles that possess characteristics of safety, high immunogenicity, efficacy, and ease of large-scale production. Additionally, the technology is readily transportable. Each pathogen is distinct, ranging from whole inactivated viruses (like cholera, polio and SARS-CoV-2) to live attenuated vaccines (such as chikungunya, SARS-CoV-2 and polio). These vaccines may also need to be manufactured at biosafety level 3 depending on

the pathogen, which might restrict the potential for technology transfer to increase the global production capability. Additional vaccinations rely on recombinant vector platforms, which can be further classified into live attenuated vectors like the vesicular stomatitis virus (VSV) vector or the measles-based vector, as well as highly attenuated vectors such as modified vaccinia Ankara (MVA) and nonreplicating vectors like adenovirus 5 (Ad5). For both the HIV and Ebola vaccines, a heterologous prime-boost (HPB) vaccination strategy has been thoroughly investigated. Additional HPB combinations combining DNA, mRNA, and vaccines based on viral vectors and proteins should be taken into consideration. HIV and Ebola vaccines have both been made using viral vectors, including Ad5, Ad26, and MVA (Pollard et al., 2020).

Significant advancements could hasten availability. These include the establishment of a no-fault compensation system for major detrimental effects associated with vaccine administration, standard indemnity as well as responsibility language that can be agreed upon by all manufacturers, the adoption of production date rather than expiration for monitoring shelf life, standardization of regulatory harmonization, and standardization of vaccine labeling are all crucial aspects of the COVID-19 vaccine response that are being worked on and must be optimized for future outbreaks.

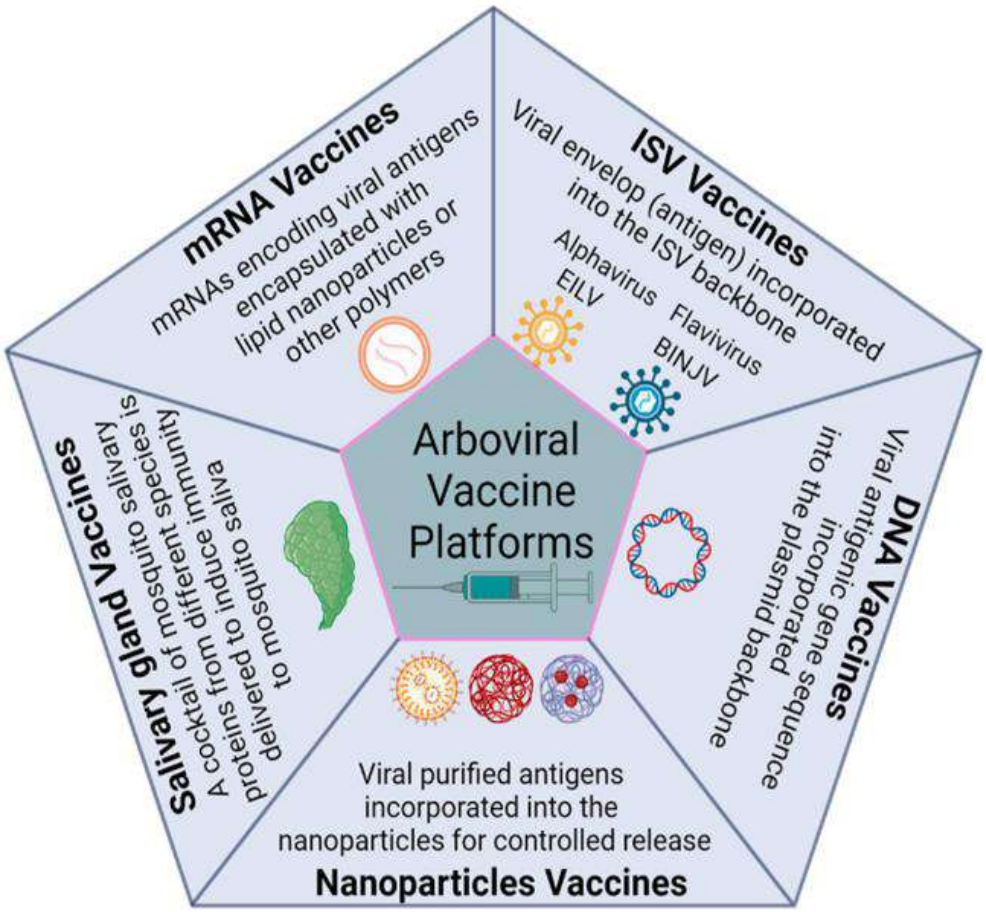

Fig. 3: Different vaccine platforms showing specific potential of each vaccination type

Vaccines for Ratavirus

Rotaviruses are the most common cause of serious gastroenteritis in infants and young children worldwide. As a consequence, severe dehydration, electrolyte imbalance, and acid-base disruption account for almost half of the fatalities in this age group each year (Parashar et al., 2006). Rotavirus vaccinations are intended to prevent moderate-to-severe sickness, imitating the defenses offered by spontaneous rotavirus infections. The two vaccines that are now authorized for use were independently designed, using distinct biological concepts, in order to attain immunity against a diverse array of circulating rotavirus serotypes. The pentavalent rotavirus vaccine (PRV) is an oral vaccination that provides live protection against stomach acid. It is kept at a temperature range of 2-8°C and comprises five strains of human-bovine reassortant rotavirus that are suspended in a liquid suspension with buffering (Cortese et al., 2009). The replication efficiency of animal-derived strains inside the human intestine is comparatively lower than that of human strains. The administration of PRV involves a three-dose oral series, commencing between 6 and 14 weeks of age. Subsequent doses are taken at intervals of 4 to 10 weeks, with the last dosage being given before 8 months of age (Cortese et al., 2009). The human strain rotavirus vaccine (HRV) is an orally administered live vaccination, including a solitary strain of the human rotavirus, namely G1P. The administration of HRV follows a two-dose regimen, commencing at around 6-14 weeks of age, and ending with second dosage at the age of 8 months (with variations in maximum age limits across different nations). It is recommended to maintain a minimum 4-week gap between each treatment. Since the release of the two key rotavirus vaccine studies in 2006, the licensing of PRV is observed in more than 90 countries,

with its inclusion in a minimum of seven national immunization schedules. Similarly, HRV has been licensed in over 125 countries and has been integrated into the global immunization initiatives of 24 countries. The World Health Organization (WHO) issued a proposal in 2009, suggesting that newborns around the globe should get immunization against rotaviruses (Dennehy, 2007; Bjur and Jacobson, 2009).

Vaccination in the 21st Century

Role of Vaccines in Addressing Antimicrobial Resistance

The escalating issue of antimicrobial resistance (AMR) is a significant global health concern. Pathogens that are resistant to treatment, such as viruses, parasites, fungi, and particularly bacteria, result in substantial illness and death. There are several justifications for seeing vaccinations as one of the most promising preventive measures to tackle the difficulties posed by antimicrobial resistance (AMR). Initially, vaccinations have the ability to directly avert infections caused by highly destructive AMR organisms. Moreover, they indirectly mitigate the use of antibiotics by diminishing the symptoms that often elicit antibiotic usage. Vaccines effectively inhibit the growth of bacteria, preventing them from reaching the required quantities to develop resistance mutations shown in Fig. 4. Another important reason for considering vaccines for antimicrobial resistance (AMR) lies in the fact that the capacity of immunizations to impact resistant pathogens is not only theoretical (Rappuoli et al., 2017). Research conducted in Africa on the impact of the pneumococcal conjugate vaccination has shown a decrease in cases of resistant invasive pneumococcal illness, as well as a reduction in antibiotic use, or both (Klugman and Black, 2018). Over the course of the previous ten years, significant progress in the domains of immunology, genetics, structural biology, and microbiology has facilitated the emergence of vaccine technologies that hold promise for significantly enhancing the likelihood of efficacy in preventive measures against infections caused by antimicrobial resistance (AMR) pathogens (Delany et al., 2014).

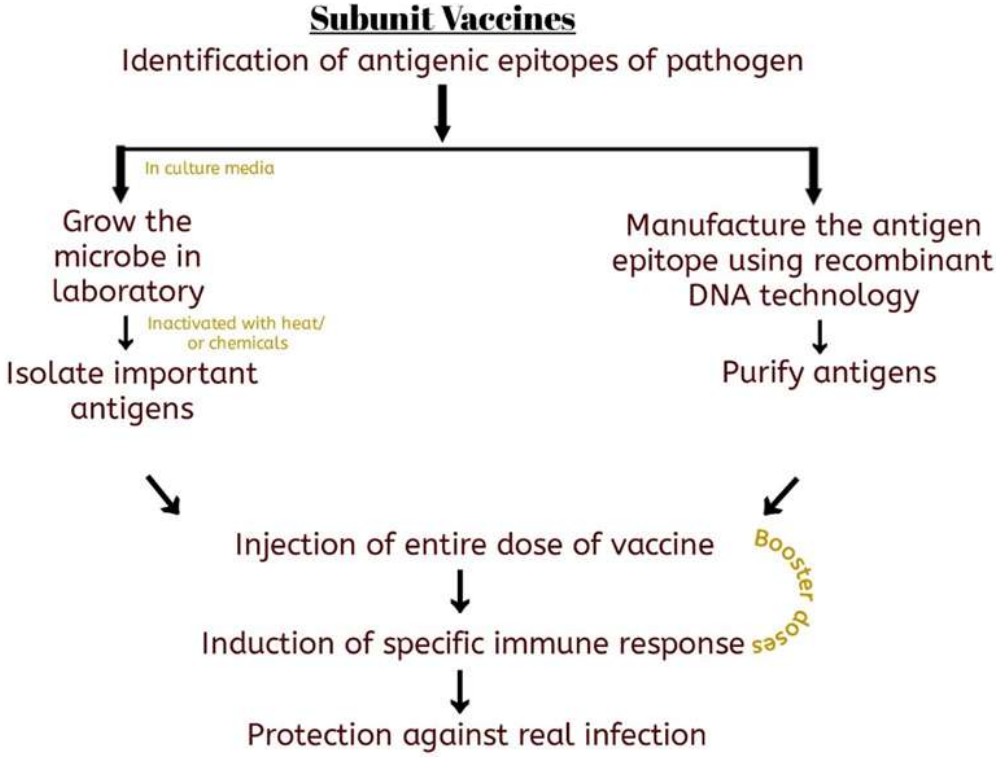

Fig. 4: Mode of action of vaccine

Future Prospects and Challenges in Vaccine Research and Development

Despite the aforementioned challenges, it is reasonable to anticipate a promising future for vaccinations. Despite the aforementioned challenges, it is reasonable to anticipate a promising future for vaccinations. This is due to the fact that in several instances, vaccination is the only efficacious medical intervention and it often proves to be more economically advantageous than treatment. The task of creating novel or improved vaccinations will include not only scientific and technical aspects but also political, economic, and societal factors. Nevertheless, it is justifiable to anticipate that the commitment to providing universal lifetime protection against vaccine-preventable illnesses would ultimately be realized via worldwide efforts (Andrey, 2001).

The encouraging achievements in the development of novel vaccine techniques provide an additional challenge, namely, the ability to forecast and evaluate the impact of vaccination on unintended immunological reactions. While there have been instances of political and social concern regarding the potential adverse effects of certain vaccines (Kesselheim, 2011), it is necessary to conduct further research to ensure the safety of their administration. Additionally, it is important to further investigate the possibility that vaccine administration may elicit nonspecific effects on host immunity (Salemi and D'Amelio, 2014). One of the concerns that raises is repeated vaccination, particularly among youngsters, who are the

primary focus of several immunization techniques that are being used or being developed. There is a need for more investigation on vaccine delivery strategies in order to effectively tackle these concerns. Furthermore, it is crucial to conduct further investigation into the impact of concurrent administration of multiple antigens and adjuvants on the effectiveness of individual vaccines against their respective pathogens. Additionally, it is essential to examine the potential adverse effects that may arise when these vaccines are administered together or separated by brief intervals.

Conclusion

In conclusion, vaccines have significantly contributed to the mitigation of the impact of infectious illnesses. The origins may be traced back to the early immunization efforts undertaken by the Chinese and Indians around five centuries ago. This practice underwent a journey that included the arrival of the Ottomans and Africans in Europe and North America. Edward Jenner's theories, which served as the basis for vaccination, aimed to ultimately eradicate smallpox. Subsequently, his journey has been difficult and filled with challenges and failures, nevertheless his aspirations were ultimately achieved when the World Health Assembly proclaimed the eradication of this ailment worldwide in 1980. The reservoir of information pertaining to the development of vaccinations has continued to expand, while advancements in laboratory methodologies have resulted in the preservation of countless lives. Moreover, the remarkable success of COVID-19 vaccinations has contributed an additional piece of data to support the efficacy of vaccines. Every vaccine has an own developmental history and examining it might provide valuable insights that could aid us in future pandemics.

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Chapter 05

Vaccine Development and Design: From Bench to Beside

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ABSTRACT

Vaccination has led to a significant enhancement in worldwide health. It has effectively saved several lives, diminished medical expenses, and enhanced the overall well-being of both animals and humans. Current conventional vaccines were developed without a clear understanding of how they affect our immune system, often relying on trial and error rather than scientific expertise. Despite the potential progress in vaccine design, there are concerns about the immune response in certain vulnerable populations, emerging and reemerging infectious diseases, pathogens with complex life cycles and antigenic variability, the need for personalized vaccinations, and the potential for vaccines to trigger non-antigen-specific responses that could lead to autoimmunity and vaccine allergies. Immunotherapy uses the body's natural defenses against illnesses such as cancer, whereas precision medicine customizes care based on a patient's unique genetic composition. These considerations have motivated immunologists to do research aimed at developing an improved strategy for vaccine creation that takes into account these challenges. The area of medicine is on the verge of groundbreaking advancements in the future, as long as challenges like data security, regulatory adherence, workforce adjustment, and ethical concerns are effectively addressed. Efficient healthcare systems optimize medical advancements to provide good patient care universally. This chapter will provide a broad understanding of vaccinations, including their historical significance and the fundamental concepts that underpin their efficacy

KEYWORDS
Vaccines, Vaccine development, Medicine, Immunotherapy, Gene editing

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INTRODUCTION

Vaccine development has significantly progressed medical science and enhanced public health by effectively managing and averting the spread of infectious illnesses (Nooraei et al., 2021). These biological preparations have revolutionized worldwide disease prevention, resulting in numerous lives saved and a reduction in the total burden of illness (Zinkhan et al., 2021). The history of vaccinations exemplifies human creativity and the constant quest for healthier civilizations. The discovery that individuals who had previously survived specific diseases were immune to future infections prompted the creation of immunizations in ancient civilizations (Link et al., 2012; Del Giudice et al., 2018). However, the idea of vaccination in the modern sense was not initially developed until the late 18th century. In 1796, English physician Edward Jenner conducted a groundbreaking experiment that changed medical research. The mild disease cowpox seems to protect milkmaids from the fatal and contagious smallpox. Jenner administered a youngster cowpox sore material, which made him immune to smallpox (Bachmann et al., 1993; Arevalo et al., 2016). The word "Vacca," meaning "cow," comes from this episode, which was the first vaccine. Jenner helped develop the smallpox vaccine, which eradicated the illness worldwide. Smallpox was the first and only infectious illness that could be eradicated by vaccination in 1980, according to the WHO. This remarkable achievement showed how immunizations might defeat deadly diseases (Heddle et al., 2017). There are some interconnected concepts remarkably indulged with the vaccination, vaccine development and design processes in the following.

Immunity

Vaccines elicit an immunological response that enables the immune system to identify and retain information about particular infections. A person who has had a vaccination can prevent the disease from progressing by having an effective immune response when they come into touch with the virus (Cao et al., 2018).

Herd Immunity

This notion emphasizes the communal safeguard that vaccines offer to entire communities. When a substantial proportion of a community is vaccinated, the transmission of illness is restricted, safeguarding individuals who are unable to get vaccines owing to medical contraindications or age. Herd immunity exerts a significant influence in diminishing the transmission of diseases (Mohsen et al., 2018).

Vaccine Efficacy

The efficiency of a vaccination is determined by its ability to prevent infection and, in some situations, lower the severity of disease if infection does occur (Vahdat et al., 2021). Vaccine efficacy rates vary and are assessed using extensive clinical trials. Vaccines are made to mimic a pathogen's presence without actually causing disease. They typically include genetic material that codes for the proteins present in the disease-causing agent, fragments of the pathogen, or attenuated or inactivated forms of the agent. When these components are administered, the immune system detects them as foreign intruders and mounts an immunological response (Beck et al., 2010; De Mot et al., 2020).

Understanding the importance of vaccines lies in their ability to train the immune system's memory cells, allowing them to remember the pathogen and provide long-lasting immunity. Vaccines have a fascinating historical legacy filled with groundbreaking discoveries and remarkable achievements that have had a profound impact on saving lives (Spohn et al., 2007; Budroni et al., 2021). Understanding and appreciating the significance of immunity, collective and vaccine efficacy requires a grasp of their underlying concepts. As we progress through this chapter, we will surely examine how vaccinations affect disease prevention, the scientific basis for immunizations, safety concerns, distribution challenges, new trends, challenges, and the potentially bright future of this groundbreaking medical intervention (Chackerian et al., 2001).

The Impact of Vaccination

Since its inception, vaccination has made a notable and significant impact on public health. It has significantly improved the quality of life worldwide, reduced death and illness rates, and helped manage and prevent infectious diseases (Cappella and Durham, 2012). A very convincing illustration of the effectiveness of vaccination is the complete elimination of smallpox. Smallpox was a highly destructive illness, resulting in significant death rates and leaving those who survived with terrible physical deformities. The smallpox vaccine, created by Edward Jenner in the late 18th century, marked the beginning of a new era in disease control. (Sonderegger et al., 2006).

These immunizations not only saved human lives, but also helped to restore normalcy during a worldwide health catastrophe. There is a wide range of rich and diverse success stories related to vaccines (Farahnik et al., 2016). Due to its ability to control and prevent infectious diseases, vaccinations have had a significant impact on public health. It continues to contribute to international efforts to control disease and has led to the total eradication of smallpox as well as a significant reduction in the death and rates of disease from several other illnesses (Foerster and Molęda, 2020). The scientific details, safety concerns, challenges, and possible future applications of vaccinations discussed in the following sections demonstrate that vaccines remain an important component of modern medicine with the potential to have a positive, significant impact on global health in the coming years.

The Science behind Vaccines

Vaccines are advanced medical instruments that strengthen the immune system to protect against infectious diseases. To comprehend the scientific principles underlying vaccinations, it is crucial to delve into their mechanisms, the diverse categories of vaccines, and the intricate procedures entailed in their creation and production (Foerster and Molęda, 2020). Vaccines contain harmless parts of the pathogen or an inactivated form of the pathogen. (Datsi et al., 2021). These components boost the immune system's response to immunization without producing disease. For example, the measles vaccine contains a weakened version of the measles virus. Antibodies to the measles virus remain dormant within the body, prepared to engage in combat with it should subsequent exposure occur (Zeltins et al., 2017). The memory response functions as the fundamental building block of immunity, offering protection against subsequent infections (Fig. 1).

Live Attenuated Vaccines

These vaccinations contain attenuated strains of living microorganisms. Vaccines include the oral polio vaccine and the mumps, measles, and rubella (MMR) vaccine. Vaccinations with live attenuated agents usually provide robust and long-lasting immunity (Gabriel et al., 2018).

Inactivated Vaccines

These vaccinations include microorganisms that have been rendered non-infectious or inactive, so preventing them from causing disease. This group includes the hepatitis A vaccine and the inactivated polio vaccine. Booster injections are supplementary doses of inactivated vaccines that are usually necessary to sustain immunity (Thrane et al., 2016).

Subunit, Recombinant and Conjugate Vaccines

These vaccines function by utilizing distinct pathogen constituents, such as carbohydrates or proteins, to elicit an immune response. The Hemophilic Influenza Type B (Hib) vaccine and the Human Papillomavirus (HPV) vaccine are two

instances of vaccinations (Zou et al., 2010; Vidal et al., 2023). They are known to be both reliable and efficient. MRNA vaccines, such as the Pfizer-BioNTech vaccine and the Moderna COVID-19 vaccine, use a trace amount of the pathogen's genetic material (mRNA) to direct cells to manufacture a harmless spike protein found on the infection's surface (Gabriel et al., 2019). The immune system responds once it recognizes this protein. mRNA vaccines have shown remarkable effectiveness and quick development. The general structure of the corona virus is depicted in Fig 2.

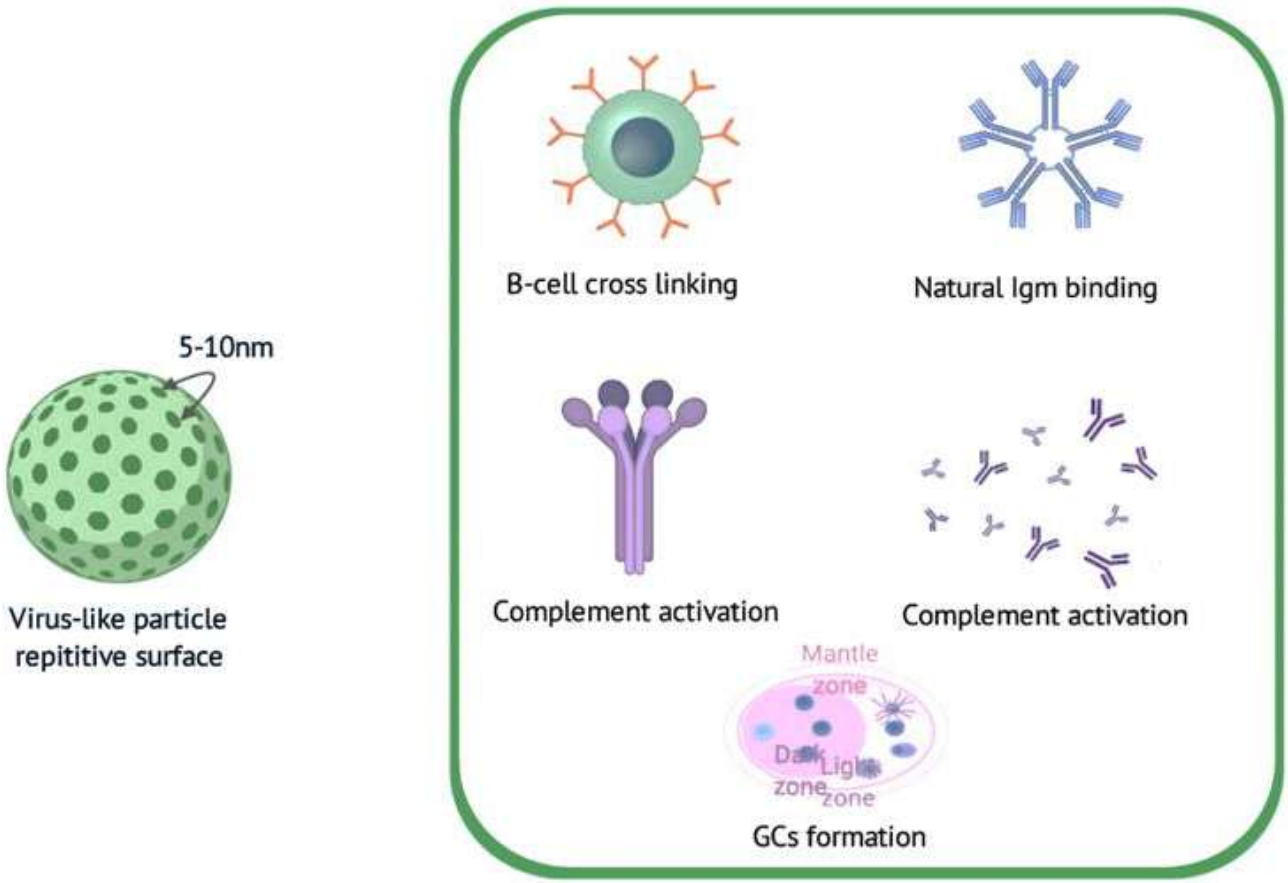

Fig. 1: Particulate antigens with repeating surface epitopes every 5–10 nm aid processing. By recognizing repeating structures as Pathogen-Associated Structural Patterns (PASPs), the immune system may cross-link B cells, bind Natural IgM, activate complement, induce high-affinity long-lived antibodies, and produce GCs.

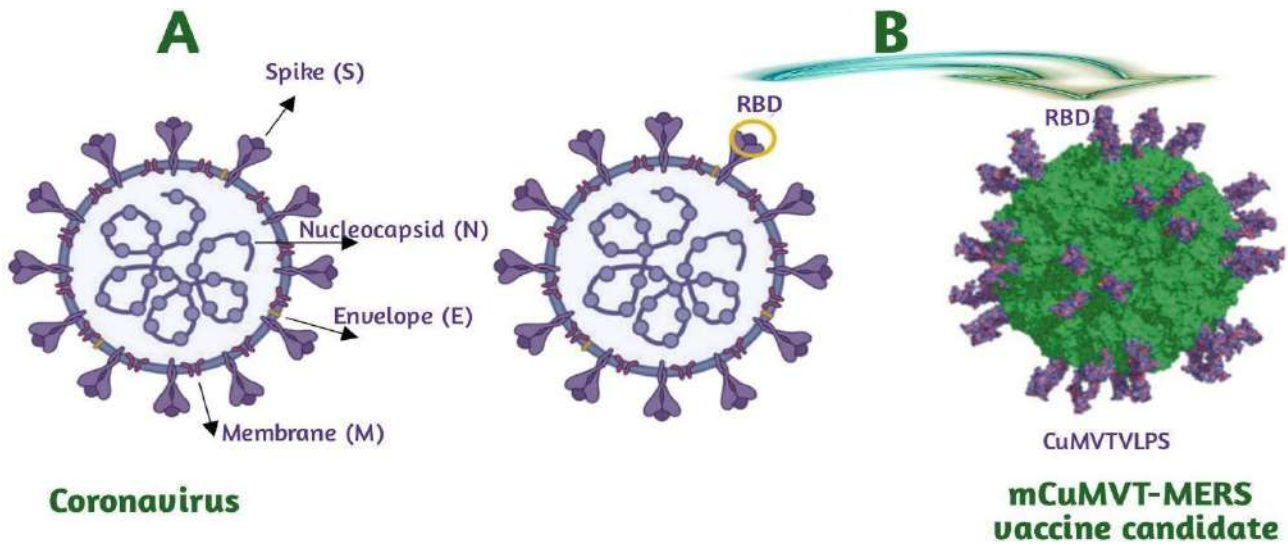

Fig. 2: An average coronavirus has four structural proteins: spike (S), envelope €, membrane (M), and nucleocapsid. B Strategy: Genetically fuse the RBD of MERS-CoV into optimized CuMVTT-VLPs with a Universal TT epitope and TLR7/8 ligand to create a mosaic VLP-based vaccine.

Clinical Trials and Regulatory Approval

Candidates for vaccines go through three stages of clinical testing on humans. Phase I involves evaluating safety in a limited group of volunteers; Phase II is on evaluating durability and dosages in a larger population; and Phase III necessitates the participation of thousands of individuals to evaluate safety, effectiveness, and any possible adverse effects (Hammad and Lambrecht, 2021). To determine a vaccination's safety and effectiveness, regulatory agencies like the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) examine trial data (Röhn et al., 2006).

Manufacturing and Post-Market Surveillance

To ensure consistency and safety, mass vaccine production requires specialized facilities and strict quality control procedures (Kurotaki et al., 2002) after immunization, ongoing monitoring of vaccination efficacy and safety is required. The emergence of mRNA vaccines in recent years has brought attention to the possibility of expedited vaccine development. MRNA vaccines possess the advantage of being able to be formulated and manufactured at a faster rate compared to conventional vaccinations (Chames et al., 2009).

Vaccine Safety

Maintaining the public's faith in immunization programs requires ensuring the efficacy of vaccinations. Throughout the entire research, approval, and post-market phase of development, vaccines are subjected to extensive testing and monitoring to identify and mitigate any potential risks (Schmitz et al., 2009). The core components of vaccination safety will be covered in this section, along with techniques for overcoming vaccine reluctance, monitoring adverse responses, and regulatory oversight that assures vaccine safety. It is crucial to stress that vaccinations have many more advantages than disadvantages. Among these advantages is the prevention of potentially lethal diseases. Several techniques are used to monitor and investigate any adverse events to ensure the safety of vaccines (Arbyn et al., 2020).

The Vaccine Adverse Event Reporting System (VAERS) in the US is a crucial tool for achieving this goal. Through VAERS, medical professionals and the general public can document any adverse events that follow a vaccine. Subsequently, health authorities scrutinize these reports to identify possible indications of safety concerns and implement suitable measures (Yousefi et al., 2022). Vaccine myths can contribute to vaccine reluctance, which is a major public health issue. Widespread misconceptions involve assertions that vaccines are responsible for causing autism or contain detrimental substances (Vicente et al., 2011; Burny et al., 2017). It is imperative to confront these misunderstandings with knowledge that is supported by evidence (Nieto et al., 2012).

Several thorough investigations have repeatedly disproved the theory that there is a connection between autism and vaccinations. There has been criticism of the initial study that suggested this correlation, and further research has not found a cause-and-effect relationship. In addition, vaccines are subjected to thorough testing to ensure their safety and effectiveness, with the contents being thoroughly assessed and monitored (Lang et al., 2009).

For regulatory bodies around the world, vaccine safety is the top priority. It is the duty of the Food and Drug Administration (FDA) in the US to ensure the effectiveness and safety of vaccines before authorizing their use. The FDA evaluates vaccination safety and disease prevention using preclinical and clinical trial data. After approval, post-market monitoring monitors a vaccine (Huber et al., 2021). To maintain the uniformity, cleanliness, and effectiveness of their vaccinations, they must comply with Good Manufacturing Practices (GMP) (Schellenbacher et al., 2009).

Table 1: List of FDA approved vaccines in USA (https://www.fda.gov/vaccines-blood-biologics/vaccines/vaccines-licensed-use-united-states)

Trade Name	Product Name	Targeted Disease	Manufacturer
Biothrax	Anthrax Vaccine Adsorbed	Anthrax caused by *Bacillus anthracis*	Emergent BioDefense Operations Lansing LLC
Imovax Rabies	Rabies Vaccine	Rabies	Sanofi Pasteur
Jynneos	Smallpox and Monkeypox Vaccine, Live, Non-Replicatin	Smallpox and Monkey pox	Bavarian Nordic
Ervebo	Ebola Zaire Vaccine, Live	Ebola virus disease caused by *Zaire ebolavirus*	Merck Sharp and Dohme LLC
Spikevax	COVID-19 Vaccine, mRNA	Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)	Moderna Tx Inc.
VF-Vax	Yellow Fever Vaccine	Yellow Fever	Sanofi Pasteur, Inc

Several steps, including vaccine research, approval, and post-market monitoring, are involved in the extensive and intricate process of guaranteeing vaccine safety. Table 1 illustrates some of the FDA approved vaccines. To discourage vaccine hesitancy, initiatives are put in place to closely monitor adverse events following immunization and disseminate evidence-based knowledge (Tumban et al., 2015). To ensure that vaccines are both safe and effective, regulatory authorities like the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) play an essential role. Vaccinations continue to be a crucial instrument due to the numerous safety measures that have been adopted. Vaccinations are essential for the control of infectious diseases as well as the health of the general public (Rumfield et al., 2020).

Vaccine Distribution and Access

Making vaccines easily available and disseminated equitably is a crucial part of global health. If vaccines aren't broadly available to all individuals, regardless of where they reside or their socioeconomic situation, they won't be able to save lives or prevent tremendous suffering. The COVAX initiative exemplifies a contemporary global endeavor to distribute vaccines. COVAX was established in reaction to the COVID-19 pandemic to provide fair and equal access to COVID-19 vaccines for all countries, regardless of their economic status (Lu et al., 2015). The objective is to deter the accumulation of vaccinations and the prioritization of one's own country by combining resources and allocating vaccines to nations that require them (Wang et al., 2007).

Vaccine reluctance can impede distribution efforts, despite their importance. Vaccine hesitancy is the act of being hesitant or refusing to receive vaccinations, even when vaccines are readily available (Vandenberghe et al., 2017). This issue is impacted by various reasons, such as misinformation, lack of trust in healthcare systems, and cultural attitudes. Tackling vaccine skepticism necessitates a comprehensive and multifaceted strategy. Healthcare providers should communicate with patients and communities on a proactive basis to ensure that they obtain accurate information about the safety and effectiveness of vaccines. Educative initiatives and public health campaigns can dispel myths and rectify misconceptions about the subject under investigation. The global magnitude of the COVID-19 epidemic has brought attention to the difficulties posed by vaccination reluctance (Martin et al., 2017).

The efforts to achieve complete vaccination coverage have been hampered by the widespread spreading of false information as well as the lack of public faith in newly manufactured vaccinations. Vaccination campaigns for big populations are meticulously planned and managed to efficiently reach huge populations. Mobile medical centers and outreach programs are frequently utilized to specifically target rural areas (Palladini et al., 2018).

Vaccine Outreach and Education

When it comes to educating communities regarding vaccines and making it easier for them to obtain immunizations, community health professionals and volunteers play an extremely important role (Ji et al., 2020).

Supply Chain Strengthening

It is crucial to have a strong supply chain, which includes cold storage and distribution networks, to ensure the quality and accessibility of vaccines, especially in areas with limited resources (Chackerian et al., 2006).

Reducing Costs

Enhancing affordability can be achieved by engaging in negotiations to lower vaccine prices and providing subsidies to cover costs for underprivileged communities (Doucet et al., 2017).

Public-Private Partnerships

Partnerships among governments, non-governmental organizations (NGOs), and the corporate sector can bolster initiatives to distribute and improve access to vaccines (Irvine et al., 2008).

Research and Development

Allocating resources towards the advancement of cost-effective and user-friendly vaccinations can enhance availability in settings with limited resources. The idea of "vaccine equity" holds that everyone should have fair and impartial access to vaccinations, irrespective of their socioeconomic status or place of residence. In addition to being morally right, achieving vaccine equity is crucial for maintaining global health security (Bach et al., 2009). The task encompasses more than just the distribution of vaccines (Zamora et al., 2006). Global vaccine programs, such as COVAX, strive to guarantee fair and impartial availability of vaccines, while it is crucial to tackle vaccine hesitancy and overcome obstacles to access to achieve success. Attaining vaccine equity is a continuous and crucial endeavor to prevent and manage infectious illnesses worldwide (Maphis et al., 2019).

Emerging trends in Medicine

The medical field is defined by its dynamic nature, as it is always changing to meet society's ever-changing healthcare needs. Recent advancements in medical technology and pioneering techniques can transform patient results, diagnosis, treatment, and the delivery of healthcare. Precision medicine is progressively being utilized in various medical fields, including neurology, oncology, cardiology and viral diseases. Using the body's immune system to target and eradicate cancer cells, immunotherapy is a cutting-edge approach to treating cancer. It is currently considered a significant breakthrough in cancer treatment, capable of delivering long-lasting and strong outcomes in certain individuals. Driven by advancements in information technology and telecommunications, telemedicine has become a game-changing idea in the provision of healthcare. Telemedicine makes remote medical care easier, especially in undeveloped or rural areas (Storni et al., 2020).

Telemedicine has become more popular as a safe and useful way to provide medical care since the COVID-19 outbreak. These days, remote diagnostics, virtual monitoring, and telehealth consultations are crucial components of modern healthcare systems (Storni et al., 2020). The reimbursement policies and regulatory frameworks are changing to

reflect telemedicine's growing importance in the healthcare industry (Nicoll et al., 2003). While telemedicine is expanding access to healthcare services, gene editing technologies provide promise for treating genetic problems. These advancements symbolize the continuous effort to get improved healthcare results and are propelling medical advancement in the twenty-first century (Wiessner et al., 2011; Burny et al., 2019).

Challenges and Ethical Considerations

The medical industry faces numerous obstacles and ethical dilemmas when it develops and utilizes novel technologies. These issues arise from the swift rate of progress, intricate healthcare systems, and the necessity to find a middle ground between progress and ethical values O'Hagan et al., 2020). Nevertheless, this advancement necessitates the implementation of stringent safety protocols to mitigate any potential danger to patients. organizations within the government tasked with overseeing particular sectors, such as the U.S. The European Medicines Agency (EMA) and the Food and Drug Administration (FDA) are crucial in assessing the efficacy and safety of novel medical interventions. Before approving new medicines, medical equipment, and therapies, they carefully think about the pros and cons of every clinical study result (Mohsen and Bachmann, 2022). There is a significant disparity in the accessibility and cost of medications and therapies worldwide, resulting in unequal health results (Loga et al., 2019). At the national and international levels, coordinated and cooperative actions are required to address these disparities. Despite the existence of initiatives such as the World Health Organization's (WHO) essential medicines list and similar programs, substantial obstacles persist that impede universal access to vital medications (Berenbaum et al., 2020).

An exemplary instance is the gene-editing tool known as CRISPR-Cas9. CRISPR has significant potential for the treatment of genetic disorders, but it also raises ethical considerations around the possibility of creating customized kids, genetic improvements, and unanticipated outcomes of genetic modification (Bertram et al., 2010). The use of compulsory licensing, a policy that permits a government to issue licenses for the production of generic copies of proprietary drugs, seeks to enhance the availability of vital treatments while upholding intellectual property rights These problems persistently dominate conversations regarding global health and ethics. Contemporary medicine encounters various obstacles and ethical concerns as it progresses (Soongrung et al., 2020). Key issues include making sure that innovation and safety work well together, fixing unfair healthcare systems around the world, dealing with difficult ethics problems, and finding fair solutions for intellectual property rights. Ethical frameworks and governing systems are important for guiding medical progress while protecting patients' rights and the honor of society as a whole. As the field of medicine progresses, ethical issues will continue to be central to the process of making healthcare decisions (Bachmann et al., 2020).

The Future of Medicine: Promising Developments

Medical science is a dynamic discipline driven by ongoing scientific discoveries, technological innovations, and human comprehension growth. In the coming years, the field of medicine has great promise for remarkable advancements that could completely transform healthcare, enhance patient results, and expand our knowledge of health and illness. These initiatives seek to eradicate the disease by administering vaccinations and implementing widespread immunization campaigns (Engeroff et al., 2018).

Challenges in the Adoption of Emerging Technologies in Medicine

Modern medical technology has the potential to change how healthcare is provided, improve patient results, and help us learn more about health and illness. However, there are certain challenges in integrating this technology into medical practice (Sani et al., 2021). With the rising digitization of healthcare, the accumulation and retention of patient data are expanding at an exponential rate. Significant amounts of private patient data are generated via wearable technology, telemedicine platforms, and electronic health records (EHRs). Ensuring patient privacy and protecting this data from breaches are critical (Del Giudice et al., 2018). Robust cyber security policies must be established and put into place by healthcare institutions to protect patient data from illegal access and internet attacks. Data breaches in the healthcare sector can have detrimental effects on an organization's brand, impair financial gains, and compromise patient privacy. Striking the right mix between security and usability is still very difficult (Foerster et al., 2020). Healthcare providers and systems face both exciting potential and difficult obstacles as a result of the use of developing technologies in the medical field. Healthcare organizations need to address several complex issues, including workforce training, regulatory hurdles, data privacy and security, interoperability, economic concerns, and ethical and legal issues. Ensuring that patients receive the best possible treatment and realizing the full potential of medical technology development require tackling these issues head-on (Datsi et al., 2021).

Conclusion

"Vaccine Development and Design: From Bench to Bedside" provides a comprehensive exploration of the journey from initial research to the administration of life-saving vaccines. It highlights the collaborative efforts of scientists, healthcare professionals, and policymakers in overcoming biological, technical, and logistical challenges. Emphasizing the importance of innovation, rigorous testing, and ethical considerations, this chapter illustrates how modern vaccine development not only safeguards public health but also prepares humanity for future pandemics. Ultimately, it serves as a testament to the power of scientific advancement and global cooperation in creating a healthier and more resilient world.

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Chapter 06

Emerging Infectious Diseases and the Role of Vaccines in Prevention

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ABSTRACT

Over the past few decades, numerous contagious viruses have arisen from wildlife or resurfaced, posing significant risks to global health and the global economy. Ebola, Marburg, Lassa, Dengue, West Nile, Zika, Chikungunya, Swine flu, SARS, MERS, and COVID-19 are zoonotic diseases that have spread globally and affected public health. The scientific community has been urged to assist to prevent and treat these emerging infections promptly. Vaccination is widely regarded as the most efficacious method for stimulating the immune system to initiate defensive reactions against infections, hence lowering both illness and death rates, as evidenced by historical data. In the context of health emergencies, it is crucial to employ novel and alternative methodologies to design and develop vaccines. This is essential to achieve rapid and extensive vaccination coverage, effectively control disease outbreaks, and mitigate the spread of epidemics. This chapter provides a comprehensive overview of vaccination techniques for emerging or re-emerging infectious diseases. It also addresses the obstacles and difficulties that need to overcome to develop effective vaccines against future infections.

KEYWORDS
Vaccines, emerging infectious diseases, Viruses, Epidemics, pandemics, Antibody-dependent enhancement, SARS-CoV-2, COVID-19

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INTRODUCTION

Throughout history, infectious viral illnesses have continually arisen and reappeared, presenting a substantial danger to mankind. The appearance of animal viruses poses a significant danger to human health due to a variety of interconnected and mutually reinforcing factors. These factors include demographic trends, urbanization with high population density, modernization enabling increased mobility through various transportation methods, large gatherings, changes in human behavior, environmental changes affecting ecological systems, and insufficient global public health systems (Condit et al., 2016). In 1918, during the height of the "Spanish flu" pandemic, global population was around 1.8 billion. Zhu et al. (2020) predicted that the global population will increase by about 25%, from 7.8 billion in 2020 to 9.9 billion by 2050.

The COVID-19 pandemic arose as a result of the abrupt spread of the new SARS-CoV-2 virus worldwide, happening in less than six months (Huang et al., 2020). The virus's elevated fatality rate had a greater impact on older persons and those with coexisting medical conditions (Wang et al., 2020). The epidemic has had a substantial detrimental impact on the global economy. Aside from lockdowns, several stringent and ambiguous procedures like as wearing masks, maintaining distance from others, imposing travel restrictions, and avoiding crowded places have emerged as the only viable option for control. With an astonishing tally of over 100 million illnesses and a remarkable 2 million deaths, it is clear that integrating vaccine(s) into current measures offers the most hopeful chance of successfully reducing the spread of the pandemic. Friedler (2021) and Gully et al. (2020) argued that the presence of these factors requires scholars and decision-makers to be cautious, reevaluate their strategies for detecting and managing emerging risks linked to infectious diseases, and scrutinize international frameworks for controlling pandemic-related illnesses.

Emerging and Re-emerging Infectious Diseases

For thousands of years, people have been cognizant of the potential emergence of new infectious diseases, even before the identification of the causative organisms (Morens and Fauci, 2020). The phenomenon of global travel and the increasing interconnectedness of nations have posed challenges in effectively managing specific infectious illnesses, despite notable progress in the field of countermeasures such as diagnostics, treatments, and vaccines. Marston et al. (2014) state that the appearance of infectious diseases (EIDs) poses a substantial risk to both worldwide security and public health. The history of pandemic illnesses helps to explain coronavirus outbreaks like the SARS-CoV-2 pandemic (Sacchetto et al., 2020).

As human civilizations expand in both population and intricacy, infectious pathogens have several chances to emerge in the vacant ecological spaces created by human activity (Prompetchara et al., 2020). A study by Hu et al. (2016) illustrate the inherent vulnerability of communities to newly identified and reemerging infections, as well as the capacity for these diseases to rapidly escalate into severe outbreaks and pandemics. Dengue and yellow fever are two prevalent viral infections transmitted by mosquitoes; yet, they possess characteristics that distinguish them from developing infectious diseases. Yellow fever, a disease transmitted by Aedes mosquitoes, has been acknowledged for millennia and is prevalent in over 40 countries within South Africa. Yellow fever outbreaks have been observed in various countries, including Nigeria, the countries affected by the outbreak since 2016 include the Democratic Republic of the Congo, Angola, and Brazil (Shaikh et al., 2020). These outbreaks have prompted significant concerns over the accessibility of yellow fever immunizations. The World Health Organization (WHO) has officially approved almost 4 live attenuated vaccines that are produced from the yellow fever strain (Klemm et al., 2018; Yousafzai et al., 2019).

The four variants of the dengue virus (DENV 1-4) are presently found in most regions where dengue is common, presenting an increasing danger to global public health (Qamar et al., 2020). The incidence of dengue infections and associated illnesses has steadily risen over time, mostly as a result of factors such as population expansion, the proliferation of habitats conducive to Aedes mosquito species, and the convenience of travel (Marchello et al., 2020). Dengue fever is prevalent in more than 100 countries globally. Around 400 million people are yearly infected with the dengue virus. Around 100 million people suffer from different diseases, and out of these cases, 22,000 deaths are caused by severe dengue. The region's most profoundly impacted by epidemics include the US, South and Southeast Asia, and the Western Pacific. Asia bears over 70% of the worldwide illness load. Andrews et al. (2019) have created a wide range of vaccinations. The Dengvaxia vaccine, created by Sanofi Pasteur and carrying the yellow fever 17D antigen, has received authorization for use in 20 nations. Nevertheless, research has shown that the present level of acceptance towards vaccination is quite low (D'Souza et al., 2010). The detection of a safety signal in individuals who had not been previously exposed to dengue prompted a global assessment of the vaccine's effectiveness, resulting in new guidelines for its usage by the World Health Organization (WHO). The Philippine government, regulatory bodies, Sanofi Pasteur, healthcare professionals who tested and administered the vaccine, and parents of vaccinated children fought over this (Lin et al., 2007).

There are two bacterial diseases that have been a persistent problem for humans. These diseases are widespread, often lead to outbreaks, and are increasingly resistant to medications. Since 1817, pathogenic strains of *Vibrio cholerae* have been responsible for a total of seven global pandemics. One of the most notable pandemics began in 1961 (Martin et al., 2008). The global mortality rate attributed to cholera infection continues to be high, primarily due to delays in the rehydration of patients (Jacob et al., 2020). Annually, the global incidence of cholera ranges from approximately 1.4 to 4.3 million documented cases, leading to a mortality rate of 21,000 to 143,000 individuals, primarily concentrated in the regions of Asia and Africa. Antimicrobial resistance to various strong antibiotics, including furazolidone, chloramphenicol, nalidixic acid, trimethoprim-sulfamethoxazole, tetracycline, and fluoroquinolones, has been observed in Asia and Africa over a period of time. The World Health Organization (WHO) has produced and prequalified several immunizations. According to Wolf et al. (2020), Gavi sponsors a global stockpile of vaccines that can be rapidly delivered in times of epidemics. Fig 1 depicts the recently identified and recurring viral illnesses throughout the timeline.

A severe sickness known as typhoid fever is caused by the Gram-negative bacteria *Salmonella enterica* subsp. enterica serovar Typhi (*S. Typhi*). There has been a consistent increase in the occurrence of antimicrobial-resistant strains of *S. Typhi*. The initial detection and subsequent spread of a groundbreaking extensively drug-resistant (XDR) strain of *S. Typhi* occurred in Sindh, Pakistan (Feldmann et al., 2018). Subsequently, this strain has been documented in various countries including India, Bangladesh, Iraq, Philippines, Nepal, and Guatemala (Gouglas et al., 2019). Enhancing the immunogenicity and efficiency of typhoid vaccinations, specifically in children below the age of 2, can be achieved through the successful development of enhanced vaccines. This process entails conjugating the Vi polysaccharides with a carrier protein. Reducing the frequency of typhoid fever cases that require antibiotic therapy, will help manage typhoid, especially in areas with extended drug resistance (XDR) (Jackson et al., 2020; Sandbrink et al., 2020).

A Model of Vaccine Development for Emerging Infectious Diseases

Considerable progress has been achieved in comprehending emerging infectious illnesses in the last twenty years (Polack et al., 2020). An examination of the SARS-CoV epidemic in 2002 shows that, although there were just a few deaths and infections, its high death rates and impressive ability to spread caused significant global disruption.

Consequently, the investigation of vaccines for SARS-CoV came to a halt, leading to a decrease in financial backing. According to Adalja et al. (2020), the Phase 1 clinical trials exclusively assessed a comprehensive inactivated vaccination and a DNA vaccine. The length needed to progress a vaccine for an infectious agent, following a traditional research and development approach, usually falls within the range of 5 to 10 years. According to Excler et al. (2019), the existing methodology does not adequately meet the demands associated with the emergence of a new disease within the framework of an epidemic. The Ebola outbreak that occurred in 2014 had a duration of nearly 24 months and led to a total of 11,325 deaths. The prolonged timeframe facilitated the development and assessment of Ebola vaccines, wherein one vaccine (among others) exhibited efficacy towards the culmination of the epidemic. Furthermore, vaccine manufacturing platforms overview is represented below in Fig. 2 (Clemens et al., 1996; Kim et al., 2021).

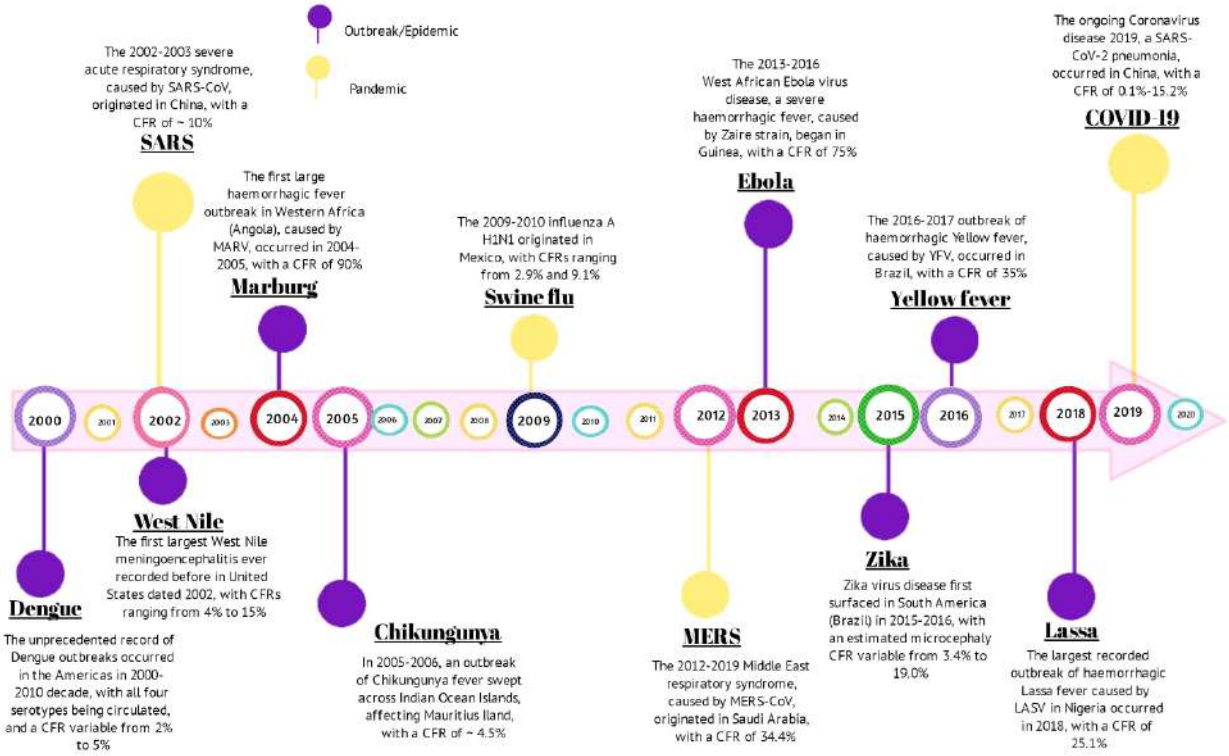

Fig. 1: Recently identified and recurring viral illnesses timeline. The timeline shows the year viral pandemic outbreaks first appeared or returned in a location. The Dictionary of Epidemiology (228) defines CFR values as the ratio of fatalities to total cases for a disease over a certain time period. SARS-CoV, SARS-CoV-2, MERS-CoV, MARV, YFV, and LASV stand for severe acute respiratory syndrome coronavirus, Marburg virus, Yellow Fever Virus, and Lassa virus.

The COVID-19 pandemic is notable for completing the research and development process in less than 300 days, from SARS-CoV-2 viral sequencing to vaccination effectiveness trial interim evaluations (Noorbakhsh et al., 2019). The WHO has called for further vaccine research to address concerns about uncontrolled transmission during the 2013–2016 Ebola pandemic in Western Africa (Antonelli et al., 2022).

Following this, Gavi proceeded to engage in an advance purchase deal. Curiously, prior to the present epidemic, there existed Ebola vaccinations that had been developed and assessed for the purpose of biodefense in nonhuman primates. Nevertheless, the previous initiatives were considered inappropriate for conducting clinical trials and did not possess the requisite financial viability to successfully conclude their development (Prasasty et al., 2019).

Various Vaccine Platforms and Vaccines for Emerging Infectious Diseases

Vaccines are the most effective way to reduce the risk of pandemics and epidemics and are crucial for managing infectious disease outbreaks (Lunardelli et al., 2021). The prompt implementation of a vaccine is directly linked to the prompt containment of a disease outbreak. As was already established, the traditional vaccine manufacturing process is not designed to meet the needs of pandemics that are spreading quickly (Barouch et al., 2017). mRNA technology was used to develop the Pfizer-BioNTech and Moderna COVID-19 vaccines. According to Pattnaik et al. (2020), the vaccines in question have exhibited both safety and significant effectiveness As a result, they have been granted emergency use approval (EUA) by the US Food and Drug Administration (FDA) and conditional marketing authorization (EMA) by the European Medicines Agency (EMA). Table. 1 provides some details of the CEPI vaccine portfolio. These details are also provided on the CEPI website (CEPI, 2021).

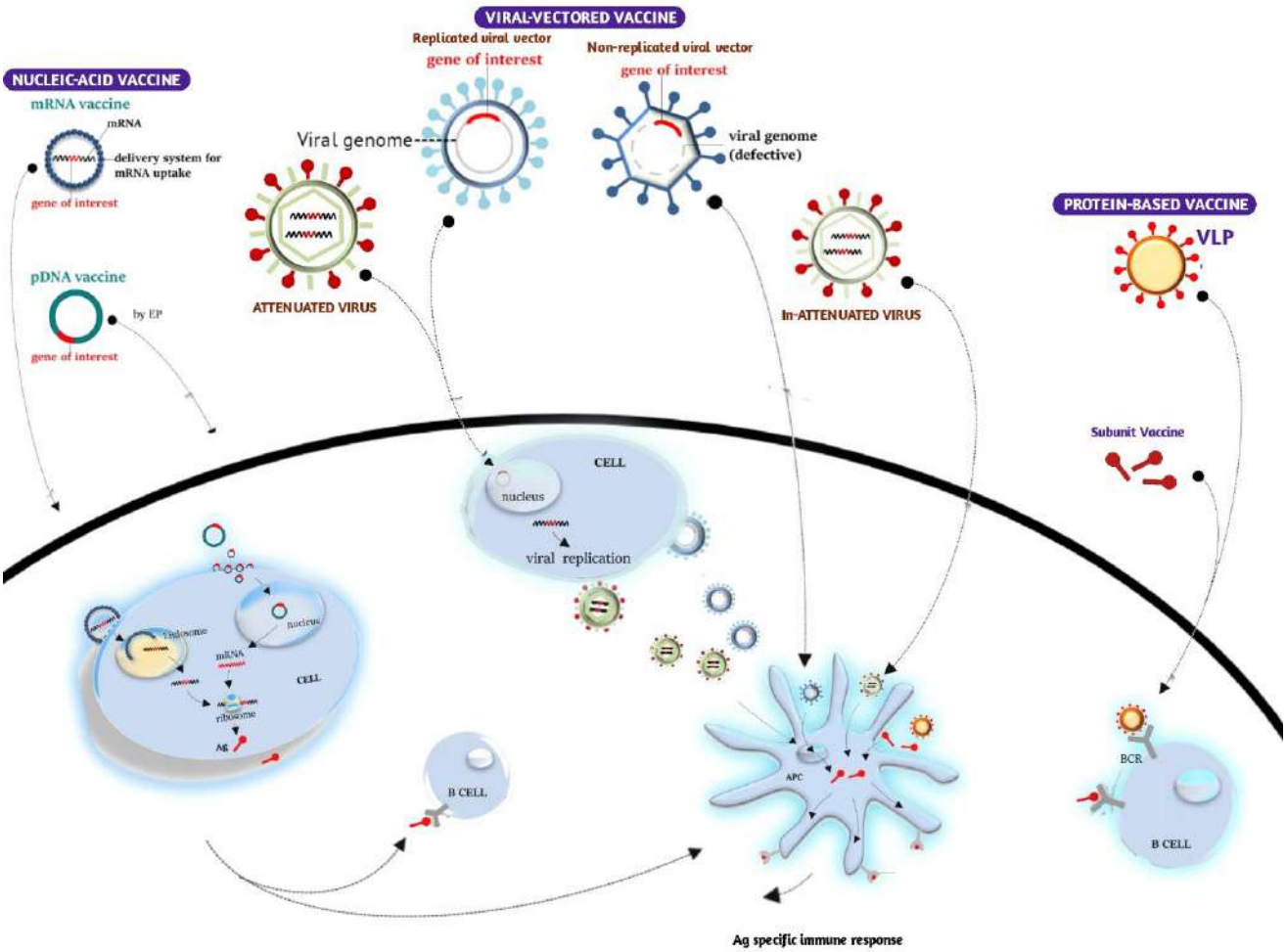

Fig. 2: Graphical vaccine manufacturing platforms overview. Nucleic acid, viral vector, protein-based, live attenuated, and inactivated vaccines are shown. Non-replicating mRNA vaccines with the intended gene sequence are nucleic acid vaccinations. The delivery technique enhances cell uptake of these vaccinations. After leaving the endosome and entering the cytosol, the host immune system turns it into the desired antigen. Plasmid DNA (pDNA) with a particular gene sequence enters the nucleus and undergoes cytoplasmic transcription and translation. Myocytes may absorb pDNA and produce antigens. Antigen-presenting cells (APCs) or naïve B cells might capture these antigens, triggering immune responses.

Table 1: The purpose of the Coalition for Epidemic Preparedness Innovations (CEPI) in the process of developing vaccines:

Discovery	Development/ License	Manufacturing	Delivery/ Stockpiling	Last mile
Academia	Industry	Industry	GAVI	Countries
Governments	Governments	BARDA	UNICEF	WHO
Welcome Trust	Regulators	CMOS	PAHO	UNICEF
NIH	Welcome Trust	Regulators	Government	Responding
IMI	NIH	Government	WHO	organization (e.g., MSF)
GLoPID-R	EC	WHO	Industry	
Industry	IMI	GHIF	Pandemic Emergency	
Regulators	BMGF		Facility (World Bank)	
Biotech	BARDA/ DTRA, etc			
	WHO			
	Biotech			
	PDs			

EPI Role as a Facilitator

Although there is potential and advancement in the use of mRNA vaccines for the treatment of many infectious illnesses (EIDs), it is not yet suitable to claim that they offer a universally applicable vaccine strategy for other EIDs, including microbial or enteric infections (Poland et al., 2019). Additional research and time are required to gain significant insights from the comprehensive deployment and efficacy investigations of COVID-19 mRNA vaccines, notwithstanding

their valuable display of potential. While many DNA vaccines have received approval for animal administration and have exhibited safety and immunogenicity in clinical trials including humans, none of these vaccines have been authorized for human use. The design of recombinant proteins for a specific disease, such as subunits or virus-like particles, demonstrates considerable variability (Langerak et al., 2019). These proteins are commonly synthesized using adjuvants; however, their maturation periods are extended. According to Wen et al. (2019), vaccines that employ virus-like particles for the management of hepatitis B and human papillomavirus possess attributes such as safety, significant immunogenicity, effectiveness, and the potential for large-scale manufacturing.

The procedure exhibits a significant level of transferability. Each illness, ranging from entirely inactivated viruses such as SARS-CoV-2, polio, and cholera to live attenuated vaccines like SARS-CoV-2, polio, and chikungunya, has unique and specific properties. Manufacturing these vaccinations, particularly for COVID-19 and polio, may require biosafety level 3 protocols, depending on the specific pathogen involved. Two main kinds of recombinant vector platforms are often used in vaccinations. One category consists of nonreplicating vectors such as adenovirus 5 (Ad5), adenovirus 26 (Ad26), and ChAdOx, which is derived from chimpanzee adenovirus. Others include highly attenuated vectors like modified vaccinia Ankara (MVA) and live attenuated vectors like measles-based vector or vesicular stomatitis virus.(Boigard et al., 2017).

The process of vector creation may encompass the integration of distinct inserts that are tailored to specifically target particular infections, or alternatively, the vector can be formulated with a range of inserts to efficiently address different diseases (Follmann et al., 2020). Additional crucial factors to take into account include the rate of progress, simplicity of production and expansion, convenience of logistics (including the presentation, storage, and management of products), transfer of technology to various other manufacturers to ensure global accessibility, and the cost of goods (Lee et al., 2020). Graham et al. (2018) have utilized viral vectors, specifically Ad5, Ad26, and MVA42, in the advancement of HIV and Ebola vaccines.

Rather than platforms, regulatory organizations are in charge of approving vaccines. There are unique characteristics of each vaccination. Lambrecht et al. (2020) notes that there has been a lot of research on using a heterologous prime-boost (HPB) vaccine approach for Ebola and HIV IV.

Currently, the COVID-19 vaccines developed by Pfizer and BioNTech, as well as those from Oxford-AstraZeneca and Gamaleya, are being assessed by ComcovStudy.org.uk. The potential for this intervention to enhance the immune response and reduce the occurrence of multidose reactogenicity or anti-vector immune responses was raised by Rémy et al. (2015).

The Pathway to EUA, Licensure and Beyond

Major pharmaceutical and biotechnology companies, with the help of groups like CEPI and initiatives like Operation Warp Speed, have carried out successful research in areas or countries where the SARS-CoV-2 patient incidence is greatest (Petersen et al., 2018). Not only that, but these organizations have promised to foot the bill for risky, large-scale industrial projects. There are now more than 60 vaccine candidates for COVID-19 undergoing clinical trials, and an additional 170 are in the preclinical research phase, as reported by the World Health Organization (Duffy et al., 2000). Candidates for vaccines that have not yet passed the first round of testing or been given the green light are especially vulnerable to this kind of uncertainty (Dumonteil et al., 2021).

The process of granting further licenses may include the implementation of noninferiority clinical trials carried out by regulatory bodies and ethics committees. These trials involve the comparison of comparator vaccines that have demonstrated clinical effectiveness. In order to expedite the implementation of such an occurrence, it is imperative to establish a widely accepted agreement concerning the immunological correlates of protection (ICP) for COVID-19, which remains undetermined at present. The equivalence of immunological responses induced by chimpanzee adenovirus, proteins, or fully inactivated viruses remains uncertain (Volz et al., 2021; Creanga et al., 2010). After the licensure of COVID-19 vaccines, a multitude of clinical trial ideas have been suggested through current phase 3 trials.

Pharmacovigilance and Surveillance

In June 2020, the group was formed via a collaboration between the COVID-19 Vaccine Safety Technical Working Group and the US Advisory Committee on Immunization Practices (ACIP). Important lessons may be gleaned from the past when new vaccinations were introduced in reaction to epidemics and pandemics (Hargrave et al., 2021). Vaccination distribution and adverse event recording were components of comprehensive pandemic preparation strategies implemented by just a few of countries during the 2009 H1N1 influenza pandemic (Kim et al., 2021). The African Vaccine Regulatory Forum collaborated with global regulators to undertake a thorough assessment of clinical trial methods and outcomes. They also coordinated the monitoring of trials and approved and distributed vaccines (Henderson, 2011). The genetic closeness of SARS-CoV-2 and SARS-CoV raises questions about the safety of antibody-dependent enhancement (ADE) in vaccine-associated enhanced respiratory illness (VAERD) (Coughlan et al., 2020).

The current reports of the SARS-CoV-2 vaccine phase 3 study do not include any information on VAERD or ADE. According to Sell (2019), no incidence of VAERD has ever been reported in animal challenge tests that have used SARS-CoV-2 vaccines to provide protection. Line developmental time and vaccination release is shown in Fig. 3.

The PREVENT Working Group has developed a roadmap that outlines the measures to address pregnant women's concerns regarding the development and execution of vaccinations for emerging illnesses. New lineages have been

documented globally, indicating that the SARS-CoV-2 virus is undergoing evolutionary changes. Compared to other lineages, the D614G lineage has demonstrated improved development in lab settings and increased transmission in small animals, leading to its global prevalence (Gao et al., 2019).

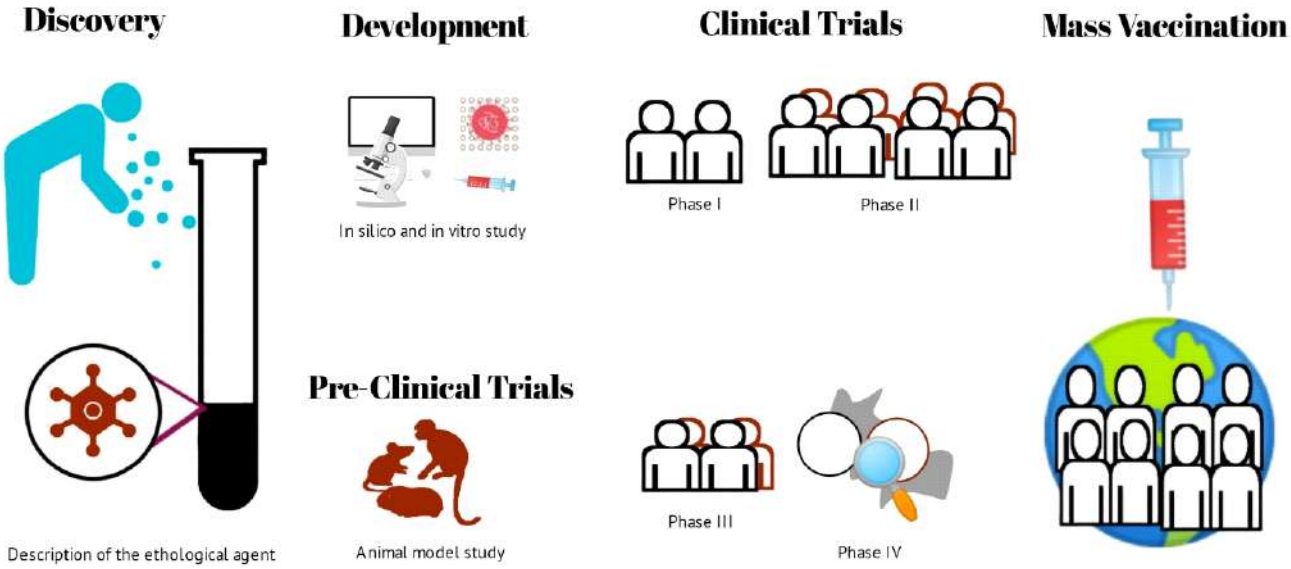

Fig. 3: Developing the vaccination prototype; Preclinical tests on animal models; Clinical trials are divided into phases I, II, and III. Approval, vaccine release, and phase IV clinical trial; Mass immunization program.

The Approval Processes for Licensure and EUA and the Risk of Speed

For the most part, the European Medicines Agency (EMA) or a country's national regulatory agency (FDA, for example) facilitates a centralized procedure for vaccine authorization. Stapleford et al. (2020) note that manufacturing companies have the option to apply for World Health Organization (WHO) prequalification after getting license clearance from a powerful or strict national regulatory body in the place of production. Among the 98 nations that finance themselves, 92 are low- and middle-income countries (LMICs), and they may choose to receive funding via the Gavi COVAX advance market commitment. The regulatory body has adopted a conditional approval procedure, EMA (Matz et al., 2019).

In situations when the public may be more accepting of a lower degree of assurance regarding the efficacy and safety of products, national regulatory agencies can refer to the EUAL (Pollard et al., 2021). The license and contract manufacturing agreements are expected to enable the manufacture of an ample number of vaccine doses, ensuring fair and equal access for persons worldwide who are at risk (Langsjoen et al., 2018).

Ensure that everyone is Included and that there is Fair Grant to Immunizations and Cure

The 2030 Agenda for Sustainable Development prioritizes inclusivity for low-income countries. COVAX, which recently announced agreements for various dosages to meet 50 targets by 2021 (Sridhar et al., 2018), seems to be moving into the US market. Alternatively, COVAX is almost ready to vaccinate. Postponing COVID-19 vaccinations will threaten the 2030 Agenda for Sustainable Development, particularly Health-Related SDG 3 (Dhama et al., 2018).

Conclusion

Controlling and preventing EID outbreaks is greatly enhanced by immunizations. But the ability to quickly design and test a vaccine that is safe and effective against the present danger is a major concern. The field of vaccine development for emerging infectious diseases (EIDs) was lacking a long-term framework before CEPI was established. This framework would have enabled successful projects throughout the entire vaccine development life cycle and efficiently coordinated efforts to address the most urgent global epidemic threats. To address these limitations and promote, fund, and organize the discovery of vaccines against EIDs, CEPI was established. This was particularly the case when market incentives failed to generate the required innovations on their own. With the goal of moving immunization ideas forward to the Proof of Concept (PoC) stage, CEPI has funded phase I and II clinical trials from the beginning. Furthermore, CEPI aims to back technological and institutional platforms that speed up the response of research and development to newly emerging infectious diseases (EIDs). To combat the spread of potentially epidemic-like emerging infectious diseases (EIDs), the WHO and the international community will maintain their tight collaboration at CEPI to fund the research and development of vaccine candidates. In addition, CEPI will set up experimental reserves of eligible individuals, keep them under strict scientific scrutiny, and test them extensively during epidemics.

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Chapter 07

Conventional and Advanced Approaches in Veterinary Vaccines

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ABSTRACT

Veterinary vaccination is one of the main effective measures to reduce and eradicate impairment prompted by infectious diseases in animals and humans. Various vaccines are used as either, prophylactic vaccines that protect body prior to pathogenic exposure, or as therapeutic vaccines that are responsible for strengthening immunity post pathogenic infections. Several effective vaccines have been developed which have significantly reduced the impact of a number of diseases, playing a decisive role in the health, survival and well-being of livestock and companion animals. Thus, vaccination has a pivotal role in the prevention, management and eradication of lethal diseases as the use of antibiotics against these infections is developing antibiotic resistance in livestock as well as in humans due to the consumption of food derived from these animals. Conventional vaccinations, such as live or killed modified pathogen, have been used for centuries to regularly immunize animals to reduce the impact of disease. However, current developments in genetics, molecular biology, microbiology, and immunology have led to the development of numerous innovative, safer, and more effective approaches to vaccine development. This chapter sheds light on the conventional and advanced approaches in veterinary industry to combat the challenges in vaccine development against veterinary diseases.

KEYWORDS
One-Health, Veterinary, Vaccination, Immune Response, Advanced Approaches

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INTRODUCTION

History of Veterinary Vaccines and Immunization

The vaccine inoculations have been started for about 500 years ago when Edward Jenner first inoculated a boy with the small pox lesions for immunization in 1796 (Barakat, 2021). After 80 years, Louis Pasteur developed the vaccine for viral disease rabies. With the advancements in the vaccine production, further research involving erysipelas and rabies investigated serial passage as a substitute method to lessen or eradicate virulence in animals or other animal-derived tissues. This immunization technique aided in the prevention of anthrax and rabies (Saleh et al., 2021). Another method of immunizing against veterinary diseases was developed by Salmon and Smith in 1886, which was based on attenuation and inactivation principles (Nooraei et al., 2023). To increase vaccine diversity, these vaccine types were expanded to include toxoid vaccinations developed by Gaston Ramen at the Pasture institute against tetanus (Conti, 2021). Thus, in early twentieth century, first toxoid vaccine was created in 1924 by chemically inactivating the toxin, which was then adjuvanted to increase its effectiveness (Gupta and Pellett, 2023).

Veterinary Vaccines and One-Health

The rise and dissemination of zoonotic infections such as COVID-19 emphasized on averting the emergence of novel and hazardous zoonoses that could have adverse effects on human health. This is especially significant as the pandemic aligns with the One Health theory, which takes into account the relationship that exists between people, animals, and the environment (Zinsstag et al., 2023). At least 75% of newly discovered diseases have a zoonotic origin, with a variety of

animal species serving as their main reservoir. Prominent instances of these zoonoses comprise pandemics and/or epidemics including the Ebola virus, H1N1, SARS, MERS, and Spanish flu (de Melo et al., 2020).

In veterinary medicine, vaccination decreases disease rate within animal populations and improves public health by targeting zoonoses. There are various diseases that are zoonotic and can be transmitted by direct interaction or contact with the diseased animals, fluids, tissues or by some vectors like arthropods, effecting both food security and public health (Udainiya et al., 2024). It is very important to secure animal and human life by preventing the transmission of diseases at animal-human interface. Hence, veterinary vaccination acts as a bridge in "One-Health" and as a barrier to pandemic and epizootics control (Entrican and Francis, 2022).

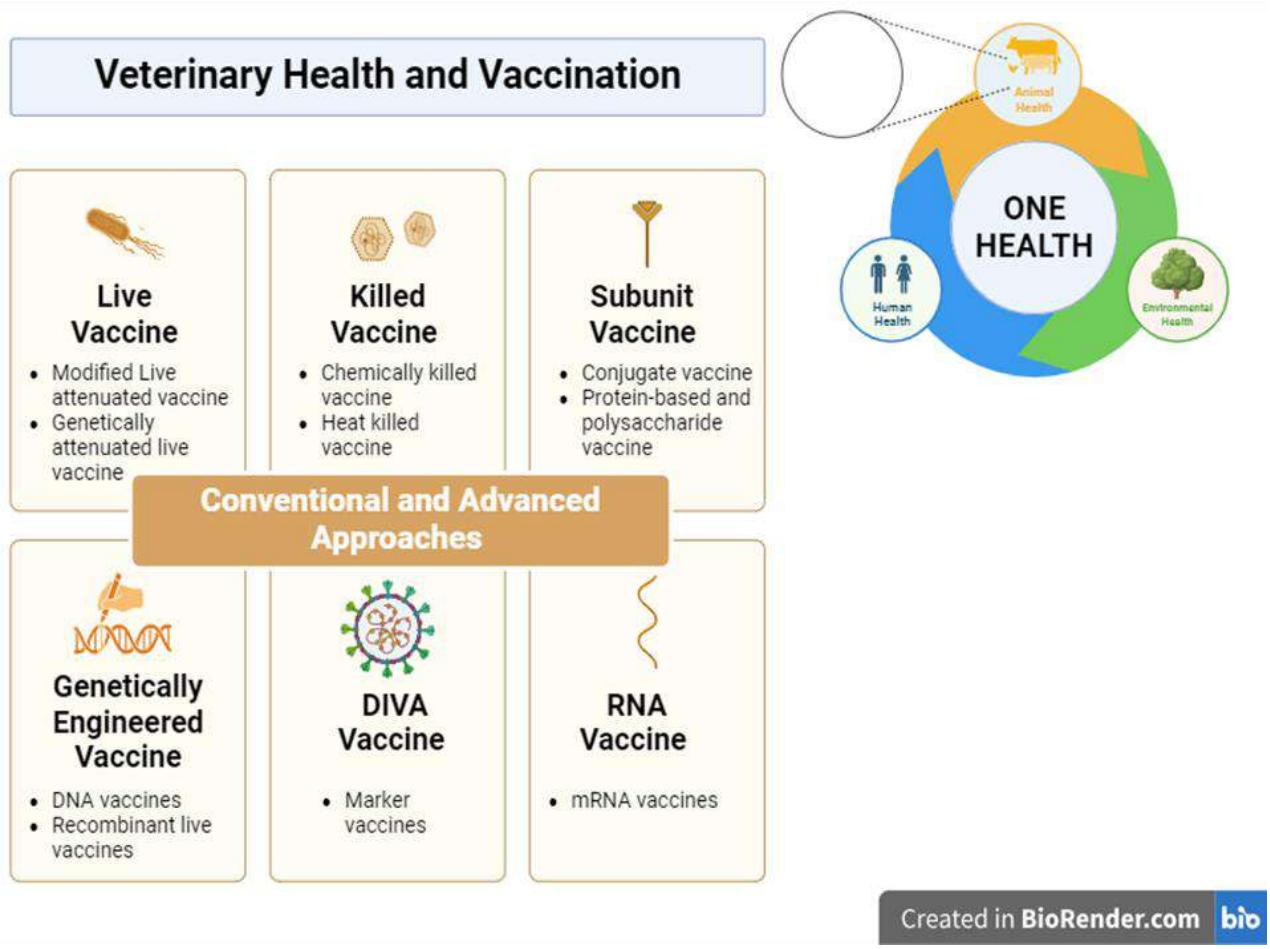

Fig. 1: Veterinary Vaccines – One Health Perspective

Development of Vaccine and Immune Responses

For many of the animal diseases, licensed vaccines produced by classical methodologies are being administered according to standard immunization schedule (Ghattas et al., 2021). Vaccines exploit two main principles, specificity and memory. Specificity is the crucial factor for the successful production of vaccines. This can be achieved by selection of whole antigen or some particular antigens that will induce the pool of memory lymphocytes as the primary response after vaccination (Brun, 2022). Ideally, the immunity would not only protect against morbidity and mortality but also prevent infection by blocking the infectious agent and its replication (Ali et al., 2023). There are several methods for developing vaccine against pathogenic microbes. These decisions are usually based on fundamental knowledge about the infectious agent, including how it infects the host cells, how the immune system responds to it, and the type of field strain in the particular region of the world (Yadav et al., 2020).

The developed vaccine should have the following properties

- It should provide long lasting immune responses including B-cell and T-cell responses
- It should be thermostable, should not be dependent on cold chains maintenance
- Easy administration of the vaccine, preferably through oral means
- It should provide protection against multiple diseases, hence be multivalent
- It should be cost effective and acceptable to country's authorities and policy (Gravagna et al., 2020).

Vaccine Design and Selection of Antigen

The vaccination methodology in past was designed empirically, but by optimizing the response of immune system,

synthesizing a rational vaccine can maximize their effectiveness. The logical design of vaccine is substantially influenced by the determination of the immunological connection with protection. This simplifies the choice of the appropriate antigens and adjuvants for vaccination to produce optimal adaptive and cell-meditated responses. Certain critical steps are involved in the potential design of modern vaccines (Schijns et al., 2021). These tactical decisions should ideally depend on targeting the immune response for designing successful vaccine, selection of the antigen, its presentation and delivery, effective immunomodulator and vaccine adjuvant.

The strategies behind successful capturing target-antigen are opting the target receptor, antigen to be administered, and antigen transporter. The APC target, adjuvant, and specific receptor work together to assemble the CD4+ T-cell response toward the Th1, Th2, and Th17 profiles in addition to selecting a specific target receptor. (Sulczewski et al., 2020). These features are mandatory for triggering immunity to combat future pathogen eradication. The mode of delivery has also a major impact on progress of the systemic or mucosal responses. The most commonly used immunization routes include intradermal, subcutaneous, intramuscular, and oral (Melgoza-González et al., 2023).

Types of Vaccines and Immune Response

There are several vaccines that helps body to cope with infectious diseases and boost the immunity. Currently, there are four types of vaccines that are available:

Type 1 Vaccines

Inactivated or killed vaccines have been used for decades and are generally being prepared by various chemicals or physical methods which results in disruption of pathogen replication ability. Chemical methods that are most widely used include formaldehyde and organic compounds based on cyclic esters (β-propiolactone) or binary ethylenimine (BEI). Some other cross-linking agents like glutaraldehyde can be used for this purpose. However, the limitation of using these cross-linking agents is their potential for disrupting the antigenic epitopes by aggregation leads to reduced immunogenicity (Jarvi and Balu-Iyer, 2021). Hence, other inactivation approaches can be opted such as hydrogen peroxide or some protonating compounds such as diethyl-pyrocarbonate (DEPC). The ability of hydrogen peroxide to inactivate both RNA and DNA viruses reduces the antigenic structural damage and minimize the effect on immunogenicity (Brun, 2022). However, such types of vaccines provide short duration of protection and induce ineffective immune response, as compared to live viral vaccines. This is why strong adjuvant is required when administering inactivated vaccinations, and it requires multiple booster doses to maintain long term and satisfactory immunity (Vashishtha and Kumar, 2024) .

Type 2 Vaccines

Live attenuated vaccines are one of the most successful kinds of vaccines with respect to providing booster immunity. The most highlighting feature of these vaccines is their ability to eliminate the virulence factors while maintaining the immunogenicity. The prominent benefit of attenuated vaccines is that it presents wider range of epitopes, leading to expression of more proteins as a result of viral replication into the infected host cell. The other possibility is its administration through natural routes of infection. Therefore, the induced immune responses are also similar to that of infections which triggers innate immune responses along with humoral and cellular responses (Torina et al., 2020) .

Type 3 Vaccines

This category of vaccine includes both subunit and nucleic acid vaccines. Subunit vaccines are superior to attenuated vaccines especially in production and safety factors. In subunit vaccines, another effective aspect is its possibility of generating virus like particles (VLPs) devoid of ribonucleoproteins by co-expressing the capsid proteins that are constituents of virions. Just like viral capsids, VPLs are also made of geometrically arranged patterns of proteins that forms the repetitive structures against which B-cell receptors or soluble antibodies can interact with high affinity. These VPL structures are good inducers of T-cell independent responses. In addition, these VPL structures can also be internalized and processed by antigen presenting cells (APCs) to trigger Th and CTL responses, showing the capacity to generate broader immune responses as compared to monomeric forms of protein subunit (Banerjee and Madhyastha, 2021).

On the other hand, genetic vaccines or nucleic acid vaccine were first discovered upon gene therapy experiments conducted by Wolff and Felgner when they were designed to transfer DNA into muscle cells using cationic lipid as carrier (Tiwari and Menghani, 2020). Generally, DNA vaccines are usually delivered by intradermal or intramuscular injections. But muscle cells can also be directly transfected and express the proteins. This can be achieved by using dendritic cells that are present in interstitial spaces, actively responsible for the uptake of soluble antigens, or can take up the cells killed by vaccines, or can be transfected directly. The advantages of these DNA vaccines are their easy design and production procedure, differentiation of vaccinated and infected animals (DIVA), natural processing of antigens, mimicking the immune response generated by virus replication as a result induce both humoral and cell-mediated immune responses. To date, the only DNA vaccine licensed have been against VHS in Salmonids and WNV in horses (Brun, 2022).

Type 4 Vaccines

Recombinant viral vectors, which make up the fourth category of vaccines, are an essential domain for the development of vaccines and for exploratory vaccination point of view. In this technique, non-pathogenic, infectious, virus

can be used to provide a system for recombinant incorporation and to express foreign genes. This technique has been used for different RNA viruses but among the DNA viruses, Poxvirus (from parapoxvirus and orthopoxvirus genus), Adenovirus, Herpesvirus and Baculovirus have been most widely used to deliver vaccine in experimental vaccine trials. The advantage of using DNA viruses over RNA viruses is the high stability of DNA genomes, containing more insertional sites and availability of BAC-DNA clones making it possible to engineer and rescue recombinant virus, a conventional laboratory task. Additional beneficial factors include, cytoplasmic expression and generation long term humoral and cell-mediated immune responses. These immune responses with immense emphasis on CD8-T-cell activation that is mediated by attenuated adenovirus and poxvirus infections (Dhakal et al., 2021).

Conventional Veterinary Vaccines against Bacterial Infections

Historically, vaccination with a range of raw antigen preparations was tried to control a number of bacterial veterinary infections. Live vaccinations are wild-type or naturally occurring variations of parental strains, whereas bacterin vaccines are prepared from complete dead cells or cell lysates (Rabie and Amin Girh, 2020). Due to the lack of knowledge about specific protective antigens and low cost of manufacturing, early vaccination followed bacterins or live cells for immunization. For sheep, cattle, and pigs, bacterin immunization showed that piliated bacteria were required for protection against foot rot, pinkeye, and scours respectively. For each of these infections, the early bacterin vaccines were unable to offer sufficient levels of host protection because of poorly piliated vaccine cultures containing only one or two potential serotypes. To overcome this problem, eight *B. nodosus* (Tizard, 2021) and twelve *E. coli* strains are currently present in the effective foot rot and scours bacterin vaccines respectively, which in each case represent all known serotypes.

The effectiveness of live-cell and bacterin vaccinations against cow mastitis has been studied. It was discovered that vaccinations against *Staphylococcus aureus* given into the mammary gland, generated protection against *Staphylococcus* infections to considerable extent, which are one of the main causes of mastitis (El-Diasty et al., 2021). Nevertheless, the large *S. aureus* bacterin doses necessary to promote protective immunity may cause lactation loss in the future. For commercial application, intramammary immunization is therefore not a viable choice. Differently from the case of killed vaccine, live *S. aureus* administered systemically to cows elicited protective immune responses because only live cells growing in vivo produce immunogenic level of capsular and toxin antigens. Which are critical for eliciting protective immunity. The cost of producing live vaccinations is higher than that of inactivated vaccines, which are also more resilient to environmental changes (Côté-Gravel et al., 2019). Since inactivated immunizations stop the spread of pathogens, they are not effective for long-term protection, even though their safety profiles are superior (Jorge and Dellagostin, 2017).

Salmonellosis and brucellosis in cattle are those infections in which the bacterial pathogen lacks both virulence elements associated with toxins and pili. Calves have been protected against oral *Salmonella* challenges by vaccination with live, virulent *Salmonella* and with a dead whole-cell bacterin; however, attenuated live bacteria are generally recommended as salmonellosis vaccine (Edrington et al., 2020).

Whereas, brucellosis caused by *Brucella* affects both humans and domestic animals including sheep, goats, cattle, and pig. Although live vaccinations including *B. suis* S2 and *B. melitensis* M5 have been widely utilized for preventing brucellosis infection in China (Li et al., 2023), the live attenuated *B. abortus* strain 19 is currently the recommended vaccine against bovine brucellosis (de Oliveira et al., 2022). The 45/20 strain of killed *B. abortus* vaccine has been used successfully in several nations with variable immunogenic properties. Originally isolated in the early 1920s, strain 19 is inexpensive and easy-to-use option for vaccination, but it has drawback of its lethal nature as a tiny portion of vaccinated animals shed the bacterium and experience chronic illnesses (Maruf et al., 2019).

Conventional Veterinary Vaccines against Viral infections

The most prevalent viral infections causing gastroenteritis in neonatal calves are rotavirus and coronavirus. In the gastrointestinal tract, local immunity is necessary to offer sufficient protection against infection. For calves, an oral vaccination has been designed to confer local immunity. Since nearly all cattle have antibodies to the coronavirus and rotavirus in their milk, the vaccine virus is swiftly neutralized by the antibodies in the milk, preventing the formation of immunity. Therefore, the vaccination needs to be given before nursing. The current vaccination contains only one serotype (serotype 6) contributing to the lack of potential action in the field. According to recent research, calves may carry multiple serotype infections, and vaccination against one serotype may not guarantee protection against heterologous serotype challenges (Geletu et al., 2021). The obstacle in the active immunization within calf is resolved by hyper immunizing the dam in mid-gestation and increasing antibody levels during the end of gestation. This methodology resulted in increased levels of milk and colostrum antibodies.

The foot-and-mouth disease (FMD) is a highly feared bovine viral infection in prevalent regions such as South America, Asia, and Africa, where immunization is the only measure of prevention. Inactivated and live-attenuated vaccines have been tested, which are mostly synthesized by inactivating viruses cultivated in tissue cultures (Kamel et al., 2019). Numerous serotypes of FMDV exist, including 0, A, C, SATl, SAT2, SAT3, and Asia 1. Apart from these seven serotypes, there is a significant level of antigenic diversity among these serotypes. Due to this antigenic diversity, it is crucial to make sure that control efforts through vaccination are being made against those specific serotypes circulating in the field. The antigenic structural analysis of the virus reveals VP1 protein as primary immunogenic location on FMDV. This localization led to the expression of FMDV VPl in *E. coli*, which was one of the first proteins to be evaluated as a vaccine in the early

1980s (Qadeer et al., 2021).

A member of the morbillivirus genus, rinderpest induces acute systemic infection that erodes the mucosal epithelium of the respiratory and digestive tracts in ruminants. Vaccination against rinderpest using inactivated viruses have been administered in regions where the disease is enzootic (Jia et al., 2020). The immune response associated with these vaccines was frequently transient, necessitating yearly booster shots providing enhanced degree and duration of immunity.

Table 1: Licensed Vaccines against Veterinary Bacterial and Viral Infections

Vaccines	Disease	Pathogen	Protected Host	Category of Vaccine	References
WEST NILE-INNOVATOR®	Encephalomyelitis	West Nile Virus	Horses	DNA vaccine	(Pereira et al., 2014)
Plowright Tissue Culture Vaccine	Rinderpest disease	Rinderpest Virus	Cattle, Buffalo	Live attenuated	(Sills and Robertshaw, 2010)
Human Diploid Cell Rabies Vaccine (HDCV), Purified Chicken Embryo Cell Vaccine (PCECV)	Rabies	Rabies lyssavirus	Rabid animals	Live attenuated	(Prevention, 2024)
Avinew	Newcastle Disease Virus	Newcastle Disease	Chickens	Live VG/GA virus strain	(Bwala et al., 2009)
AE-Poxine	Avipox virus, Avian encephalomyelitis virus	Fowl pox, Avian encephalomyelitis	Chicken	Combination modified live virus	(Islam et al., 2008)
Tetanus toxoid	*Clostridium tetani*	Tetanus	Equines	Subunit vaccine	(Manual, 2021)
Bovilis Vistal Once SQ	*Mannheimia haemolytica, Pasteurella multocida, BHV-1, BVDV, BPI3V, BRSV*	Bovine respiratory disease, IBR, Bovine viral diarrhea, Bovine parainfluenza 3, viral pneumonia,	Cattle	Modified live virus vaccine	(Purtle et al., 2016)
Nobivac Lepto	*Leptospira canicola, Leptospira iterohaemorrhagiae*	Leptospirosis	Dogs	Bivalent inactivated vaccine	(Health, 2023)

Advancements in Veterinary Vaccines

Despite accessibility of innovative classes of vaccines, the same fundamental technologies continue to be the backbone of vaccine manufacture in the contemporary era of vaccination use. The fundamentals of manufacturing, registration, and developmental research still adhere to the magnificent heritage methods. The majority of these vaccines were created in local research facilities. When Waldman and others employed large-scale, regulated procedures to create FMD antigens in Germany in the 1930s and 1940s, the processes started to become industrialized (Abbas et al., 2022). In the 1950s and 1960s, primary cell lines were developed, followed by clean cell lines (Szkodny and Lee, 2022). Moreover, significant developments in the field of vaccination have been made possible by advances in inactivation technologies, antigen concentration, purification, bulk antigen storage, enhanced aluminum gels, and oil suspension as adjuvants used in the preparation of polyvalent antigens (McVey and Shi, 2010).

The area of vaccine technology with the quickest rate of growth is DNA vaccination. DNA vaccinations cause the host to produce antigens through a plasmid containing a gene for a protein, found in mammals or for a virus, bacteria, or parasite that can be generated in mammals (Shafaati et al., 2021). Innate and adaptive immunity are both stimulated by DNA vaccination. The innate immune system can be triggered by identifying the dsDNA of the plasmid backbone, whereas the adaptive immune response comprises the processing of antigen and its presentation in class I and class II MHC molecules to CD8+ and CD4+ T cells, respectively (Gómez and Oñate, 2018).

Attenuated live vaccine Attenuation via extended tissue culture could be considered an early form of genetic engineering. While molecular genetics technique modifies an organism's genes to cause irreversible attenuation and helps pinpoint certain virulence genes (Antoine et al., 2021). This can be achieved by introducing many mutations or even eliminating the entire gene, depending on the pathogen. Using this methodology, it is possible to create a vaccination that is both safe and affordable instead of utilizing traditional techniques. To change the attenuation level, the appropriate gene or gene set(s) should be removed or modified (Dolan, 2020). The capacity to deliver live vaccines that have been genetically attenuated that resemble their actual infection pattern is the primary advantage of this technique. Therefore, they should elicit an immunological response same as induced by highly pathogenic field isolates of the pathogen (Gutiérrez-Álvarez et al., 2021).

Live recombinant vaccines are based on a genetically modified live virus or bacterial vector that expresses a variety of foreign antigens in the cytoplasm of target cells. As a vaccination, the recombinant organism itself may be used. The most frequent viruses utilized as a vector for the creation of live recombinant virus vaccines are adenoviruses, herpesviruses, and

poxviruses (Kamel and El-Sayed, 2019). Poxviruses with large, stable genomes that allow it relatively easy to insert a new gene, such as vaccinia, fowl pox, and canarypox, have been the most commonly used viruses in the design of live recombinant vaccines (Lee et al., 2012). The rinderpest vaccine is made up of a capri pox or vaccinia vector that has the rinderpest virus's fusion (F) or hemagglutinin (H) genes (Minhas et al., 2016; Teffera, 2021). Live recombinant vaccines can induce robust humoral and cell-mediated immune responses that can result in immunological memory. It can also encode for many antigens from different diseases. There is a chance that one vaccination could soon be available for several diseases. One of the drawbacks (Francis, 2018) of live recombinant vaccine is its live-attenuated nature (either bacteria or viruses), so there is potential for reversion to virulence form of the pathogen.

Short polypeptides can be chemically synthesized to create subunit vaccinations. While solid phase peptide synthesis is not a novel technology, advancements in the last ten years have reduced costs and raised process efficiency (Ferrazzano et al., 2022). The methodology involves precisely sequencing DNA to pinpoint protective antigenic determinants on antigens and availability of monoclonal antibodies to identify these epitopes. The FMDV was among pioneer viruses to be tested for determining the viability of exploiting synthetic peptides as a vaccine candidate. Unfortunately, antigenic variation can occur in many viruses, including FMDV. Therefore, prior to the development of a single broad coverage synthetic peptide vaccination, it is necessary to identify important conserved epitopes on the virus. Furthermore, to enhance the efficacy of these vaccines, genetic engineering methods are followed. For instance, all rotavirus serotypes share a proteolytic cleavage site in VP4, which is present in all rotaviruses (Hoxie and Dennehy, 2020). It has been demonstrated that a synthetic peptide vaccine targeting the VP4 cleavage site can produce protection against several other serotypes from different species. These molecular approaches have resulted in new strategies for creating novel vaccines against infectious, parasitic, or metabolic diseases in addition to improving knowledge of the genes causing virulence and making it easier to identify the factors influencing protective immune responses.

Subunit Vaccine comprise of one or more pure or semi-pure antigens from the target pathogen. These subunit vaccines are being produced in huge quantities using a variety of expression systems including, prokaryotic systems, and eukaryotic systems, such as yeasts, filamentous fungi, algae, mammalian and insect cells. To enhance the immunogenic response against multiple serotypes of the same pathogen, it is advantageous to immunize an animal with a vaccine comprising several defensive proteins from several serotypes. This can be accomplished by creating chimeric proteins, which are proteins that combine protective epitopes from several organisms into a single protein. (Karch and Burkhard, 2016). One example of subunit vaccine which makes use of recombinant DNA technology, incorporates the fusion and hemagglutinin-neuraminidase proteins of rinderpest into the vaccinia virus, resulting in the development of a strain that is resistant to heat (Cid and Bolívar, 2021) .

Marker vaccines enable serological distinction between vaccinated and infected persons. The basis for this distinction is lack of one or more microbial proteins within vaccine formulation that are found within wild type microbe. Consequently, an antibody response against those particular protein or proteins can be found after infection but not after immunization. Thus, it is possible to differentiate between vaccinated and infected patients using a protein-specific antibody test. Through conventional methods as well as recombinant DNA technologies, marker vaccines against infections caused by the pseudorabies virus (PRV) and the bovine herpesvirus 1 (BHV1) have been created (Zheng et al., 2022). It has been demonstrated that these vaccinations are effective in lowering clinical symptoms following infection, replication of the wild-type virus following infection, and transmission of the wild-type virus in both the laboratory and the field.

mRNA vaccines are becoming focus of research in recent years because of their high efficacy, speed of production, potential for low-cost manufacture, potential for safe delivery, and as a replacement for conventional vaccination methods. mRNA vaccines do not create or incorporate infectious particles into the host cells; therefore, their genome remains unaltered. They can express complex antigens without being limited by packing restrictions and can be used to transport antigens for in-situ expression without any requirement to breach the nuclear membrane barrier (Nitika et al., 2021). By utilizing the machinery of the host cell to convert mRNA into the proper antigen in vivo, the mRNA vaccine imitates a natural infection and elicits robust humoral and cellular immune responses (Zhang et al., 2019).

Conclusion

Vaccination in veterinary sector is the most economical method for preventing and controlling newly emerging and reemerging infectious diseases. Improving animal health acts as a tool in improving public health and welfare, and maintaining the balance of One-Health triangle. New and more potent vaccinations have been created as a consequence of recent developments in molecular biology and genetic engineering. Novel methodologies within vaccine development can revolutionize the fate of vaccine industry through low-cost, high production rate, safe delivery, and high efficacy.

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Chapter 08

Vaccine Based Cytokines and their Role in T Cell Proliferation

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ABSTRACT

In the present context of immunological research, the interaction between vaccines and cytokines is an essential element of understanding immune responses, especially with regard to T cell proliferation. This also aims to understand the activation mechanisms of T cells as well as to explore the central role cytokines play in determining immune outcomes. It particularly stresses the importance of cytokine immunotherapies for conditions that are diverse indeed, from infectious diseases to cancer and autoimmune disorders. It also provides an overview of new technologies in vaccine development, emphasizing in particular the potential carried by innovative delivery systems and adjuvants for enhancing T cell responses. At the level of clinical application, ethical issues and regulatory umbrella of cytokine-based approaches serve as Achilles heels. Therefore, safety first Informed consent is necessary for any proposed campaign or intervention. Last, but by no means least, the research aims to bring about perspectives from personal usage of cytokine therapy. This is the area seen as giving eminent possibilities for the future of vaccine immunology research and cytokine research.

KEYWORDS
Vaccine, Cytokines, T cell proliferation, Immunotherapy, Immune responses.

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INTRODUCTION

In the dynamic world of immunology, the mutual interaction of vaccines and cytokines stands as the linchpin of the contemporary medical science. Vaccines are the foremost symbols of the immune system, and their induction of cytokines of various kinds that convoke, differentiate, and proliferate T cells is without doubt the correct paradigm of how immunity is first and last regulated. Understanding of vaccine-induced cytokines and derived signals of T cell proliferation is of fundamental order for understanding immune responses and has great consequences for vaccine design and therapeutic interventions. This book spans the structures, mechanisms, implications and potential therapy that is unfolded in study of vaccine-based cytokines and their possible role in T cell proliferation (Reens et al., 2021).

The research and technology work on vaccines allowing human beings not to contract a contagious disease entirely. On one hand, as occurs in vaccines, such work on viruses has have been the civilization´s most remarkable accomplishments. The virus has an extremely simple structure: it is just a shell with the genetic material enclosed, which in some cases can be RNA or DNA. It has been calculated down to the dot after 10 years of work where a state-of-the-art evaluation system has been used, two major groups of tests on volunteers and another one has also been done on animals, conducted with this system. Immunity to such vaccines results from the complex interplay between Antigen Presentation and Cytokine signaling pathways (Farmer, 2016).

Studies demonstrate that the hierarchical immune response following vaccination is orchestrated by cytokines small molecules secreted by a variety of immune cells that regulate the activation, proliferation and differentiation of immune cells, e.g. T lymphocytes. To learn and/or optimize vaccine efficacy it is essential to mechanistically understand the interplay between vaccines and cytokines (Croci et al., 2007)

Vaccines have shaped modern health care more than any other medical advances, and they have played a key role in preventing innumerable infectious diseases and saving millions of lives across the globe. Acting as immunostimulants,

vaccines present a weakened or killed pathogen or pathogen components to the immune system, which responds by learning to recognize and remember specific antigens and conferring immunity upon re-encounter with the pathogen. This process, known as vaccination or immunization, orchestrates the orchestrated, careful interaction of antigen-presenting cells, including dendritic cells and macrophages with diverse immune cells such as T lymphocytes (Farmer, 2016).

The immune response to vaccination is guided in part by cytokines, small proteins secreted by immune cells, which capture the general environmental conditions that these cells sense. One family of cytokines, which includes interleukin-12 (IL-12) and interferons (IFNs), is produced by so-called "antigen-presenting cells" in response to the components of a vaccine that activate these cells. Cytokines from this family serve as a potent "third signal" that is required for the activation and differentiation of T cells. They are essential for "priming" naïve T cells so that they differentiate into the effector and memory T cell subsets that are responsible for controlling and eliminating pathogens (Pollard and Bijker, 2021).

Vaccines and cytokines have a complex interplay that is critical to the optimization of optimal vaccine efficacy, and the design of novel immunotherapeutic approaches. Cytokines have pleiotropic immunomodulatory properties that can be harnessed to design next-generation vaccines capable of eliciting robust and durable immune responses against a host of pathogens and/or other immune system modulated disorders (Chabalgoity et al., 2007).

Mechanisms of T Cell Activation and Proliferation

T cells serve as a critical arm of the adaptive immune response, unmasking infected or aberrant cells through processes known as cell-mediated immunity. T cells become activated after recognizing antigens bound to antigen-presenting cells such as dendritic cells. Activation involves a series of steps that commence with TCR-mediated recognition of antigen-MHC complexes which is followed by co-stimulation and signal transduction (Faget et al., 2019).

When the T cells are functionally activated, they undergo a clonal expansion. It is very important to find such an immune response which is both strong and also prolonged. The cloning process engages the expansion of T cells that reproduce themselves while producing Effector cells which can either act directly on the target cells or they can produce cytokines to avoid the original immunity. The latter phase of this process is a very part of it. Regulates in this manner cytokines like interleukin-2 (IL-2). These cells differentiate into many growth factors which promote the division of T cells and also aid to keep these lymphocytes alive (Bevan, 2004).

Therefore, T cell activation and proliferation are tightly controlled in a very highly ordered regulation to ensure that any immune response takes place within a reasonable level of inflammation and damage. Dysregulation of T cell activation pathways leads to many different types of immune disorders, e.g. autoimmune disorders, hypo immunity (Figure 1). In this light, the molecular basis promoting the T cell activation and also proliferation function should be keenly explored, as it is a sine qua non condition for designing new therapies for immune-related diseases (Izcue et al., 2006).

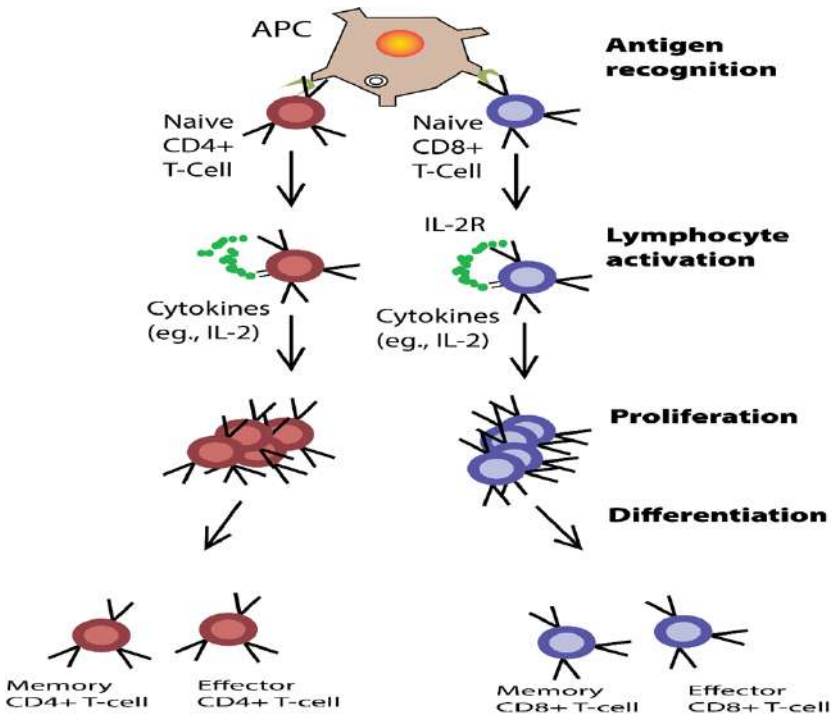

Fig. 1: Mechanisms of T Cell Activation and Proliferation

Role of Cytokines in Modulating T Cell Responses

This class of tiny signal molecules has wide-ranging effects on T cells, which include influencing the T cell 's activation, differentiation and effector functions. Among the many cytokines that change T cell responses interleukin (IL), its child interferon (IFN) and its grandchild cell necrosis factor (TNF) have an important role in regulating T lymphocyte proliferation, survival or allocation to effector lineages (Izcue et al., 2006).

Formerly identified as T cell growth factor, interleukin 2 (IL-2) serves an integral part in promoting the proliferation and survival of T cells. Once it has been captured by its receptor, IL-2 triggers signaling pathways that move cascade mechanisms forward promoting passage of the cell cycle (rather than apoptosis, survival elements). Thus, it assists the expansion of antigen specific T cell clones. In addition, IL-2 has also been found to be involved in the generation of regulatory T cellsTregs, a specialized type of T cell engaged in sustaining the body's immune system and maintaining tolerance among cells (Malek, 2003).

Interferons are another type of cytokine's study have shown hold back the immune system's responses. They have powerful immunomodulatory impacts on T lymphocyte activity. Interferons play vital roles in antiviral, immunomodulatory and antiproliferative activities, affecting various aspects of T lymphocyte function such as antigen presentation, T cell activation and effector differentiation. IFNs also make significant contributions to enhancing the cytotoxicity of T cells and promoting their development into effector subsets competent at destroying infected or malignant cells. This is an invaluable asset in our fight against section oddities (Trinchieri, 1994).

Vaccine Strategies Targeting T Cell Proliferation

Vaccines generally need to coax in addition be T cell proliferation, from immunopathological mice to rabies-free human beings, in order that the quicker and string immunity immune system usually elicit will not fall short against pathogenic germs. Traditional vaccine development has concentrated on prompting an immune response by stimulating the body's production of antibodies. However, recent advances in this process have demonstrated that there is also a role for T cell reactions to occur as well. vaccine strategies directed at T cell Proliferation aim to improve antigen presentation, co-stimulation and cytokine signaling so as to prompt the activation and expansion of antigen -specific T cell populations (Pulendran and Ahmed, 2006).

One way to boost T cell responses is by using adjuvants. Adjuvants are substances incorporated into vaccines that increase the immune response. Innate Reprogramming of immune responses by adjuvants such as aluminum salts and toll-like receptor agonists not only lead to more antigen uptake but also more efficient presentation and activation of T cells. With adjuvants promoting T cell division, they are crucial in boosting the effectiveness and long-term response characteristics of vaccines (Di Pasquale et al., 2015).

A new strategy to make T cells proliferate is to develop innovative vaccine delivery systems. These systems are intended to enhance antigen presentation and T cell activation by specifically targeting immune cells or tissues. Viral vectors, nanoparticles, and liposomes are counted among these (Figure 2). Not only do they bear antigens and adjuvants efficiently to antigen-presenting cells (APCs), they also induce strong T cell responses (Fan and Moon, 2015).

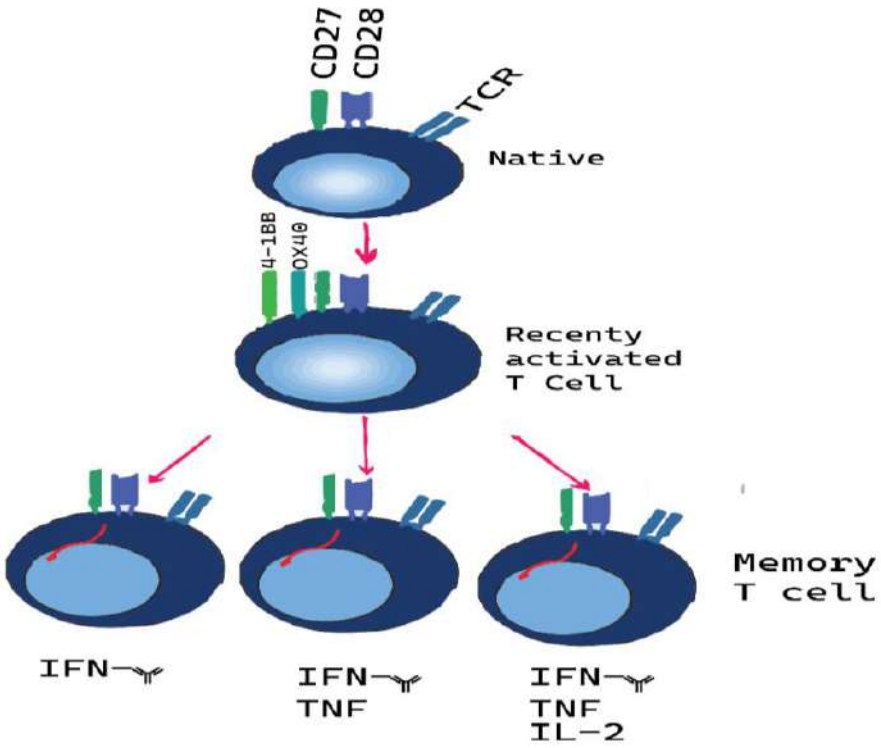

Fig. 2: Vaccine Strategies Targeting T Cell Proliferation

Cytokine-Mediated Immunotherapies

Cytokines are integral players not only in the shaping of immune responses but also as therapeutic targets in a multitude of immune-related disorders. The cytokine signaling pathways that have been harnessed to manipulate immune responses for therapeutic benefit include those the enhance T cell proliferation and function. Recombinant cytokines have been used to augment T cell responses in conditions as varied as cancer and autoimmune diseases (Salvador et al., 2021).

An early form of cytokine-based cancer treatment--Il-2 therapy represented the earliest type used for treating cancer and has been around since 1992. The intention of high-dose Il-2 administration is to mobilize and increase cytotoxic T cells and natural killer cells, leading to the death of tumor cells. While being effective in certain cancers, IL-2 treatment also carries significant toxicities, driving the development of new, safer cytokine-based immunotherapies (Sunga et al., 2023).

As other cytokines, interleukin-12 (IL-12) and interferons (IFNs) have also been considered for their potential in immunotherapy. IFN-based therapies have efficacy in treating viral infections, autoimmune diseases, and particular cancers by promoting T cell-mediated immune responses and actively opposing viruses or tumors--while they are antiviral drugs themselves Similarly, therapy with IL-12 tries to make cytotoxic T cells and natural killer cells active, stronger and more deadly: their ultimate object is the elimination of infected or cancerous cells (Colombo and Trinchieri, 2002).

Emerging Technologies in Vaccine Development

With the development guided by technology advances, as Liu (2019) says that in very start of this paper-these new vaccine platforms target T cell proliferation much more precisely and effectively than the old vaccine types Advancing technologies, include nucleic acid-based-vaccines mRNA and DNA vaccines, have a number of advantages in terms for example their ability to be manufactured at speed, brought from small pilot batches up to production scale and versatility. This sort of vaccine delivers genetic material encoding antigens directly into host cells. That leads to antigen expression on the cell surface and an increase in T cell activity (Ajah and Nweke, 2019).

A different successful way is to develop a viral vector vaccine; this vaccine uses vectors of viruses as delivery agents for antigens. The vectors carrying these vaccines can bring antigens very precisely to place in front of immune cells at their workstation; there they will produce T cells and ask in earnest for more. Viral vector vaccines offer unique advantages to the host; they might allow for many different types of immunizations, even against multiple targets with one dose (Ura et al., 2014).

With novel delivery systems and adjuvants, nanotechnology embarks on a quest of big promise in vaccine development. Their other advantage is T cell responses: How to Achieve a Great T Cell Response with Nanotechnology Nanoparticle-based vaccines can achieve efficient delivery of antigens and immunomodulatory factors to immune cells, thus directly promoting antigen uptake, presentation and T cell activation. In addition, nanotechnology permits the creation of multivalent vaccines aimed at several antigens simultaneously--thus, it stimulates a more vigorous immune response than if just one such monovalent vaccine was given (Wilson-Welder et al., 2009).

Table 1: Some Emerging Technologies in Vaccine Development

Technology	Description	Reference
mRNA Vaccines	Utilize messenger RNA to instruct cells to produce proteins that trigger an immune response.	(Jonuleit et al., 1997)
Viral Vector Vaccines	Use a modified virus to deliver genetic material from the target pathogen into the body.	(Seow and Wood, 2009)
DNA Vaccines	Introduce genetically engineered DNA molecules encoding antigenic proteins to stimulate immunity.	(Liu, 2011)
Nanoparticle Vaccines	Use nanoparticles as carriers for antigens or adjuvants, enhancing immune response and delivery.	(Mody et al., 2013)
Virus-like Particle Vaccines	Mimic the structure of viruses without the genetic material, eliciting an immune response.	(Nooraei et al., 2021)
Synthetic Peptide Vaccines	Utilize synthetic peptide fragments of pathogens to stimulate specific immune responses.	(Hilchie et al., 2013)
Self-amplifying RNA Vaccines	Employ RNA that can replicate within the host, potentially leading to stronger immune responses.	(Li et al., 2020)
Inactivated Vaccines	Utilize pathogens that have been rendered non-infectious but still elicit an immune response.	(Köhl, 2001)

Clinical Applications and Future Perspectives

Integrating vaccine development and clinical applications for use medicinally to better human health is also on the card's Infectious disease, cancer, and autoimmunity are thus the target. Clinical trials to test whether vaccines administered with cytokine enrichment really do stimulate better T cell responses have likewise shown that they perform better as vaccines, e.g. Amherst *et al* investigation APIs Interleukin-12 (IL-12), an immunological modulator, and granulocyte-macrophage colony-stimulating factor. (GM-CSF) have demonstrated enhanced T-cell responses to vaccines and improved vaccine efficacy. These results constitute the foundation on which It is hoped that cytokine-adjuvanted vaccines will be developed for a range of infectious diseases and cancer therapy projects (Vellingiri et al., 2020).

In addition to infectious diseases and cancer, T-cell modulation employing cytokine-driven immunotherapies also bodes well for the treatment of autoimmune diseases like those caused by tuberculosis. Biologic agents interfering with cytokines such as TNF-α and IL-6 can effectively control diseases such as rheumatoid arthritis both in the long and short term-inflammatory bowel disease. true research will focus on optimizing the therapeutic effects of such treatments in autoimmune diseases and reducing side effects (Möller and Villiger, 2006).

As for the future, research underway has an objective to discover how host immune systems and vaccines interact by means of cytokines. For instance, that involves learning about the conditions that cause cytokines to be produced, and it means research on signaling pathways so as in turn to study their effect on T cell activation growth added wisdom. Further work through the techniques used in immunogenomics and systems biology will offer valuable insights into vaccine strategies which are tailored to individual immune profiles--indeed, by creating a method for precision medical treatment and in immunotherapy design itself (Lunney et al., 2016).

Challenges and Considerations in Vaccine Development

While vaccine cytokine - method holds great promise, but also several questions and considerations to consider, in order to achieve optimal efficacy of its safety and effect. One question is how to get a deeper understanding of collapse biology, and its intricacies in managing immune reactions. Cytokines have pleiotropic effects and may beneficial or harmful to the immune system, so it is important to fine-tune cytokine-based treatments (Berraondo et al., 2019).

In addition, to attain both an efficient cytokine delivery and better target ion of immune cells another obstacle is in optimizing the delivery system and formula of vaccines. Improved bioavailability of cytokine, raising tissue-specific delivery and stability are all antecedent requirements for achieving maximum immunomodulatory effect with minimal off-target response. In particular the former two will alleviate safety concerns. At another level, vaccine safety also has to be considered including cytokine-induced inflammation and immunopathology in preclinical and clinical studies (Bolhassani et al., 2011).

Furthermore, in order to ensure equitable global vaccine coverage considerations for vaccine access, affordability and distribution are absolutely essential Addressing socioeconomic disparities as well as vaccine hesitancy and logistical problems in vaccine delivery is essential for maximizing the public health impact of cytokine-based vaccines and immunotherapies. Once again, in order to ensure vaccine safety the possibility of cytokine-induced inflammation or immunopathology should be carefully considered in preclinical and clinical studies (Wouters et al., 2021).

Ethical Considerations in Cytokine-Based Vaccines and Immunotherapies

Convenience claims the development and implementation of cytokine-based vaccines as well as immunotherapies bring an inevitable chance to make ethical quandaries related to its safety, fairness and even whether participants are fully informed about what they are letting themselves in for. Safeguarding the life and health of participants in clinical trials is a primary concern, meaning companies should go through thorough preclinical tests and comply with ethical guidelines. In addition, the need for equal access to cytokine-based therapies must be pursued both out of concern for social justice and to ensure that all will profit from potential discoveries (Bocchia et al., 2000).

In the conduct of clinical trials in which cytokine-based interventions may occur, informed consent is indispensable. Participants must be given information enough about risks, benefits, and other options to enable them to decide autonomously whether to take part. Furthermore, keeping the lines of communication open with participants and respecting their autonomy throughout all phases of testing is also a basic ethical principle.

It is a matter of ethics even more so to kick the conference center of life-saving vaccines and immunotherapies. Such treatments should be made available to all low and middle-income countries by means of international collaboration and partnership, thereby addressing the injustice of global health inequalities. Hence, open communication and dealing with local communities to gain trust both are important aspects of this effort also important for solving social and cultural problems as well as economic ones in attempts to carry out vaccination programs. Any vaccination campaign should take up not only the scientific evidence but also those of an emotional and human nature (Fig. 3).

Regulatory Considerations and Approval Processes

To ensure the safety, effectiveness, and quality of proteins such as vaccine immunotherapies regulation is essential. Before coming to market, these interventions have to undergo rigorous assessment by regulatory bodies as a process authorized by governments including the Food and Drug Administration (FDA) in United States and the European Medicines Agency (EMA) in Europe. They examine preclinical and clinical data from these agencies in order to evaluate the risks and benefits of cytokine-based interventions; and to determine whether it meets requirements for approval (Jawa et al., 2020).

The pre-filing operation can be considered the start of the regulatory process. The real research starts from preclinical testing and continues into phase 1, II and III trials bioequivalence studies under post – marketing surveillance procedures. Preclinical immunologic safety data on the safety of immunity with cytokine-based therapies originates from animal models. This early test of potential efficacy confirms for researchers something which will be of interest to drug companies. follow-up Clinical trials Studies of the safety and efficacy, as well the dosage to be used in human subjects. Generally, phase III takes place at a time when large-scale evaluations of its effectiveness can be made unfettered by experimental bias (Sokal and Gerstenblith, 2010).

After clinical trials have been concluded successfully, the accumulated data will be reviewed by these agencies to make an informed final judgment as to whether or not cytokine-based interventions should be approved. Conditions they look at during their review include profiles of safety, efficacy results from dialogue partner discussions with researchers in all pharmaceutical disciplines; once these have been finalized and scaled up to industrial levels However, if the benefits of

intervention more than outweigh its risks, regulatory authorities may also grant approval to market and sell it. Moreover, after regulatory approval, post marketing surveillance continues to monitor for long-term safety and efficacy of cytokine-based interventions (Waselenko et al., 2004)

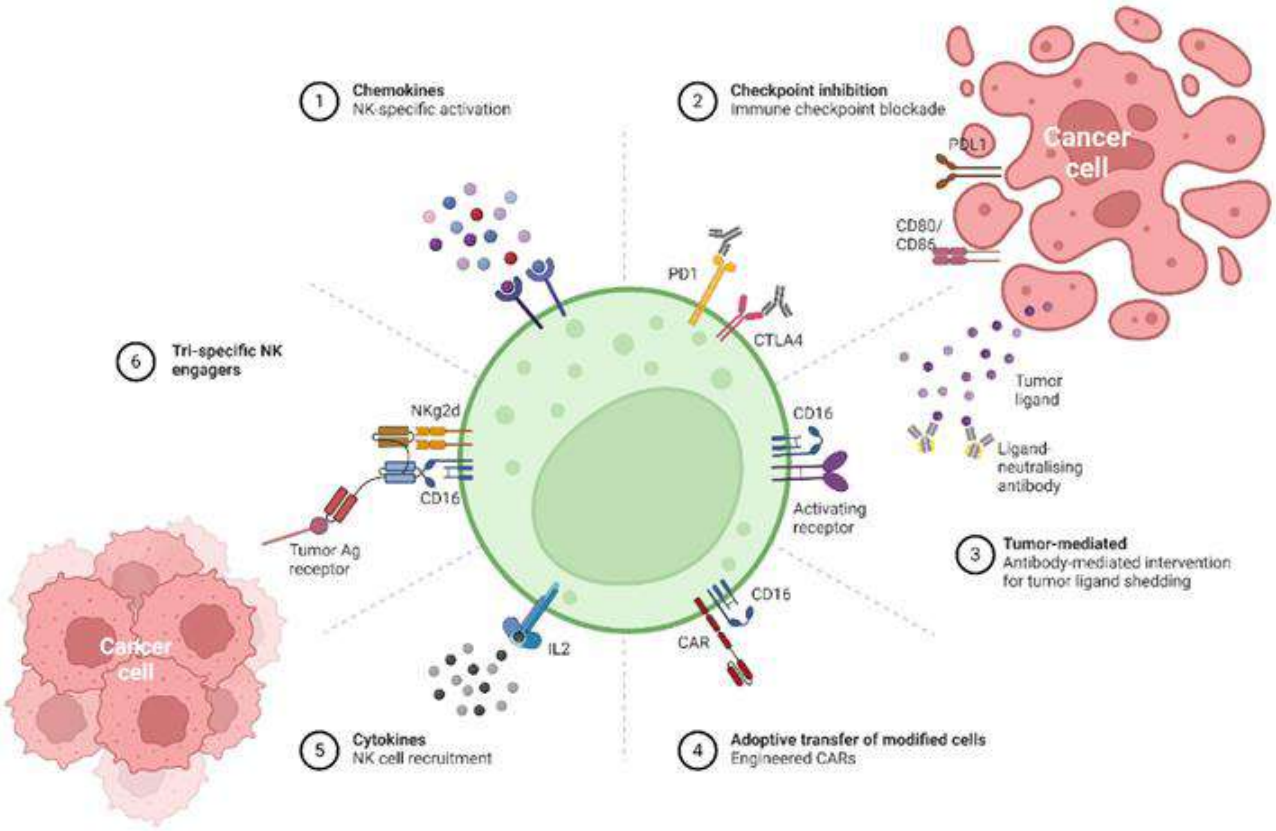

Fig. 3: Cytokine-Based Vaccines and Immunotherapies

Future Directions and Emerging Trends

Looking ahead, right now there are so many exciting new routes that could further the cytokine-based vaccines and immunotherapies fields immensely. One such trend in this is the boom of personalized cytokine therapies that are designed according to your body's very own immune structure. It is hoped that by taking advantage of recent progress in immunogenomics and computational modeling, scientists can create cytokine-based interventions that are carefully matched to individual patients 'genetic characteristics (Liu et al., 2023).

Away, by combining cytokine combated vaccinations with other immunomodulatory methods such as adoptive T cell therapy and immune checkpoint inhibitors assurances of synergy in its therapeutic effects can be given. Therapies targeting multiple immune checkpoints are designed to do more than simply disrupt an overall tumor defense mechanism: they also encourage anti-tumor immune responses which are able keep up with various physical distances involved. so, it can gain more profits for patients with carcinoma of the lung (Guha et al., 2022).

Not only that, the latest delivery techniques, such as nano-particle carriers and microneedle patches, offer fresh ways to increase the efficacy, stability, and scaling capacity of cytokine vaccines. These innovative techniques for delivery targeted administration of antigens and controlled release of cytokines result in enhanced immunity and fewer side effects over the entire body (Hossain et al., 2020).

"What's more, current research in systems biology and computational model is starting to reveal the intricate relationships between cytokines, immune cells, and the tumor microenvironment. With the aid of these complex networks, researchers hope that one day they will be able to identify new targets for therapies and develop high performance interventions which take into account cytokines produced in a new way that has not yet been repeated and therefore requires correct dosing." (Norton et al., 2019)

Conclusion

In conclusion, vaccine-based cytokines have emerged as pivotal orchestrators of T cell proliferation, fundamentally shaping the adaptive immune response. By leveraging the inherent immunostimulatory properties of cytokines, vaccines can enhance T cell activation, differentiation, and clonal expansion, ultimately leading to robust and enduring immunity. The strategic incorporation of cytokines like IL-2, IL-12, and IL-15 into vaccine formulations has demonstrated significant potential in preclinical and clinical studies. These cytokines, delivered alongside vaccine antigens, create a

microenvironment that promotes T cell survival, proliferation, and effector function. However, the optimal cytokine combinations, dosages, and delivery mechanisms remain areas of active investigation. Future research endeavors will likely focus on refining cytokine-based vaccine strategies to maximize T cell responses while minimizing potential adverse effects. A deeper understanding of cytokine biology, combined with innovative vaccine platforms, holds the promise of developing next-generation vaccines that elicit potent and durable T cell immunity against a wide range of infectious diseases and cancers.

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Chapter 09

Role of T-Helper Cells in Generating Effective Humoral Immune Response in Vaccinated Cows

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ABSTRACT

Effective vaccination is vital for the protection of bovine populations against infectious challenges. The complex interplay between antigen presentation, B cell activation, and formation of antibodies is the focus of current thinking. It looks closely at how vaccines activate B cells to make antibodies. The mechanisms that control B cell receptor signaling, formation of germinal centers, and the process by which antibody potency develops and produces humoral response. Upon encountering vaccine antigens, naive helper T cells differentiate into Th cells, these Th cells interact with B cells, promoting their proliferation, differentiation, and antibody class switching. Influence factors such as age, breed, and nutritional status of the animal affect the efficacy of the vaccine formulation employed, stressing the importance of each vaccination strategy to improve cow's health. The final goal of all vaccinologists is to induce robust and life-long immunity. This provides information essential to vaccine development, attempting to improve cow's health and the dairy industry.

KEYWORDS
Vaccination, Dairy cows, Helper T cells, Humoral response, Germinal centers

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INTRODUCTION

Vaccines have transformed the way diseases are prevented in cows, protecting them from many different infectious diseases (LeBlanc et al., 2006). The key to this achievement is an extraordinary immune reaction producing a focused group of antibodies meant to disable the intruding pathogen. However, the intricate coordination of immune cells would not be achievable without the essential role of the helper T cell (Santana and Rosenstein, 2003). Vaccines expose the cows (Figure 1) immune system to weakened or inactive pathogens or specific antigen fragments (Eckel and Ametaj, 2020). This regulated inoculation stimulates the formation of a reservoir of antibodies, enabling the immune system to quickly and effectively respond to the actual pathogen in the future. Helper T cells play a vital role in adaptive immunity, coordinating various responses against pathogens. Humoral immunity is produced in our body by T helper cells in many ways (Kara et al., 2014). These lymphocytes communicate with other immune cells such as B lymphocytes and macrophages, providing stimulatory or inhibitory signals to activate pathogen-specific defenses (Basset et al., 2003). When we introduced a vaccine in the cow the immune system recognized it as a foreign particle and mounts an immune against it. The antigen may come from an actual pathogen and maybe from the vaccine administered in the cow (Bowersock and Martin, 1999). The role of T helper cells is to recognize the antigen and present these antigens on their surface with the help of antigen-presenting cells (APCs) (Weaver and Unanue, 1990) The activation of these cells produced antibodies against it. T cells contain a subset of T helper cells (Th) and T cytotoxic cells (Tc) which are essential for immune responses. T helper cell is associated with the CD4+ receptor and is presented by the Class MHC-II molecule whereas, the T cytotoxic cell is associated with the CD8+ receptor and is presented by the Class MHC-I molecule (Frasca et al., 1998).Without these cells, the humoral immune response could not be generated, B cells could not produce the antibodies necessary to disable pathogens, nor could macrophages engulf and phagocytose foreign invaders. Even cytotoxic T cells require helper T cells, as they help these killer cells eliminate any cells harboring intracellular pathogens (Kägi et al., 1996). Although antibodies directly combat and deactivate pathogens, their creation heavily depends on the direction provided by helper T cells. These adaptable immune components serve as a link between the first encounter with an antigen and the later release of a specific antibody response (Kaattari and Piganelli, 1996).

Fig. 1: Administration of vaccine in a cow, resulting the production of antibodies.

This way, helper T cells assist in creating the immunological memory required to avoid repeat infections. The cells of these pathogens remain in our body after the first encounter. If the cow is exposed to that pathogen again memory cells recognize it immediately and robust an immune response (Bronzo et al., 2020). In vaccination T helper cells are important for protective immunity. This chapter explores the intriguing realm of helper T cells and their crucial involvement in coordinating a strong immune response (antibody creation) after cows are vaccinated (Mak et al., 2013). We will investigate how these specialized immune cells interpret the signal from a vaccine, mobilize the B cell, and guarantee lasting immunity.

The Basics of Vaccination in Cows

Immunization is the keystone of preventive veterinary medicine by shielding cows from infectious diseases that can be costly and hurtful to an individual animal or to a whole herd (National Academies of Sciences and Medicine, 2016). The process of immunization consists of inoculating a cow with a vaccine consisting of live pathogens that have been weakened and can partially multiply. This restricted reproduction induces a powerful immune reaction, which involves triggering helper T cells. Nevertheless, there is a possibility of the weakened pathogen returning to a stronger form in animals with compromised immune systems (Tizard, 2017).

Inactivated vaccines consist of decreased pathogens or their constituents. Although they are safe, booster vaccinations may be needed to sustain long-lasting immunity because the immune response could be less powerful than with live attenuated vaccines (Ghattas et al., 2021). Recombinant Subunit vaccines include precise, purified antigens derived from the pathogen. They are both safe and efficient, although they may also need additional doses to ensure long-lasting immunity (Hansson et al., 2000). Viral Vector Vaccines employ a changed virus to transport protective antigens from a different pathogen (Ura et al., 2014). Immunization educates the immune system to recognize the agent if it comes in contact again and produces a rapid and effective response that either prevents the disease from occurring or reduces the severity of the infection.

Cows are typically vaccinated using a variety of routes and types, including subcutaneous injection, intramuscular injection, or intranasal administration, depending on the specific vaccine and its pathogen target (Bowersock and Martin, 1999). In the choice of vaccination pathway, factors that must be taken into account include the vaccine's stability, immunogenicity, and ease of administration. Moreover, vaccination times can differ depending on factors such as the cows age, health status, and the risk of exposure to certain pathogens. Regional disease incidence and management practices influence them (Garvey, 2022).

In animals, good vaccine programs not only protect individuals but also improve herd health and productivity. Vaccination reduces the incidence of infectious diseases within herds and lowers morbidity and death rates. It reduces the need for antibiotics and thereby promotes sustainable, welfare-friendly livestock farming (Ulf Magnusson et al., 2021). For

one thing, it minimizes the virus spread. In addition, it also reduces survival rates and recovery times. Along with that, avoiding disease outbreaks can lead to economic benefits from decreased production losses due to lower milk yield and reproductive performance, as well as reduced veterinary expenses (Hogeveen et al., 2019).

The Role of T-helper cell in Humoral Immunity

T helper (Th) cells are important in producing humoral immune responses in vaccinated cows (Baldwin et al., 2020). They serve as coordinators, instructing other immune cells to form a specific defense (Peña-Romero and Orenes-Piñero, 2022). This collaboration is critical for effective immune responses.

The cells serve a role in activating B cells, which are the immune systems antibody makers.The Th cells help B cells to increase antibody production. When a B cell recognizes an antigen, it delivers it to Th cells (Rajewsky and Eichmann, 1977). In response, Th cells produce cytokines such as interleukin-4 (IL-4) and interleukin-21 (IL-21), signaling B cells to multiply and develop into plasma cells. These plasma cells are antibody factories, manufacturing particular antibodies designed to neutralize the invading pathogens (Tangye, 2014).

A vital component of humoral immunity is the antibodies that plasma cells manufacture. By attaching themselves to disease antigens and designating them with more immune cells, they function as molecular warriors (Singh et al., 2022). This procedure creates immunological memory in addition to aiding in the removal of existing infections. The cells coordinate this enduring defense by ensuring that B cells generate the appropriate antibodies for efficient defensemechanism (Roy et al., 2022).

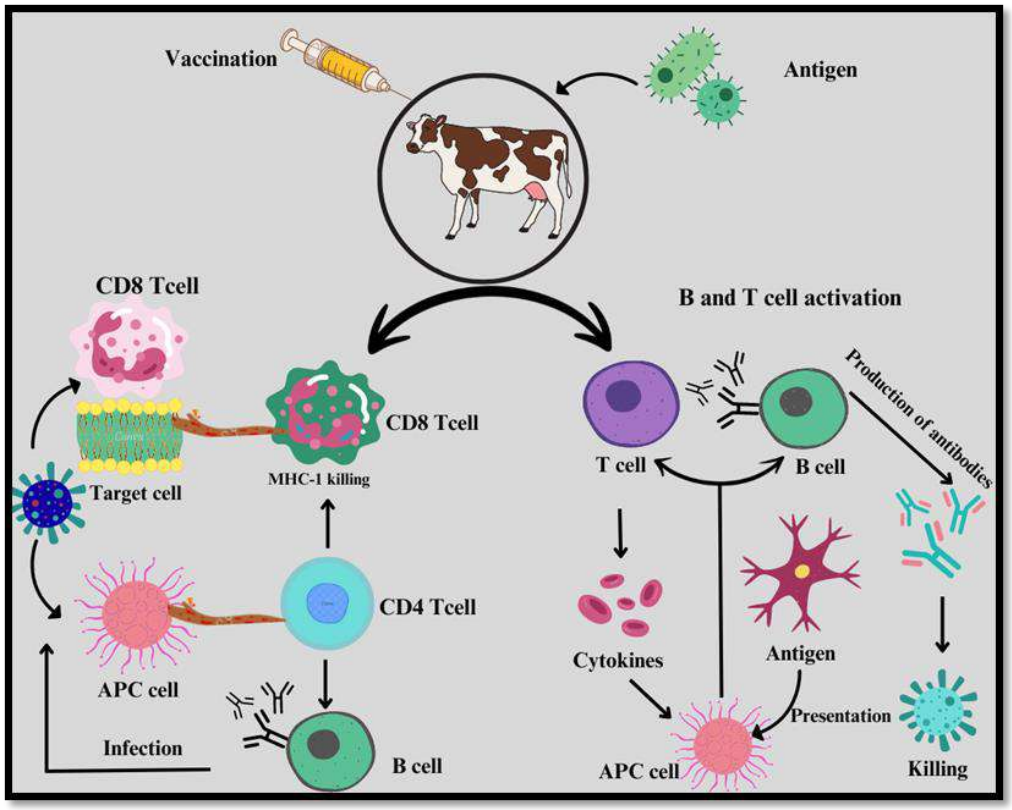

Fig. 2: Biotechnological approaches to vaccine development involve isolating antigen genes. These genes are then expressed and extracted from a protein-production device or directly expressed in the vaccine recipient using engineered plasmids or live vectors. Prime-boost techniques enhance immune responses through various antigen distribution methods. Attenuated vaccines are produced via cell passage, while inactive vaccines use heat or chemical reagents.

The Function and Importance of T-Helper Cells in Cows

T helper cells are a white blood cell that takes on a very important role in determining and regulating cow immune responses. As in (Figure 2) the T cell's function is to recognize antigens, production of cytokines, and activate the immune system (Santana and Rosenstein, 2003). The health of cows is dependent on the immune system. Helper T cells play a role in managing the immune system and protecting it. Having healthy cows means fewer medications and, an increase in productivity. Helper T cells improve the cows capacity to produce specialized immune responses meant to counteract various infections by stimulating B cells and encouraging the creation of antibodies. Helper T cells are essential targets for studies aimed at understanding bovine immunity, infectious diseases, and vaccine development. Researchers that study the role of helper T cells in cows may discover new therapeutic targets produce innovative vaccines, and optimize vaccination strategies to improve bovine populations health and wellbeing of animals.

Recognition of the Immune Response

The immune system does not directly recognize pathogens. Instead, it relies on specialized antigen-presenting cells (APCs) to bind these peptide fragments on MHC (major histocompatibility complex) molecules present on the surface of cells (Yadav, 2022). In cows, Bovine Leukocyte Antigen (BoLA) is the MHC equivalent. The antigen is presented on APCs

such as macrophages, and dendritic cells. Phagocytes are professional APCs like macrophages and dendritic cells (Hume, 2008). They engulf pathogens and break them into fragments, then present these fragments to MHC class II molecules on their surface. The B Lymphocytes can also act as APCs. B cells internalize the antigen fragments, process them, and then express them to MHC class II molecules on their surface (Kotsias et al., 2019). However, their primary role is antibody production. Following the recognition of antigens, the immune response is initiated. T helper cells are activated upon encountering antigens by Toll-like receptors (TCR) the immune response is initiated (Schnare et al., 2001). The T helper cells respond by secreting cytokines, which are the signaling molecules that assist in the activation of other immune cells (Santana and Rosenstein, 2003). The immune cells are activated by these cytokines to enhance the immune response.

Role of Subsets of Helper-T cells

The differentiation of T helper cells into subsets Th1 and Th2. Th1 is associated with the cell-mediated immunity. They release cytokines interferon-gamma (IFN-γ) to produce cellular immunity and Th2 is associated with humoral immunity (Desmedt et al., 1998). They release cytokines interleukin-4 (IL-4) to produce humoral immunity.

Certainly, T-helper cells are working with B-cells to stimulate the humoral immune response with B-cells together. As demonstrated by the concept T-helper cells also offer essential signals to support cytokine production and thereby higher-affinity antibodies produced by B-cells for future immobilization of specific pathogens or toxins that might come along such as little-known immune systems in cows occasionally (Alsén, 2018). Both T helper cells and the B lymphocytes are vital and active and give cows security against infectious diseases.

T helper cells are fundamental coordinators of immune responses against pathogens in domestic farm animals, including the cow, via stimulation of B lymphocytes for enhanced antibody production (Bannan, 2014). Therefore, taking advantage of the knowledge of how different T helper subsets coordinate B cell responses and antibody production could improve the efficacy of vaccines against many common infectious diseases of livestock (Booth and Toapanta, 2021). A greater understanding of these intricate and delicate interactions may guide the development of enhanced vaccination strategies and improve overall animal health (Kumru et al., 2014).

The Role of Antigen Presentation in T-Helper Cell Activation

The activation ofHelper T cell begins with the interaction of antigen by TCR and its receptor and is broken down into small fragments (Noelle and Snow, 1991). When a naive T cell interacts with the Th cell on APC along with its co-stimulators CD80 and CD86 it becomes activated (Mir). These small fragments are presented by the Major histocompatibility class (MHC). Antigens from outside are presented by class MHC II molecules while the body own's cells are presented by class MHC I molecules (Rammensee et al., 1993). CD4+ which makes a complex with naive B lymphocytes on the surface of antigen-presenting cells. Naive T cells are stimulated by all APCs but most importantly it is triggered by Dendritic cells (DC) (Ni and O'neill, 1997). The TNF (tunor necrosis factor) receptors family is responsible for co-stimulatory signals in the immune system of cows (Werling and Coffey, 2007). Receptors including toll-like receptors(TCR), and NOD-like receptors recognize pathogens and activate dendritic cells. Th1 lineage is associated with CD8 receptor and Th2 lineage is associated with CD4 receptor (Zhang et al., 2014). The Th cell releases various cytokines after this activation and initiates a cascade pathway resulting in the proliferation and differentiation of the Th cell (Luckheeram et al., 2012).

Differentiation of T-helper cell

Naive T cells are stimulated by all APCs but most importantly it is triggered by Dendritic cells (DC). The TNF receptors family is responsible for co-stimulatory signals in the immune system of cows (Puddu et al., 2011). Receptors including toll-like receptors, and NOD-like receptors recognize pathogens and activate dendritic cells. During this differentiation cytokines including Th1, Th2, Th17, and Treg cells are released (Duan et al., 2022). After this interaction, CD3 starts its signaling pathway which differentiates these cells into effector cells. It releases of IL-12 and 1L-6. IL-4 started signaling which initiates the production of signaling including GATA-3 which helps in Th2 differentiation (Jacobson, 1997). They increase cytokine production help in proliferation and involve suppression of Th1 differentiation (Zhu et al., 2006). The differentiation of Th2 is disturbed when GATA3 is absent. The differentiation of Th2 is also responsible for other factors like IL-2, and IL-16 respectively (Zhang et al., 2014). Th2 releases some interleukins like IL4, and 1L-5 which stimulate the production of antibodies inside cows (Immunivt, 1998). Overall, T helper cell division is a highly regulated process and is driven by the cytokine environment and antigen signals, resulting in the development of discrete T cell subsets with specialized tasks in immune response regulation.

Activation of B cell

When a B cell, comes into contact with an antigen that matches its B cell receptor (BCR), a chain reaction of events occurs. This antigen could originate from any other foreign substance that penetrates the immune system (Zitron, 2000). Consider the B cell which is ready to detect and respond to intruders. The attachment of the antigen to the BCR triggers a signaling cascade within the B cell, indicating that it has discovered a possible threat (Sharma, 2023).

B cells are not activated without T helper cells. After the interaction, Th cells activate and release various cytokines like IL-4 and IL-21 as shown in (Fig. 3) that help B cells memorize the same pathogen (Ettinger et al., 2008). The B cell is encouraged to proceed with its immunological response by this interaction (Perelson et al., 1976).

By T helper cells and cytokines, activated B cells proliferate and differentiate rapidly. It converts into plasma cells, that are responsible for making antibodies (Nutt et al., 2015). After that B cells differentiate into memory cells. These memory cells are long-lived and can respond to future encounters with the same pathogen. As a result, the immune response upon second exposure is fasted.

Humoral Immunity and Its Significance in Cows

Humoral immunity is one of the main defensive mechanisms for a body to stay away from viruses and bacteria (Kaattari and Piganelli, 1996). It is determined mainly by the presence in the blood and body fluids of immunoglobulins, also known as antibodies. Most importantly, humoral immunity in cows serves as a major defense against organisms that enter the body and neutralizes germs before they can harm (Vlasova and Saif, 2021).

Additionally, in cattle, humoral immunity provides a way for the body to remember a pathogen, so that the immune response will be quicker and stronger the next time around (Mukherjee et al., 2023). Memory B cells persist in the body if they are generated during the primary immune response, and can differentiate rapidly into plasma cells when reposed in the future. (Cancro and Tomayko, 2021). This increased degree of rapidity leads to a faster antibody response and lower incidence of reinfection, both of which are especially abundantly effective in cows. By measuring the amount of antibodies in blood serum, serological testing can be used to evaluate a cows humoral immunity (Adone and Ciuchini, 1999). Veterinarians and livestock farmers use these tests to assess the quality of vaccination programs and to keep an eye on the immunological state of herds.

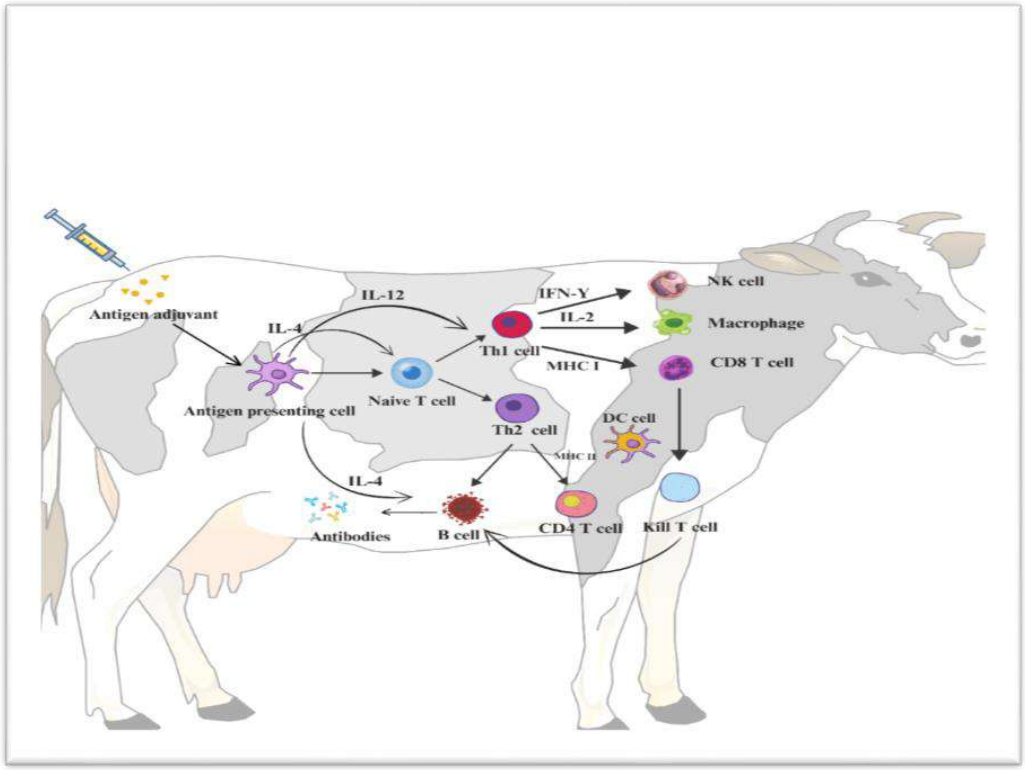

Fig. 3: The immune mechanism involving adjuvants includes increased antigen presentation, T cell stimulation, and promotion of both cellular and humoral immunity. Adjuvants enhance immune responses by activating various pathways, leading to antibody production and targeted cell killing.

The Impact of T-Helper Cell Dysfunction on Humoral Immune Response

Dysfunction is a process that leads to inadequate B cell activation and reduced antibody production. Due to irregulating or dysfunction of T helper cell causes the immune system to attach self-cells. This can damage the body's own cell and result in severe conditions. T cell is very important in generating an immune response if its function is interrupted there is a lack in the production of antibodies (Romagnani, 2006). This can also influence the class switching of antibodies which disturbs the balance of antibodies class switching. Dysfunction also affects cytokines production resulting in dysregulation of signaling molecules resulting in injury and inflammation (Koneczny et al., 2022).

Dysfunction also leads to an autoimmune disease that is characterized by dysregulated humoral immunity helper cells that play a role in memory response dysfunction also weakens this response and the cow immune system is susceptible to more pathogens (Jo et al., 2014). Reconsidering the T helper cell dysfunction in the humoral immune response is necessary if new approaches are to be identified. These will combat a variety of immunological disorders such as infections, autoimmune diseases, and immunodeficiency (Bach, 2001).

Strategies to Enhance T-helper Cell Immunity in Vaccinated Cows

To enhance immune protection in cows upon vaccination as well as vaccine efficacy, it is critical to maximize T-helper cell mediated antibody production. The production of high-affinity antibodies. The way to increase immunity in vaccinated

cows is the use of adjuvants. Adjuvants a substances that are added to the vaccine to enhance immunity (Heegaard et al., 2011).

Another approach to improving vaccine potency is through the manipulation of the formulation and route of vaccination. It is generally accepted that an increasing understanding of these facts will result in an increased ability to enhance the humoral immune response of Th-cell-mediated vaccines (Romagnani, 1999). When adjuvants and antigens are combined in a vaccine, as well as with delivery systems that facilitate their targeting to specific immune cells or tissues, as well as the uptake, processing, and presentation of antigens, this will trigger improved Th-cell activation (Rapaka et al., 2021). Vaccination strategies, such as nanoparticle-based vaccine delivery systems, and mucosal vaccination, including both oral largely avoided in adult cows as with the oral route, rectal administration does not produce the same degree of gastrointestinal upset and can still trigger an IgA response and all of these have been demonstrated to tipping the balance toward a robust antibody response.

Moreover, maintaining and promoting T cell memory formation is another critical aspect to support the durability of vaccine-induced protective immunity in immunized cows. The Th memory cell response observed with vaccination strategies such as prime-boost regimens and with heterologous vaccine platforms can support both immune memories as well as the development of a response that provides more lasting immunity against long-term pathogens (Ghattas et al., 2021).

Future Challenges and Directions

The key to future research is to understand the complex mechanism of helper T cell regulation of the humoral immune response in vaccinated cows. There is still a need to improve the efficacy of vaccines in cows. The mechanisms involve the formulation of vaccines, adjuvants that are added to enhance their efficacy, and the method of administration of vaccines in cows (Burakova et al., 2018). This ensures further investigation into the molecular complexity concerning activation and interaction between helper T cells, B cells, and so on. The specific signaling pathways and cytokine signaling are being elucidated. In addition, the development of vaccines that are the most

effective in inducing an enhanced helper T cell response in cows (Thakur et al., 2012). Understanding the connection between animals, humans, and the environment one health approach is necessary to maintain a balance. Moreover, looking at the dynamics of long-term T and B cell populations in vaccinated animals will be critical to designing vaccines capable of providing sustained protection. Future challenges include focusing on the complexities of the immune system and developing vaccine strategies according to challenges.

In advancing bovine studies, directed research was also necessary to elucidate the role of helper T-cells in stimulating humoral immune responses against important pathogens. However, moving forward in this field poses numerous challenges (Waters et al., 2014). Animal welfare and scientific integrity must both be maintained, and researchers need to consider, species-specific variability, the hurdles of vaccine design, field validation, and even ethical issues (Gjerris et al., 2023).

Conclusion

In short, the importance of T helper (Th) cells in organizing an effective immune response after cows are vaccinated cannot be overemphasized T helper (Th) cells play a crucial role in orchestrating immune responses post-cow vaccination. They regulate B and cytotoxic T cells, driving immunity against targeted pathogens. Antigen-presenting cells activate T cells, initiating a cascade of immune events vital for durable immunity. Th cells aid B cell activation, enhancing humoral responses, and support cytotoxic T cell proliferation for cellular immunity. Subsets like Th1, Th2, and Th17 finely tune immune responses, balancing protection and immunopathology. Understanding Th cell-mediated humoral immunity is vital for effective vaccination strategies in cows, ensuring health and livestock yield. Continued research in veterinary immunology is essential for unraveling T cell biology mysteries and designing better vaccines, crucial in the global fight against cow diseases. Collaboration, innovation, and scientific dedication can leverage Th cells to safeguard cow populations worldwide.

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Chapter 10

Processing Mechanism of Vaccinal Antigen in the Host for Production of Effective Immunity

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ABSTRACT

The process by which the vaccines prompt the immune system of the host to develop a rapid and powerful response is one of the most comprehensive and delicate mechanisms that shows the effectiveness of the vaccination process. This chapter focuses on explaining all the various aspects of the particular process between antigens and the host's immune system. It has been established that after taking the vaccine, the antigen-presenting cells help in degradation of the antigens and these afterwards binds the antigens of the T cells through MHC molecules. This results in activation, and division of T cells. At the same time, B cells become transformed into plasma cells and form antibodies that are compatible with the antigens. Subsequently, this synchronized immune response causes the formation of memory T and B cells, resulting in long-term immunity. Knowledge of these modes is important for the development and implementation of preventive measures through the use of vaccines against infectious diseases. Vaccine antigens interact with the host's immune system for development of immunity. Understanding of these mechanisms is crucial for the development of new vaccines and consistently improving the efficacy of existing vaccinations to reign in infectious diseases.

KEYWORDS
Vaccine, Antigen, APCs, Immune response, MHC

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INTRODUCTION

The specific components of vaccine that elicit an immune response in host is vaccinal antigens. These antigens can be a whole protein, polysaccharides, live or attenuated pathogen (Strugnell et al., 2011). Vaccinal antigens whenever deposited in the body activate several reactions to form antibodies, and established stored memory cells to prevent future infections (Singh, 2021). To achieve this goal, it is crucial to understand the various processes that are employed in the manipulation and presentation of vaccinal antigens. It not only gives us the remarkable understanding of T cell receptor specificity but it also offers great benefit to the goal of improving the target vaccine design and development (Edsall, 1966). Furthermore, understanding the various mechanisms involved in antigen processing can help in vaccine development by revealing new potential antigens and improving vaccine designs. By understanding these features, this chapter is to provide you with an overview of vaccinal antigens and the faces in this process, which determines both immunogenicity and efficacy (Graham et al., 2019). We first discuss the different types of vaccinal antigen and differentiate on the basis of source. We then proceed through a path in the explanation of various events on the cellular and molecular levels that deal with antigen processing and presentation (O'Hagan and De Gregorio, 2009). This also involves reviewing the functions of the antigen presenting mode which is the dendritic cells as well as the macrophages in capturing, processing and presenting of the antigens to the T cells (Hilligan and Ronchese, 2020). We also learn about the significant role of MHC in the presentation of the processed antigen on the surface to T cell receptors that leads to the activation of adaptive immunity (Germain, 1994).

This chapter gives us full knowledge on vaccinal antigens, ways through which they are processed and the general factors that dictate their immunogenicity. It will not only broaden their education for basic immunology but also allow

them to assess current vaccine approaches and development, and be engaged in search for more efficient and safe vaccines (Levine et al., 2004).

The Journey of Vaccinal Antigen

When vaccine is administered the journey of vaccinal antigen in the host starts a cascade of reaction. This reaction initiate an immune response in the host (Kang and Compans, 2009). When entered in the body these antigens as mentioned in (figure 1) interact with antigen-presenting cells (APCs) like dendritic cells, macrophages and B cells (Unanue, 1984). This step is crucial in capturing and processing the vaccinal antigens and present them on the surface of T cell. The presentation of antigens on the surface of T cell with MHC (major histocompatibility complex) (Kotsias et al., 2019).Upon activation, T cells proliferate and differentiate into effector T cell subsets like helper (Th) or cytotoxic cell (Tc), also undergoing clonal expansion. Th cells guide the function of the rest of the immune system via cytokine secretion, which influences other immune cell populations, and Tc cells kill infected target cells directly (Basu et al., 2021).

T cell activation is only the beginning of the immune reaction. In the context of vaccinal antigens they also stimulate B cells and generate plasma cells (den Haan et al., 2014). Specific antibodies to the vaccinal antigen are produced by these specialized antibody factories (Petrovsky and Aguilar, 2004). They attach to the pathogen, neutralizing and flagging it for other immune cells to destroy. Cellular and humoral immunity work together in this coordinated response, resulting in the establishment of immunological memory that confers long-lasting immunity on subsequent infection with the same pathogen (Plan and Additives, 1999). Appreciating this convoluted path of vaccinal antigens is not as simple. The implications are profound for vaccine development (Smith et al., 2022). Describing the pathways of antigen processing and presentation, as well as understanding dynamic changes in responses to immunity help to design vaccines that activate specific immune pathways appropriately which ultimately increases vaccine efficacy and safety (Slifka and Amanna, 2014). In addition, identification of novel vaccine targets and potential for personalized vaccines will be facilitated by this event (Poland et al., 2018).

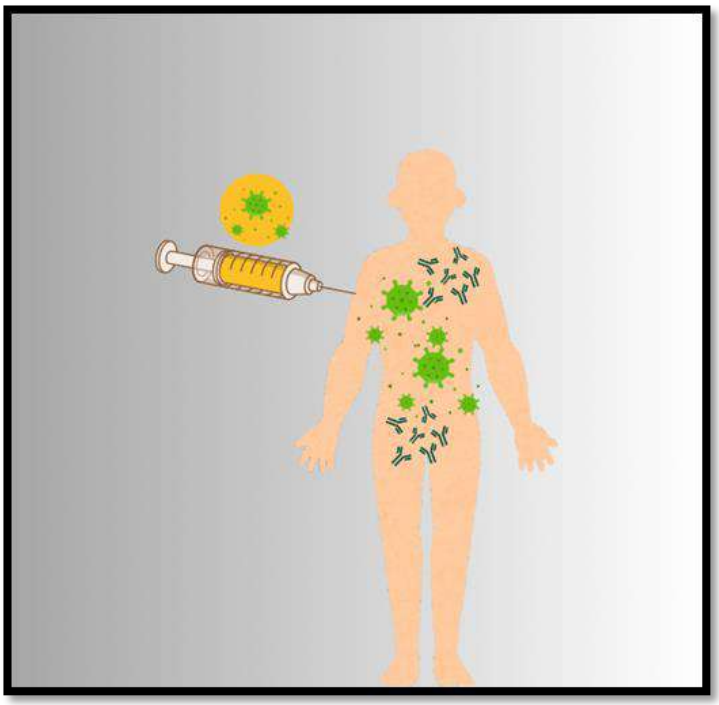

Fig. 1: Immunize the individual by vaccination.

Routes of Vaccine Administration

Vaccine can be administered by common routes such as intramuscular, subcutaneous, and oral administration (Makoschey, 2015). Intramuscular and subcutaneous injections deliver the antigen to muscle or fatty tissue, as there is slowly released and absorbed by local immune cells (Viola et al., 2018). Despite the greater difficulty of intradermal injections to administer, they are able to deliver antigen directly into skin. Oral vaccines in contrast, confront the highly specialized mucosal immune system that use unique mechanisms for antigen uptake and processing. The route of administration is generally determined by the nature of the antigen, immune requirements and convenience (Karim, 2015).

Mechanisms of Antigen Uptake by Antigen-presenting cells (APCs)

Antigen uptake by APCs involves the following processes,

Antigen Encounter and Recognition

The vaccinal antigens start their journey as they are being applied in the host and beginning the cascade of complex

immune processes, however the antigen and the immune system first meet often varies depending on what route of administration is taken (Varadé et al., 2021). Vaccines can be given by a number of different routes, each with unique benefits and consequences on antigen processing and downstream adaptive immune responses (Gause et al., 2017).

Antigen Presentation via MHC Molecules

Antigens are presented on the surface of T cell with MHC complex.MHC molecules are glycoproteins that help in antigen presentation (Pishesha et al., 2022). MHC molecule consist of two classes. Class I MHC molecule are expressed on the surface of nucleated cells and contain CD^{4+} receptor whereas class Class II MHC molecule are expressed on APCs (antigen-presenting cells) and contain CD^{8+} receptor (Neefjes et al., 2011). The loading of MHC with antigenic peptides is a complex process that occurs across different cellular compartments and therefore enzymatic cascades (Watts, 1997). The peptide-MHC complex is subsequently shuttled to the exterior of the cell and presented by T cells. The peptide MHC-complex is formed and T cells initiate an immune response (Pamer and Cresswell, 1998).

TCRs recognize precise molecular motifs provided by the combination of a peptide bound to MHC molecule. In fact, upon recognition of a pathogen-derived peptide in the context of major histocompatibility complex (MHC) molecules on APCs this single T cell can activate and even proliferate to form effector T cells suitable for a variety of functions including cytokine production, cytotoxicity, or B cell activation (Weaver and Sant, 2009). The way vaccinal antigens encounter the immune system is a complex scenario featuring by different routes of administration and uptake mechanisms by APCs and the genetic elements for antigenic presentation are needed to set up vaccines (Leo et al., 2011).

Immune Cell Activation and Response

Vaccine initiates the encounter of vaccinal antigens and antigen presenting cells (APCs), leading to activation and migration of adaptive immune response. Activated T cells are activated to divide, B cells differentiate into plasma-cells and mass-produce antibodies with a wide range of effector functions (Nigam and Knight, 2020).

Activation and Proliferation of T cells

Peptide-MHC recognition by the TCR results in a complex cascade of intracellular signaling events that culminate in T cell activation (Wu et al., 2021). Activation also involves by the engagement of costimulatory molecules and the expression of cytokines that maintain T cell survival, proliferation, and differentiation (Santana and Esquivel-Guadarrama, 2006). As describe in (figure 2) when naive T cells are activated, they clonally expand leading to a huge population of effector T cells that can detect and kill the pathogen (Pennock et al., 2013)**.**

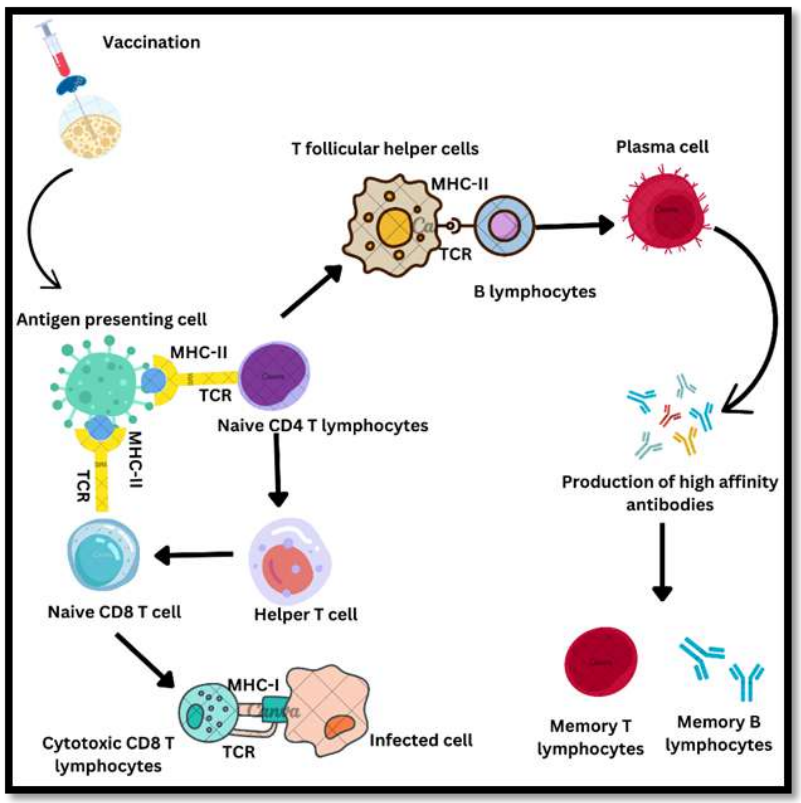

Fig. 2: When vaccine is inserted in an individual antigen presenting cell taken is as a foreign particle and present it on the surface with the help of MHC complex and toll like receptor after it shows an immune response and kill the pathogen by making antibodies**.**

T cell differentiation

Effector T cells differentiate into subsets that possess discrete functions. CD^{4+} expressing helper T cells (Th cells) are central orchestrators of the immune response (Kara et al., 2014). They also secrete many cytokines that are able to activate

other immune cells including B cells, macrophages and cytotoxic T cell (Tc) (Bao and Cao, 2014). CD^{8+}expressing Tc cells are specialized killers that kill infected cells directly by the release of cytotoxic granules containing perforin and granzymes (Janeway Jr et al., 2001). The Th cells and Tc cells both are involved in clearance of pathogens from the body.

Differentiation of B cells into Plasma Cells

Vaccinal antigens also acts on B cells that differentiate into plasma cells producing antibodies. B cells are stimulated by antigens in two signals; binding of the antigen to its specific B cell receptor (BCR) and interaction with activated Th cells (Rodríguez-Pinto, 2005). Upon activation, B cells proliferate and their BCR genes produces clones of high-affinity antigenic specific antibodies. B cells which can differentiate into plasma cells that secrete large amounts of antibodies specific for the vaccinal antigen (Schroeder Jr et al., 2019).

Production of Antibodies and Effector Functions

Antibodies are proteins that have a Y shape and attach to particular antigens, usually with high specificity. These are involved in mediating a number of effector functions, such as neutralization, opsonization and complement activation (Lu et al., 2018). Neutralizing antibodies attach to pathogens and provide prevention from getting disease (Casadevall et al., 2004). Opsonization get coated the pathogens in which will then lead to more phagocytose by macrophages and neutrophils (Uribe-Querol and Rosales, 2017). Complement-activating antibodies initiate the complement cascade (a series of enzymatic reactions) that result in membrane attack complexes that destroy target cells (Xie et al., 2020). These effector functions work in concert to clear pathogens and resolve infection. To determine the activation and systemic response of immune cells to vaccinal antigens is a very complicated process. This describes the importance of integrated action of T and B cell, accompanied by antibodies at all stages to produce an optimal protective immunity against many infections (Zhang et al., 2012). Realizing these complex, interlinked pathways allows scientists to create vaccines that stimulate powerful and permanent immune responses to shield people and populations from the horrifying results of infectious diseases (Meyer and Zepp, 2022).

Immunological Memory Development

Vaccination is basically a strategy to enable the host generate long-life immunological memory so that on second encounter of same pathogen, the response will be rather quick and stronger (Schnaack, 2022). This is achieved as a result of the generation, continuity and memory recall by memory T and B cells that together with humoral and cellular responses account for immunologic memory.

Formation of Memory T and B cells

After immunizing with antigen, fewer than 10% of the cells specific for that antigen become memory during the primary immune response (Storni et al., 2005). Memory T cells are long-lived cells that can colonize lymphoid tissues and peripheral organs, responding rapidly to traces of antigen (Farber et al., 2014). This predisposes them to respond quickly if they are ever re-exposed to that same antigen. Memory B cells, however, express high levels of surface immunoglobulins and are located in lymphoid follicles. They can differentiate into long-lived plasma cells upon re-challenge with the antigen thus generating diversified high-affinity antibodies (Merlo and Mandik-Nayak, 2013). Memory T and B cells develop through an intricate series of cellular and molecular steps, which include the upregulation of anti-apoptotic molecules as well as changes in epigenetic modifications and transcriptional programs (Tsai et al., 2019). These processes do result in the survival and continued function of memory cells, however, allowing them to persist for years or, in some cases, even decades.

Maintenance and Recall of Memory Responses

Immunological memory is maintained by pro-survival factors such as interleukin-7 (IL-7) and IL-15, which need to be constantly in contact with the antigenic exposure. Memory cells move into body looking for any further signs of reinfection (Harty and Badovinac, 2008). Presented with the same antigen a second time, they will expand clonally at high speed and be corrupted to produce large quantities of effector T and B cells capable of rapidly clearing the infectious agent.It has a shorter lag time, higher magnitude and greater affinity than the primary response (Stewart and Weir, 2012).

Factors Influencing Vaccine Efficacy

The efficacy of a vaccine, the extent to which it prevents disease is determined by many factors. These influence the antigen itself, the vaccine formulation, and specific host factors. Insights into this intricate relationship are essential for the design of potent vaccines and to maximize vaccination policies (Andre et al., 2008).

Antigen Characteristics

Immunogenicity of an antigen is largely determined by the inherent properties of the antigen itself. This, in turn will be based on antigen size, molecular structure and stability (Graham et al., 2019). Larger antigens are usually more immunogenic than smaller, simply because of their increased size and complexity and the presence of multiple epitopes, specific sites that antibodies or T cell receptors (Livingstone and Fathman, 1987). The way the antigens expressed, either

coating o or outer membrane could also be impact their association with immune receptors and thus their immunogenicity by modulating the antigenic structure form like conformational changes post-translational modifications. In addition, the antigen must be stable enough to not simply disintegrate before reaching its target tissues and persist long enough for an immune response to develop (Roth et al., 2022).

Adjuvants and Vaccine Formulations

The substances added to vaccine that enhance the immunity of vaccine is adjuvant. They work by different mechanism like prolong the antigen persistence, activation of our immune system and other immune cells (Pulendran et al., 2021). Adjuvant increase the efficacy of vaccine. Some common examples of adjuvant including Toll-like receptor (TCRs), aluminum salts etc. (Milicic et al., 2022).

Host Factors

The immune system of host plays an important role in vaccination. Age, genetics, nutritional status of host are some of the factors that involve in efficacy of vaccine. Young children and infants do not have as strong immune system as in adults (Calder et al., 2020). The people of older age group has weak immune system which can also influence the efficacy of vaccine. Immunocompromised patients and certain genetic factors like polymorphism as shown in (figure 3) can also influence the immune response to vaccine (Córdova-Dávalos et al., 2022). The pre-existing immunity present in the body in response to infection or through vaccination can also effect the efficacy of vaccine.

However, the efficacy of vaccine is dependent upon vaccine formulation, antigen characteristics and vaccine formulation. These factors are responsible for maintain the safe and effective vaccination that are responsible to provide protection to individuals against infectious diseases (Salisbury et al., 2006).

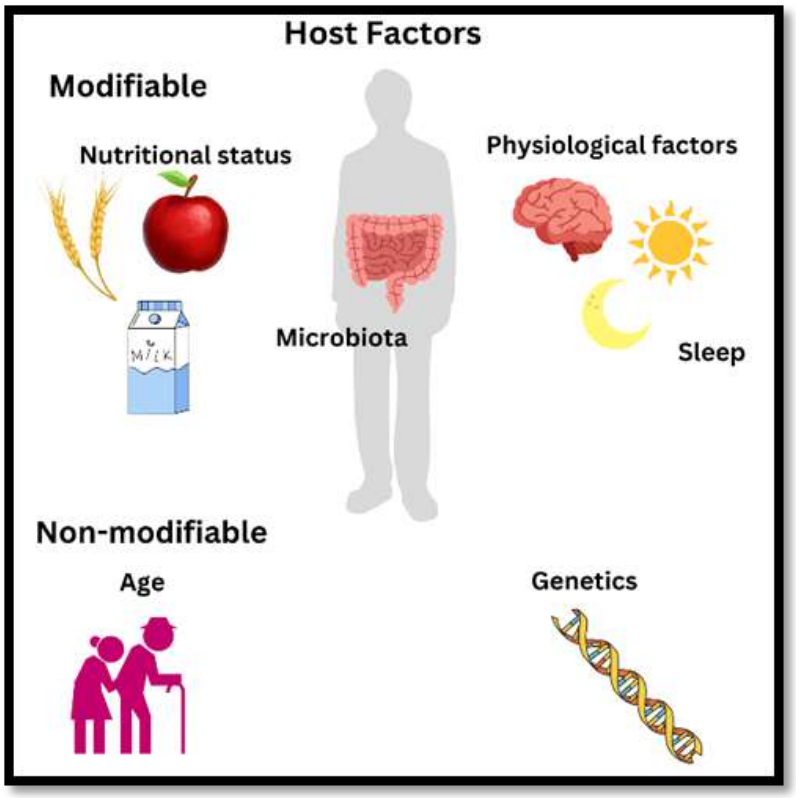

Fig. 3: Several factors affecting the immune system of host including its nutritional status, age, genetics**.**

Intracellular Signaling Pathways in Immune Cell Activation

The receptors present on T cell are TCRs and receptors present on B cell are BCRs when antigen bind to TCRs they start a cascade of reaction that stimulate the immune cells activation (Cano and Lopera, 2013). The signaling pathways activates various pathways like certain transcription factors, protein kinases and adaptor proteins (Whitmarsh, 2007). These are involved in cell differentiation and proliferation. The activation of tyrosine protein kinase Lck and ZAP-70 kinases by toll like receptor leads to the activation of different pathways like MAP kinase pathways (Krzyzowska et al., 2010). These pathways help in activation, proliferation, cytokine production of T cell calcium-calcineurin-NFAT signaling pathway.

Cytokine Networks and their Roles in Coordinating Immune Responses

Cytokines are chemical messengers which are small proteins that produce unique functional effects on the function of immune cells (Peters, 1996). They serve as key players in orchestrating immune responses due to both their positive and negative effects on the activation, proliferation and differentiation of numerous immune cell (Lu and Rudensky, 2009).

However, their network is very complex, many cytokines are also mutual, which means different cytokines can have direct interaction with each other. e.g., interleukin-2 (IL-2) produced by activated T cells drives the proliferation and survival of both T cells, and B cells (Gaffen and Liu, 2004).These contain IFN-gamma. It is a product of T-cell and it also induces macrophage for activating it and then increases its microbicidal activity. The cytokine network is essential for elucidating the mechanisms of vaccine-induced immunity and for the discovery of molecular intervention targets (Pezeshki et al., 2019).

Targeting Specific Immune Pathways for Enhanced Efficacy

Others will specifically target immune pathways (e.g. Th1, Th2, or Th17 pathways) using the vaccine to elicit one type of immune response over another, which would not only provide a built-in adjuvant, but would also be most efficacious at combating the particular pathogen for which the vaccine is used (Burdman, 2012). That includes vaccines for intracellular pathogens such as viruses and intracellular bacteria (which are often managed through potent Th1 responses, activated by production of IFN-γ and by cytotoxic T cell activity) (Igietseme et al., 2004). Vaccines that target extracellular parasites, certain bacterial infections, such as typhoid, cholera, and tuberculosis are able to induce Th2 responses, where the production of IL-4 and IL-5 result in activation of B cells. The adjuvant employed may also modulate vaccine-induced immune response profile (Ciabattini et al., 2016).

Novel Vaccine Delivery Systems and Technologies

Conventional vaccine administration routes, i.e. intramuscular or subcutaneous injections, are inconvenient and fail to induce local mucosal immunity (Hettinga and Carlisle, 2020). To understand these obstacles novel technologies and delivery systems for vaccines are being developed and designed. Collectively, these observations demonstrate that needle-free delivery systems, including microneedle patches, and oral vaccines are appealing alternatives regarding both convenience and patient preference (Marshall et al., 2016). One approach for improved vaccine stability and targeting, and controlled payload release are nanoparticle-based vaccines that encapsulate antigens and adjuvants inside biodegradable nanoparticles that can overcome the rapid antigen clearance and limit the side-effects usually generated by the alum adjuvants (Leleux and Roy, 2013). Vaccine design and optimization for the prevention of infectious diseases is a constantly changing and evolving field with many promising directions, including the use of viral vectors, DNA vaccines, and mRNA vaccines based on the findings from preclinical and clinical data review (Trovato et al., 2020).

Clinical and Public Health Implications

The clinical and public health dimensions of vaccinal antigens influence the health of populations. Vaccines equipped with their antigenic specificity, have modernized public health obviating millions of infections and pathological function (Lu et al., 2021). Vaccines prompt the body to develop immunity to specific diseases, making subsequent exposure less likely to lead to infection or lessening the severity of infection (Schiller and Lowy, 2010). Vaccination helps not only to protect individuals but also to generate herd immunity in a community.

Closing Remarks on the Significance of Understanding Antigen Processing for Effective Immunity

Describing the complex interplay of vaccine will allow us to manipulate the immune system in order to achieve our highest power over vaccinal antigen recognition, processing and presentation (McNeela and Mills, 2001). Let us focus on the vaccinal antigens and shape their evolution and catalyze responses within immune systems. As we move forward the vaccines are not just an instrument of preventing disease, but also symbol of human cleverness and persistence in defending the health and wellness of generations yet unborn (Rappuoli and Vozza, 2022).

Impact on Vaccination Strategies and Policy-making

The elucidation of the vaccinal antigens and their immunological mechanisms has had a great influence on vaccination strategies and policy making. This insight has led to new and better vaccines, including conjugate vaccines which link a weaker antigen with a carrier protein to boost its ability to induce an immune response (Slifka and Amanna, 2014). It has also provided insights that have resulted in the schedule of vaccine administration, in timing doses and giving them at particular morbidity intervals to maximize their efficacy and safety. Moreover, the understanding of vaccine-mediated adaptive immunity has been instrumental in development of mass vaccination campaigns that have controlled and eradicated a number of infectious diseases.

Future Directions and Challenges in Vaccine Development

Future promises and challenges for vaccine development breakthroughs in the host antigen processing mechanism could potentially provide promising strategies to improve vaccine efficacy. But, to bring this potential into reality, a bunch of key issues need to be tackled (Wu et al., 2013). Crucial lines for future research include understanding the interactions between various immune cell populations during antigen processing. Identification of specific pathways in antigen-presenting cells and its interaction with adaptive immune components may reveal new targets that could be used to generate or improve vaccines. In addition, the discovery that next generation vaccine platforms seems positioned to be able to address these issues represents exciting new territory in vaccinology. Advancements like nucleic acid based vaccines, viral vectors and nanoparticles drug delivery system provide great potential in the antigen delivery and immune

activation. Equitable access to vaccines has also been and is an urgent issue that highlights the necessity for global partnerships in vaccine distribution among at-risk populations globally (Excler et al., 2021).

Finally, the competent management of regulatory landscapes as well as building public confidence in vaccination programs are crucial aspects for successful vaccine creation and distribution (Keith et al., 2013). Strategies to combat vaccine hesitancy and misinformation need to be multifaceted and incorporate educational, transparent communication strategies with community engagement.

Conclusion

Conclusively, the comprehension of processing mechanisms of vaccinal antigens in the host leading to the generation protective immunity is essentially important for further advancements in vaccine development and immunization strategies. The induction of strong immune responses necessary for protective immunity against pathogens is mediated by vaccines, through the delicate mechanisms such as antigen uptake, processing, presentation and the recognition by immune cells. This chapter detailed the various routes in which this can happen, including the contribution of antigen presenting cells such as dendritic cells (DC), macrophages and B cells in shaping immune responses. Finally, the relevance of major histocompatibility complex (MHC) molecules in antigen presentation and T cell activation was highlighted. Studies that illuminated this cross-talk between innate and adaptive immune responses gave us a deeper insight into how vaccines induce memory to confer lasting protection. Furthermore, it provides the importance of vaccine formulations, adjuvants, and delivery systems in enhancing antigen processing and immunogenicity.

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Chapter 11

Vaccine-Based Immune Reactions in Dairy Cows

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ABSTRACT

Vaccination is an important strategy for managing viral and bacterial diseases in dairy cows, preserving the animal's output and health. With an emphasis on Pakistan, this chapter examines the dynamics of vaccine-based immune responses in dairy cows. Pakistan's cattle industry makes a substantial economic contribution to the country, underscoring the need for efficient disease prevention strategies. Several infectious illnesses, such as foot-and-mouth disease, brucellosis, hemorrhagic septicemia and mastitis, are serious risks to dairy cows and cause financial losses for producers. Numerous vaccine types, such as multi-epitope, oil-based adjuvant, live, killed, and nucleic acid vaccines, are used in vaccination regimens against various diseases. However, because of issues including poor vaccine quality, age-related immune responses, and insufficient serotype matching, vaccination failure continues to be a problem.

KEYWORDS
Dairy cows, Vaccination, Disease prevention, Vaccination challenges, Infectious diseases

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INTRODUCTION

The livestock sector is the backbone of agriculture in the country, sharing about 14.0 % of the national GDP (Wasim, 2005). Livestock rearing is a significant revenue source for rural residents, providing nutrients like milk, meat, eggs, and cheese. Pakistan, with a cattle population of 53.4 million, ranks 4th in milk production. However, the country faces endemic viral and bacterial diseases, necessitating vaccination strategies in both public and private sectors. Infectious diseases of dairy cows such as foot and mouth disease (FMD), hemorrhagic septicemia (HS), black quarter (BQ), bovine viral diarrhea (BVDV), anthrax, brucellosis, leptospirosis, rabies, mastitis, etc. are not only life-threatening for the animal but also poses a huge economic loss to poor livestock farmers and these diseases are being controlled by routine vaccination at farm level (Singh, 2014).

The immunization of dairy cows is considered successful when the body responds to the antigen at the humoral and cellular levels. Unfortunately, there are chances of disease occurrence after vaccination in a herd due to vaccine failure, which may be due to the vaccine, the body of the host, and an untrained vaccinator. (Shkreta et al., 2004).

Vaccination of Dairy Cows against Infectious Diseases

Mastitis is a complicated and extremely harmful disease that leads dairy farmers to face significant losses (Mekonnen et al., 2019). The use of antibiotics for mastitis treatment is restricted because of resultant antibiotic residues in milk and may lead to antimicrobial resistance (Gao et al., 2012; Nosanchuk et al., 2014). *Staphylococcus aureus* is one of the most prevalent causative agents of clinical and sub-clinical symptomatic mastitis in dairy cows (Sampimon et al., 2009). A novel strategy is to employ a multi-epitopes vaccine rather than a single-unit vaccine candidate since it consists of affordable vaccinations with exceptional specificity and durability in various settings and offers cows long-term protection (Pathak et al., 2022). *Pasteurella multocida*, a highly contagious bacterial disease, primarily affects cows and water buffalo but can infect other domestic and wild ruminants. As of 2022, hemorrhagic septicemia (HS) only accounts for losses of USD 12.4 million annually in the Punjab province. It is among the most economically significant diseases of cattle in Pakistan, and it is estimated that a 50% reduction in incidence will be sufficient to reduce the gap between demand and supply for dairy (Ahmad and Muhammad, 2008). The HS can be controlled by vaccination. However, in the current investigations, an epidemic of HS emerged among buffalo calves despite the administration of an inactivated HS vaccine manufactured with an oil-based adjuvant. Although the process takes 250 days, the commonly employed oil adjuvant immunization showed that it can prevent young buffalo calves from being affected by HS (Zamri-Saad and Annas, 2016). There are four different

forms of HS vaccines: inactivated, live, subunit, and nucleic acid vaccines. Additionally, to improve the host immune responses, commercial vaccinations include adjuvants such as alum-precipitated vaccines, aluminum hydroxide gel vaccines, oil adjuvant vaccines, and broth bacterins (Almoheer et al., 2022). Animal health officials in Spain have promoted inactivated vaccinations to prevent paratuberculosis in sheep and goats. This suggestion was made to minimize production-related losses based on the disease's clinical manifestations (Juste et al., 1994). However, their use in cattle has been restricted due to concerns that these vaccinations may interfere with testing for identifying *Mycobacterium bovis* (Garrido et al., 2013). The regional animal health department decided to investigate the effectiveness of a novel heat-killed MAP vaccination in dairy cows naturally infected with *Mycobacterium avium subsp. Paratuberculosis* (MAP) due to the rapidly decreasing prevalence of bovine tuberculosis in the Basque Country of northern Spain and the high incidence of clinical cases of paratuberculosis (Alonso-Hearn et al., 2012). Significant losses in productivity in cattle firms are caused by the trematode parasites *Fasciola hepatica* and *Fasciola gigantica*, causing severe diseases in sheep and cows. Different strategical approaches are currently employed to remove intermediate snail hosts with *molluscicides* (Dawes and Hughes, 1964). However, losses are still anticipated to exceed US $2 billion per year globally despite the availability of such management measures (Boray, 1985). Several USDA-licensed BVDV vaccines or combinations are available, primarily combining BVDV with other bovine respiratory and reproductive microbes. Previously, most of these vaccines were prepared using BVDV1 strains only, but due to the diversity in antigens, both killed and live vaccines are now easily available (Dorneles et al., 2015). The global fight against bovine brucellosis has been significantly aided by vaccination against *Brucella abortus*. To prevent abortion in cattle, strains RB51 and 19 are widely used against *B. abortus* under license (Ferrari et al., 2014). Foot and mouth disease (FMD) is the most prevalent and economically important infectious disease of livestock in Pakistan (Ali and Hussain, 2020). FMD influences the duration of milking cows. The cows' average lactation length is decreased by 45 days due to the illness (Venkataramanan et al., 2006). The high mortality in young animals and morbidity in adults caused by this disease is the main source of economic loss to sub-sectors (Selim et al., 2010). A key strategy for preventing the spread of FMD is vaccination. The demand for developing safe and effective FMD vaccines was prompted by the limited safety and disease protection associated with traditional (killed or live attenuated) vaccinations. A large-scale antigen preparation, treatment of the virus to lose its virulence, and using adjuvants to boost the immune response are the requirements for producing the FMD vaccine (Deghaidy et al., 2002). Bovine TB is caused by *Mycobacterium bovis*, which infects various hosts, including domestic animals, wildlife, and humans. It is a significant global health and economic issue. According to estimates, 50 million cattle are infected with *M. bovis* globally, causing yearly economic losses of around US$3 billion (Steele, 1995). The Bacille Calmette Guerin (BCG) vaccine is associated with variable efficacy in humans and cattle (Fine, 1989). Numerous vaccination regimens in cattle that combine the BCG vaccine with other vaccinations, such as adjuvanted subunit, virus-vectored, or DNA vaccines, have been shown to increase vaccine effectiveness in comparison to the BCG (Table 1) vaccine alone (Buddle et al., 2011)**.**

Table 1: Vaccine Schedule of Dairy Cows in Punjab, Pakistan (Mushtaq, 2023)

Disease Type	Diseases	Disease-causing agent	Effected host	Vaccine Type
Bacterial	Mastitis	*S.aureus*	Cattle and buffalo	Multi-epitotes vaccine
	HS	*P.multosida*	Cow and water buffalo	Oil-based adjuvant vaccine, Live, Killed, Nucleic acid vaccine
	Paratuberculosis	MAP	Sheep, goat, cattle	Heat-killed MAP vaccine
	Brrucelossis	*B.abortus*	Cattle, sheep, goat	RB51,19
Parasitic	*Fascioliasis*	*F.hepatica* and *F.gigantica*	Cow, sheep	Candidate vaccine
	Bovine Viral Diarrhoea	BVDV	Cattle	Live and killed vaccines
Viral	Foot and mouth disease	*Aphthovirus* genus of the family *Picornaviridae*	Cattle	Live and killed vaccines

Foot and mouth disease (FMD) is a well-known infectious disease affecting cloven-footed animals. It occurs practically everywhere and seriously threatens the cattle business worldwide (Kesy, 2002)Many countries have achieved free status based on robust surveillance mechanisms, geographic isolation, vaccination programs, and control approaches. (Backer et al., 2012). In general, FMD vaccination is not used. Only when requested or when a free vaccine is available are vaccinations given to animals (GORSI et al., 2011). Along with the FMD immunization program, surveillance work was done throughout these two consecutive years (Table 2). This intervention aimed to compare disease frequency before and after the vaccination (Mushtaq et al., 2014).

Table 2: Comparison of the frequency of Foot and Mouth disease before and after vaccination (Mushtaq et al., 2014)

Parameters	Disease burden of FMD before mass vaccination (June 2008 to May 2009)	Reduced disease burden after first year of vaccine (June 2009 to 2010)	Reduced disease burden after the second year of vaccination (June 2010 to May 2011)
Animals	3573	3269	3350
Morbidity	27 %	5.38 %	2.90 %
Mortality	0.98 %	0.15 %	0.06 %
Case	4%	3 %	2 %

Bovine mastitis is difficult and expensive to prevent and cure, especially when staphylococci are involved. The success has eluded attempts to use vaccination in prophylaxis. The authors of earlier investigations found several significant barriers (Craven and Williams, 1985). The absence of a *Staphylococcus aureus* protective antigen for chronic infections is a significant issue. Antibody levels drop during lactation due to dilution and specific antibody class selection in the mammary gland. A staphylococcal vaccination containing toxoids sought to overcome the difficulty of producing and maintaining elevated antibody titers within the mammary gland, essential for a successful mastitis vaccine (Rainard et al., 2022). Experimental studies on cows revealed that alum-adjuvanted vaccines produced fewer circulating antibodies than simple infusion into the udder, indicating a potential therapeutic value. However, despite efforts to promote local antibody synthesis by antigen infusion into the mammary gland, the increases in antibody concentrations, which are the consequence, were minor and temporary, having inadequate influence on the course of spontaneous mastitis brought on by the *staphylococci* (Rainard et al., 2018). Mastitis quarters displayed higher antibody levels, possibly due to serum-derived antibody transfer, contradicting the idea of local antibody synthesis within the mammary gland. Dairy operations exhibit significant diversity in feeding practices, management styles, healthcare strategies, and facilities, leading to variable stress levels, disease susceptibilities, and pathogen exposures (Waldner et al., 2007). Universal vaccination is impractical, so tailored programs are needed for each operation. Common vaccine types include inactivated and modified-live vaccines. Killed vaccines are liquid, while MLVs are dry and require reconstitution. Pregnant animals should be cautious. Annual boosters are recommended, and proper handling and storage are essential. The calves should receive 6 quarts of colostrum within 24 hours of birth (Rients, 2022).

A heifer's vaccination schedule from birth to six months should consider suppressed immunization early on due to colostrum-derived antibodies. The vaccination with IBR-Pia may be suitable during this period. At weaning, calves should receive a modified-live IBR, BVD, Pb, BRSV vaccine, and 7-way clostridial bacterin-toxoid, with other vaccinations as appropriate (Bagley, 2001). Heifers aged 6-10 months should receive modified-live vaccines for diseases like leptospirosis and brucellosis (Table 3). A comprehensive program should be designed for herds, including adult cows and bulls. Collaborative design, considering herd health, management practices, and biosecurity, is crucial for successful vaccination strategies (Table 4).

Table 3: Recommended vaccination schedule for dairy heifers from birth to 6 months of age (Bailey et al., 2009)

Vaccine Therapy	Disease	Age
Colostrum	HIV/AIDS	0-6 hours
7-way Bacterin/toxoid	*BVD-BRSV, IBR-PJ*	Six weeks
Modified live vaccine	*Clostridial spp.*	
RB51	*Brucellosis*	4-6 months
5-way Bacterin	*Leptospirosis*	Six months
7-way Bacterin/toxoid	*Clostridial spp.*	
Modified live vaccine	BVD-BRSV, IBR-PJ	

Table 4: Recommended vaccination schedule for adult dairy cattle (Lacasta et al., 2015)

Vaccine Therapy	Disease	Age
Bacterin/toxoid	*E. coli* + *Clostridium* perfringens, type C and D	
Killed vaccine	Rota and Corona virus	
Bacterin	*Vibriosis* (optional)	40-60 days prior to calving
	Calf scours:	
5-way bacterin	*Leptospirosisb*	
Killed vaccine	BVD-BRSV, IBR-PJ	
Bacterin/toxoid	*E. coli* + *Clostridium perfringens*,	Three weeks prior to calving
Killed vaccine	Rota and coronavirus	
Bacterin	*Coliform mastitis*	Follow label directions

Immune Reactions against Invading Microbes

The immune system eliminates microbes entering the host body through two types: innate and adaptive. Innate immunity is conserved and provides a fast response, while adaptive immunity provides a late response. The interaction between pattern recognition receptors (PAMPs) present on the surfaces of the microbes and toll-like receptors (TLRs) present on the surfaces of the host immune cells induces the innate immune response (Akira et al., 2001; Janeway Jr and Medzhitov, 2002). It causes further upregulation of co-stimulatory molecules and major histocompatibility complex classes I and II. The inflammatory cytokines are also released, which stimulate T-cells more effectively and direct the ensuing adaptive response (Medzhitov and Janeway, 1997). The type 1 helper-T (Th1) cells activate macrophages through cell-cell contact and interferon-gamma (IFN-g) secretion (Duffield, 2003; Monney et al., 2002). The Th2 cells activate eosinophils through cytokine release, and B-cells secrete antibodies to activate the cascade of complement proteins, phagocytes,

natural killer (NK) cells, and mast cells (Figure 1) (Foster et al., 1996; Keatings et al., 1997). The vaccine activates humoral immunity by producing antibodies specific to invading microbes, such as IgG and IgA. These antibodies neutralize microbial toxins and initiate a reaction cascade, preventing microbial attachment and destroying pathogens like *Salmonella, Brucella*, and *Mycobacterium*. In the case of a virus replicating in the cell's cytoplasm, it is needed to activate cytotoxic T-lymphocytes(CTL) and destroy the infected cell. (Spickler and Roth, 2003).

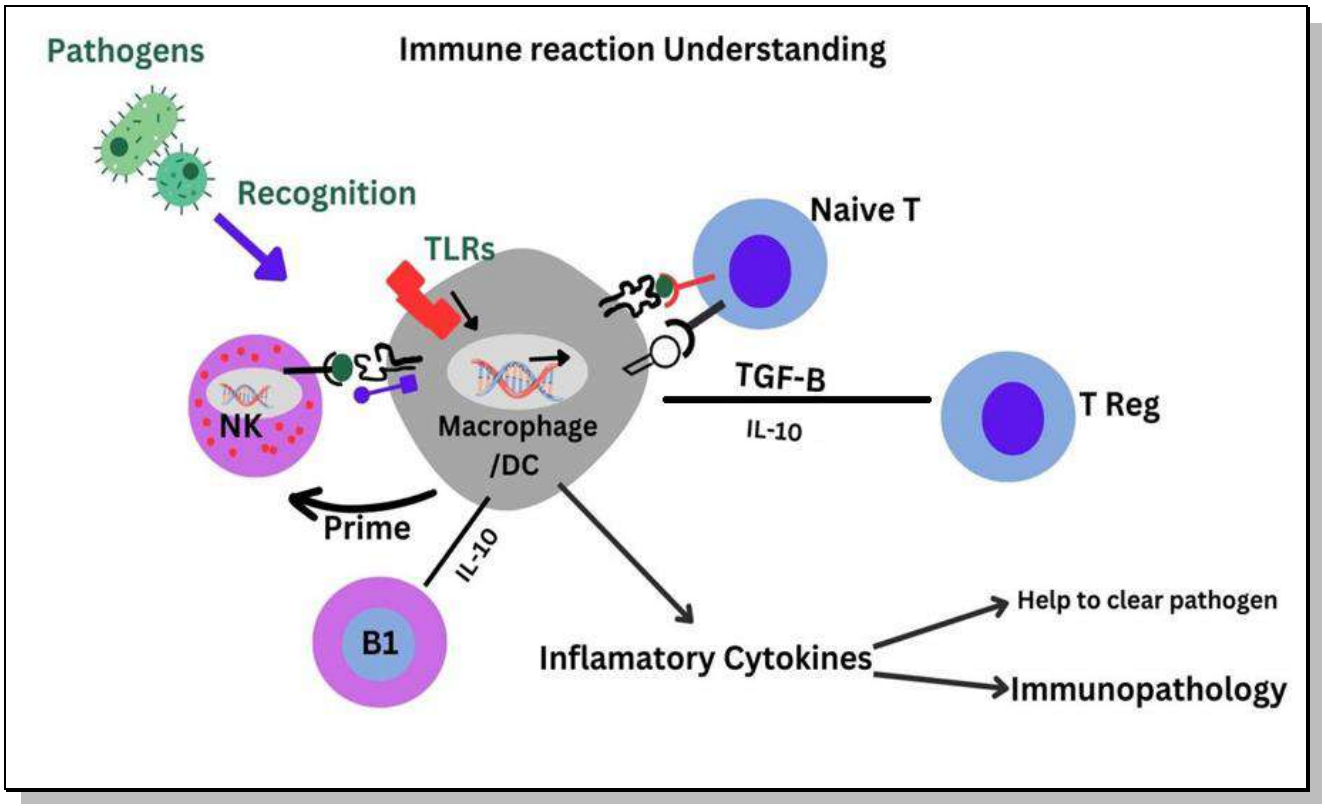

Fig. 1: A schematic diagram of Immune reaction against microbes

The immune system destroys microbes through innate and adaptive responses, including cell-mediated immunity (CMI) and humoral immunity. Humoral immunity produces antibodies against foreign agents while sub-neutralizing antibodies can promote viral infection. Antibody-dependent enhancement (ADE) occurs when non-neutralizing or sub-neutralizing antibodies promote cell entrance, affecting FcεR-dependent viral uptake. The FcεR on myeloid cells, including monocytes, macrophages, dendritic cells (DCs), and certain granulocytes, interacts with the projecting antibody Fc of the antibody-virus immunocomplex to cause phagocytosis, which results in a rise in the number of cells that are infected, or extrinsic ADE (Hawkes, 1964). The antibodies support several antiviral defense levels to destroy the virus and minimize infectivity efficiently. They negate transmission by attacking viral glycoproteins (GP) of enveloped viruses or the protein shell of non-enveloped viruses. In viruses with envelopes, they may inhibit virion binding to cellular receptors, obstruct the fusion machinery, or combine virus particles; in non-enveloped viruses, they can either stop the viral genome from being released from its coating in the endosome or encourage cytosolic destruction of forthcoming virions via a method involving the protein 21 proteasome, which contains a tripartite motif (Mallery et al., 2010). In a 1964 investigation on the neutralization of arboviruses by antiviral antibodies, the initial description of ADE was issued (Hawkes, 1964). When tested using diluted forms of their relevant anti-sera, several types of viruses from the Flaviviridae and Togaviridae families showed increased infectiousness. The subsequent studies using partitioned sera provided more evidence that this improvement was mostly due to IgG antibodies, which served as a molecular link between the virus and cells expressing FcR and were able to recognize the Fc component of IgG (Hawkes and Lafferty, 1967).

Vaccine based Immune Reactions

Throughout the history of science and medicine, vaccines have undoubtedly played a significant role in human and animal health, saving almost six million deaths globally and tens of billions of US dollars annually (Ehreth, 2003). When a disease-causing pathogen invades the host body, it induces a signal that stimulates the immune responses. The modified live vaccines also work like this invading microbe and can provide this signal. Although these vaccines are very effective, they are not encouraged due to potential risks and undesirable for some diseases. (Roth, 1999). The live attenuated vaccine contains a huge risk of coming back in virulent form through mutation (Muskett et al., 1985) Especially RNA viruses, which have a high rate of mutation to reverse into a virulent form (Lee et al., 2012). The killed vaccine is

encouraged as an alternative in this case. The pox virus causes lumpy skin disease (LSD). The vaccinations against the lumpy skin disease virus (LSDV) using the capripox virus have been linked to incomplete protection and adverse reactions. A local response at the injection site is a recognized side effect of the South African *Onderstepoort Neethling* strain of LSDV. However, with the introduction of suitably attenuated LSDV vaccinations, no significant systemic or widespread responses have been documented. (Weiss, 1968). The resulting clinical signs in dairy cattle resembled those seen with the natural LSD infection and included fever, skin lesions, and decreased milk production (Yeruham et al., 1994). However, lambs and goats have been successfully exposed to this strain without experiencing any serious or widespread responses in Kenya. (Kitching et al., 1987).

BCG vaccination may increase sensitivity to tuberculin skin tests in domestic animals and may not be 100% effective, but it may not prevent its use in wildlife. The main characteristic of an effective wildlife TB vaccine would be preventing the infection of *Mycobacterium bovis* from spreading to other animals or domestic cattle (Buddle et al., 2011). Not only must a sufficient amount of live bacteria be administered at the beginning to enable BCG-vaccinated animals to establish an efficient cell-mediated immune response, but the bacteria must also remain and replicate in situ inside the lymphatic system. The orally given BCG has been demonstrated to localize preferentially to the mesenteric lymph nodes in investigations involving mice and possums. However, it is also identified in the regional nodes draining the brain (Buddle et al., 2011).

Causes of Vaccine Failure

Ineffective vaccine strains, improper storage, and manufacturing errors reduce efficacy. Individual factors such as weakened immune systems, age, and underlying health conditions can also impact vaccine response (Figure 2).

Different Serotype

The vaccine strain must have a structural resemblance to the prevalent circulating viruses to produce a protective state of immunity. Due to the strain differences and a greater incidence of viral changes, immunization may be ineffective in protecting against changing field strains (Diaz-San Segundo et al., 2016). The antibodies produced during vaccination may vary in their ability to neutralize antigenically different clusters. Therefore, it is essential to conduct regular studies of vaccine compatibility to determine whether a potential vaccine effectively provides immunity to all common strains (Rweyemamu et al., 1978). Antigenic discrimination and field isolate matching are crucial for understanding disease epidemiology, selecting appropriate vaccination strains, identifying immunity gaps, and determining their suitability for specific situations.

Vaccine Potential

Vaccines must have A substantial antigenic load to trigger an immune reaction successfully. Lacking strict regulation, vaccines may not have this capability. According to the OIE, a vaccine's effectiveness is defined as its "concentration of the immunologically operative constituent (Lyons et al., 2016)Vaccine producers commonly evaluate potency, as stated here, by measuring an antigen. As a result, each vaccination dosage delivers a specific antigenic payload (Doel, 2003)The accepted method for evaluating vaccine effectiveness is rigorous testing of vaccinated and controlled individuals. Although these evaluations differ from the OIE's definition of potency, they are frequently called "potency tests." The first of these tests measures the PD50, or 50% protective dosage, a measure recommended by the European Pharmacopeia. The amount of vaccination needed to protect 50% of the people exposed to a certain challenge regimen is known as the PD50 (Pattnaik et al., 2012).

Vaccine Quality

The manufacturer's recommendations for storing and administering vaccinations must be strictly followed. The effectiveness of the vaccination may be jeopardized by elements including UV radiation exposure, chemical agents, medications, and adverse temperatures. Since the inactivated vaccine's formulation is susceptible to temperature changes, it must be stored in a maintained cold chain environment between 2 and 8 degrees Celsius until given to the patient (Doel, 2003). A decrease in the antigenic load, such as the 146S viral particle, within the vaccine due to cold chain failure compromises the immune system's response that is induced. Nevertheless, post-vaccination serological monitoring detects inadequate vaccine quality (Singh et al., 2019).

Immune Response

Different species produce antibody diversity through completely distinct processes, which makes it difficult to develop a useful laboratory model for evaluating the effectiveness (Robinson et al., 2016). Locally and systemically, numerous IgM antibodies help the first T-cell-independent clearance of the viral infection. According to research, inactivated vaccines can also prime an immune response by activating B-cells (Pega et al., 2015). Additionally, non-neutralizing opsonic antibodies have demonstrated possible functions in which they aid in ingesting viral fragments attached to them by dendritic cells, which act as strong immune-modulating mediators (Lannes et al., 2012)Recent scientific knowledge does not clearly define the specific role of the T-cell-dependent immune reaction in the early stages of viral infection (Langellotti et al., 2012). According to the current limited information, bovine gamma-delta T-cells have regulatory abilities similar to those of

murine and human Foxp3-positive T regulatory cells. These results also show that these cells significantly inhibit the *in-vitro* proliferation of CD4 and CD8 T lymphocytes specific for the FMDV (Guzman et al., 2008). Scientific efforts are essential for thoroughly understanding crucial immunological processes, such as the CD8 T-cell mediated response. This information serves as the foundation for improving vaccination formulations that maximize the induction of an immune response (Patch et al., 2013).

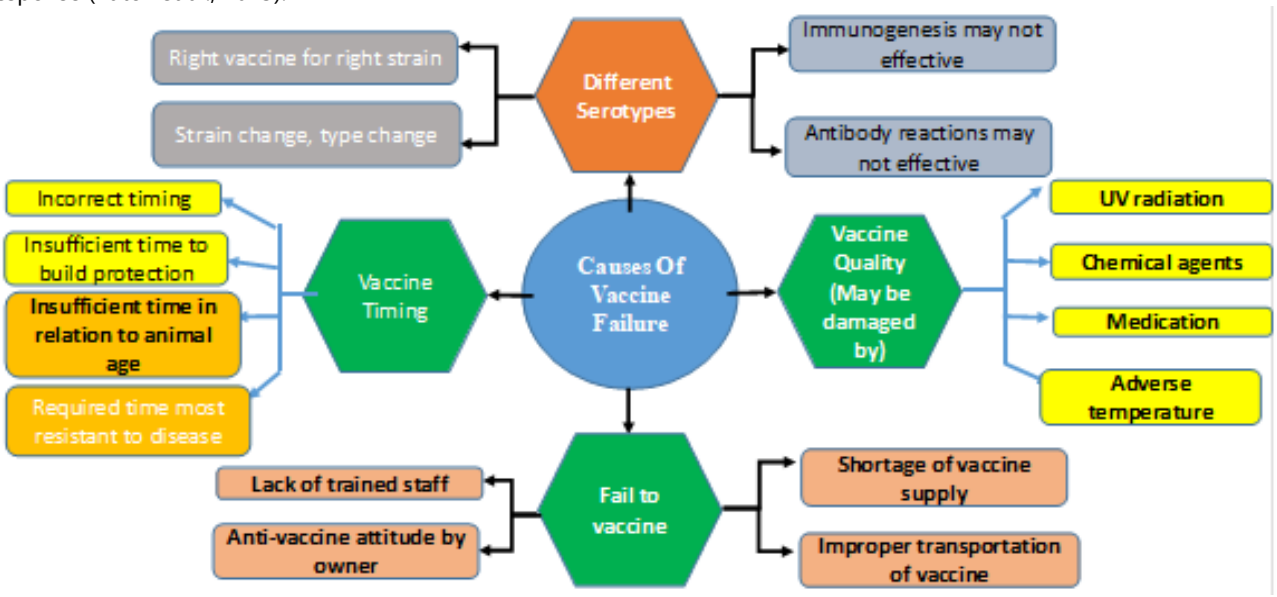

Fig. 2: Diagrammatic illustration of Causes of Vaccine Failure

Age of Animal

Infant animals lack immune maturation, elderly animals may have immune deficits, and colostrum-fed young animals absorb antibodies from mothers, preventing their immune response to vaccinations for one to two months (Firth et al., 2005).

Administration Route

The manufacturer's research indicates that muscle injections are the optimal vaccination method, and your veterinarian should be engaged to ensure the correct vaccine selection and usage (Tizard, 2021).

Failure to Vaccinate

Low vaccination rates can spread diseases due to factors like lack of supplies, transportation, training, and anti-vaccine sentiments. Milk cow owners face barriers to immunization due to decreased milk output. Post-vaccination sero-monitoring is recommended to monitor vaccination coverage and identify high-risk areas for remedial action (Park et al., 2021).

Vaccination Timing

Administering vaccines at the right time is crucial for optimal immunological response, considering factors like disease resistance, protection development, and age-related relationships (Ciabattini et al., 2018).

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Chapter 12

Biological Effects of Complement Activation in Vaccinated Dairy Cows

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ABSTRACT

In the dairy sector, the well-being of the cows is critically important as it determines the amount of milk production, animal care, as well as farm economics. Vaccination of dairy cows against infectious diseases is a critical measure for herd health, but behind this method is a complex set of mechanisms. This chapter elaborates on one of the most unique aspects of the immune system, complement activation, an extraordinary immune response triggered by the immunization process. We focus on these ever-changing proteic cascades that battle against pathogens, direct inflammation, and also deregulate milk production. We expose some advantages, like the increased resistance to mastitis, and fascinating ways of influencing the complement activation that could be utilized to act as developers of cow health and milk quality. This chapter constitutes a fascinating journey, which is a combination of immunology, veterinary science, and dairy production through which the bovine immunity gets revealed.

KEYWORDS
Bovine Vaccination, Complement System, Immune Response, Dairy Cow Health, Inflammatory Response

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INTRODUCTION

Healthy dairy cows are essential for a healthy global food chain, with their well-being directly impacting milk production and economic stability. Vaccination remains a cornerstone preventative measure against infectious diseases in these animals, but its effectiveness goes beyond just the specific antigen (Lee et al., 2012). The intricate dance of the adaptive and innate immune systems is also critical. It directly destroys pathogens, and opposites (this is when cells enhance the process of opsonization and pathogens engulfing and destroying), and generates inflammation. The innate immune response is indeed a complex mechanism one of which is the complement system. It is an effective first-line immune barrier (Castellheim et al., 2009).

Current studies demonstrate that complement activation also greatly impacts the effectiveness of vaccination in dairy cows. Through the ability of complement activation to facilitate antigen presentation and control adaptive immune responses, long-term memory immunity generation may be made possible (Nielsen et al., 2000). Discovering the exact mechanisms by which the vaccine caused complement activation and the subsequent biological events playing on the cow's immune system is very relevant.

This chapter also explores the complexities of the complement system in these animals, the different pathways of complement activation that are triggered by vaccination, and the varied biological effects it has on the immune system. We will also deliberate about the elements that can influence the extent and kind of the activation of complement after the vaccination. This chapter aims to extend the current research knowledge on the mechanism of complement system activation in vaccinated dairy cows. By investigating the intricate relationship between vaccination and the complement system, we can thereby open a door to more accurate and efficient vaccination that helps to maximize immune responses and finally makes dairy cows healthier.

The Complement System in Dairy Cows

Components of the Complement System

The complement system in dairy cows is powerful with a multipurpose immunity arm as an innate defensive mechanism. It works as a well-coordinated catena of proteins that interact with each other to destroy pathogens, stimulate inflammation,

and clear up the immune sensibilia. In contrast with the highly specific immune system, the complement system is non-specific signaling that can be used to protect against a broad spectrum of invading microorganisms (Krauss et al., 2010).

Historical Perspective

Research into the complement system has been on for many centuries (Table 1) right from the time antiquity civilizations noticed the body's immune responses till now. From the mid-20th century onwards, further research categorized the activating phases and identified inactivation pathways within the complement system (Walker et al., 2014). Currently, the complement system remains an actively investigated and also an important academic field relevant to the disciplines of immunology, infectious diseases, and therapeutic intervention.

Table 1: Historical Perspective of Complement System

Sr. No.	Year	Discovery	Reference
1.	1900s	Discovery of antibodies.	(Schmalstieg Jr and Goldman, 2009)
2.	1950s	Development of a vaccine for dairy cows.	(Biggs, 1990)
3.	1980s	Identification of complement system in dairy cow.	(Lewin, 1989)
4.	2000s	Advancement in vaccine technology.	(Hajj Hussein et al., 2015)
5.	2020s	Understanding the specific immune system in dairy cows.	(Sheldon et al., 2011)

Complement Activation Pathways

Complement can activate three principle pathways: alternative, lectin, and the classical pathway (Figure 1). The complex network is composed of individual pathways each consisting of a unique set of proteins that could be activated by specific molecular patterns, like damaged cells or pathogen signatures (Ketelut-Carneiro and Fitzgerald, 2022).

Complement Effector Molecules

Upon a pathway activation, a cascade of biological examples of reactions starts; this leads to the powerful effector substances. These are specifically produced by C3b (Figure 1) which enhances phagocytosis by the phagocytotic cells of immune systems, in addition to C5b-C9 membrane attack complexes that directly kill pathogens (Vandendriessche et al., 2021).

Regulatory Proteins

To prevent unsuitable activation and subsequent tissue damage, the complement system is closely controlled by a series of proteins. These proteins act at various points in the cascade to limit or terminate the complement response (Atkinson et al., 2019).

Functions of the Complement System in Dairy Cows

Enhanced Phagocytosis

Complement activation results in the opsonizing of pathogens and immunocomplexes and makes these molecules more relevant for phagocytic cells (Figure 1) to engulf. It triggers various events including antibody production and antigen presentation to lymphocytes which is necessary for increasing the adaptive immunity activity (Primorac et al., 2022).

Inflammation and Immune Cell Recruitment

Release of complement activators together with inflammatory mediators that involve marine cells to the location of vaccination. This area-specific inflammation serves as a boost for the adaptive immune system response (Bosisio et al., 2022).

Direct Lysis of Pathogens

The MAC complex, a strong effector molecule (Figure 1) formed during complement pathway activation, primes to kill certain bacteria and trigger their destruction (Bjanes and Nizet, 2021).

Mechanisms of Complement Activation after Vaccination

Vaccination in dairy cattle is akin to a role on the stage that involves both adaptive and innate immune systems. Vaccines stimulate the antigen-specific production of antibodies that strengthen the adaptive immune response while also indirectly activating complement, a key element of the innate immune response (Schenten and Medzhitov, 2011). The subsection addresses what pathways vaccination of dairy cows can activate and how the complement system is turned on.

Classical Pathway

The classical activation pathway of the complement system is a widely understood mechanism that is usually initiated by immunocomplexes. During vaccination, particular antigens interact with B cells after injection, so specialized antibodies are created. These antigen-antibody complexes may bind to complement component C1q which (Figure 2), in turn, activates a cascade of enzymatic reactions killing bacteria or forming holes in its membrane (Whaley et al., 2012). While the classical

pathway plays a role, recent research suggests that alternative pathways might be more dominant in vaccine-induced complement activation in dairy cows.

Alternative Pathway

The alternative pathway represents another significant mechanism for complement activation following vaccination. In contrast to the former, it does not involve the formation of such antibody-antigen complexes. However, it is not LPS nor the viral carbohydrates responsible for the TLR activation during vaccine stimulation. In the absence of particular antibodies, the alternative route is initiated spontaneously by the decomposition of complement component C3 (Figure 1), which acts as a first line of defense against infections by constantly examining the body and starting complement activation (Barnum, 2017).

Lectin Pathway

The lectin pathway is another secondary mechanism of complement activation that may play a role after inoculation. This pathway takes lectin molecules (Fig. 1) that bind to carbohydrate moieties on pathogens or vaccine components (Mason and Tarr, 2015). Nevertheless, the actual role of the lectin pathway in forming complement activation in dairy cattle through vaccines is currently being investigated.

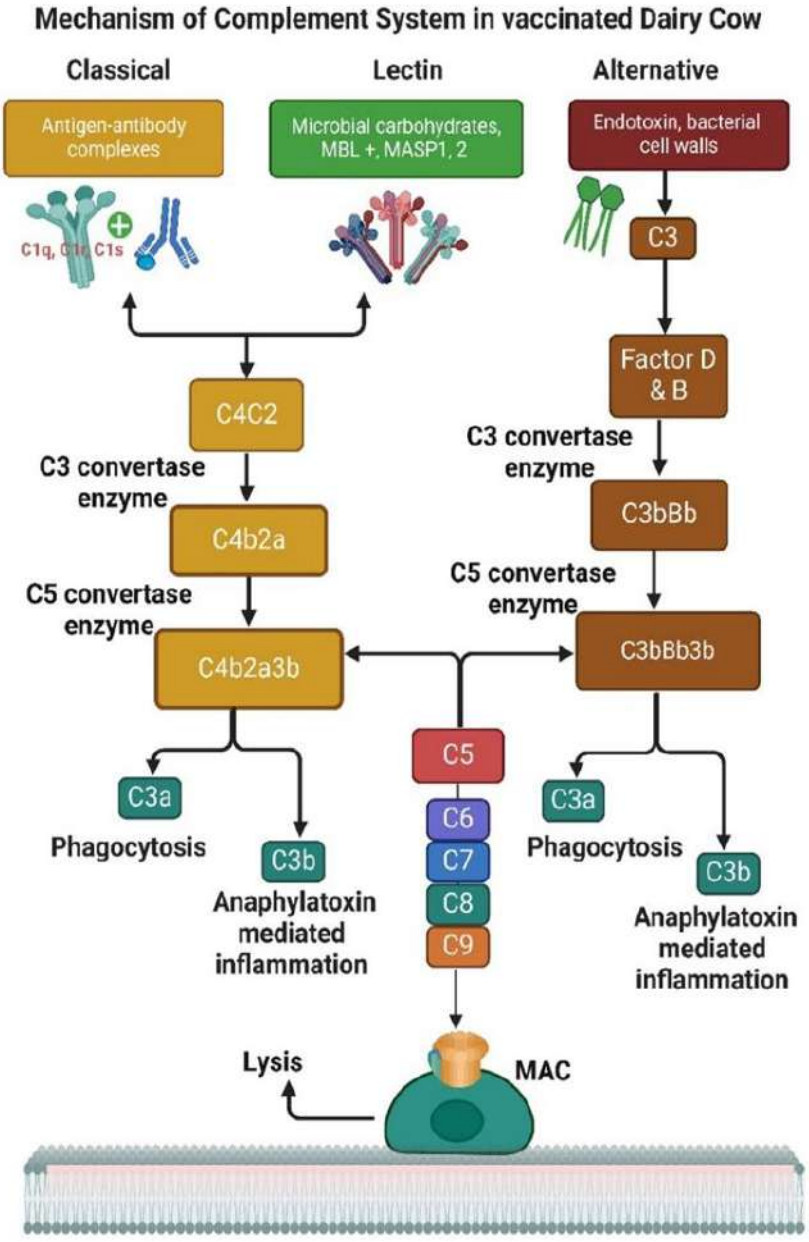

Fig. 1: The complement system has three activation pathways: crop-based varieties that are unique. The three pathways merge at the C3 cleavage site producing C3a inflammatory agent and C3b opsonizing or C5 convertase proteins. The classical pathway mechanism involves antigen-antibody complexes and a specific combination, whereas the lectin pathway binds with sugars. The alternative, or side, pathway is characterized by a low-level activation with a surplus activator loop that would amplify the other pathways. Finally, the membrane-attacking complex, which in turn leads to cell lysis, is formed as a result of the membrane-attack complex formation.

Assessing Complement Activation after Vaccination

Complement activation plays an important role in dairy cow vaccination and it is very important to understand its completeness and nature it to improve the vaccination (Herry et al., 2017). Here we discuss different strategies used for the determination of activation of complement post-immunization.

Classical Pathway Activation

Haemolytic Assays

The assay used in these studies measures the ability of human serum from vaccinated cows to lyse red blood cells coated with antibodies and complement. A decrease in hemolytic activity indicates complement consumption due to activation (Johnstone 2001).

ELISA for Complement Components

The purpose of enzyme-linked immunosorbent assays (ELISA) can be the measurement of specific complement components (i.e., C3, C4) in blood serum (Mayes et al., 1984). A decrease in these components suggests their consumption during complement activation.

Alternative Pathway Activation

Flow Cytometry for C3 Deposition

Fluorescently labeled antibodies specific for C3b can be used to detect complement deposition on opsonized particles or pathogens, indicating alternative pathway activation (Boero et al., 2023).

Emerging Techniques

Mass Spectrometry

Sophisticated mass spectrometry methods are being investigated to determine and measure complement activation components in serum samples after vaccination which will provide a better image to understand the complement response (Milewska et al., 2020).

Microfluidic Platforms

Microfluidic devices are an attractive method of swift detection of complement factor activation and could be used for immunoprophylaxis screening at the farm level (Galat et al., 2018).

Biological Effects of Complement Activation on Vaccinated Dairy Cows

Vaccinating dairy cows is a crucial step in the animals' protection from different infectious diseases. However, the complement system of the immune system sets off a remarkably complex interaction process upon the vaccination (Dunkelberger and Song, 2010). Complement activation significantly influences the success of vaccination in dairy cows by impacting various aspects of the immune response (Table 2).

Enhanced Phagocytosis and Antigen Presentation

Complements (like C3b) act like tags on pathogens, signaling them to phagocytes (the immune cells that engulf intruders) so that they can recognize them (Figure 2) and remove them. This complement improves the efficiency of opsonization, a critical first step in the activation of the adaptive immune response, that results in increasingly efficient and targeted attacks against the pathogen (Wibroe et al., 2014).

Modulated T-Cell Activation

These T lymphocytes are specific for antigens and are properly targeted by the T cells after they are activated by the complement opsonization during antigen presentation (Heesters et al., 2016). T-activated cells direct a coordinated multi-stage attack against the pathogen (Figure 2) thus the immune defense is stronger and more durable.

Potential Influence on Antibody Production

The key role of complement remains to be defined although it has been suggested that it might indirectly modulate the antibody production by B cells (B lymphocytes). Studies also mention it as producing antibody groups like IgG which might become dominant and important in long-term immunity (Megha and Mohanan, 2021).

Potential Influence on Milk Production

The mammary gland, responsible for milk production in cows, is highly susceptible to the effects of complement activation (Figure 2). The bovine mammary gland means that milk is produced and this part of the cow's body is a target for complement activation (Megha and Mohanan, 2021).

Mastitis Risk

The complement system is provoked by the mammary gland which finally results in mastitis, which is the implication of severe inflammatory disease. It has been suggested by researchers that the complement system may be a precondition leading to the injury process during mastitis (Katsafadou et al., 2019). This disease is the primary cause of lowering the quality of milk and undermining production and animal welfare.

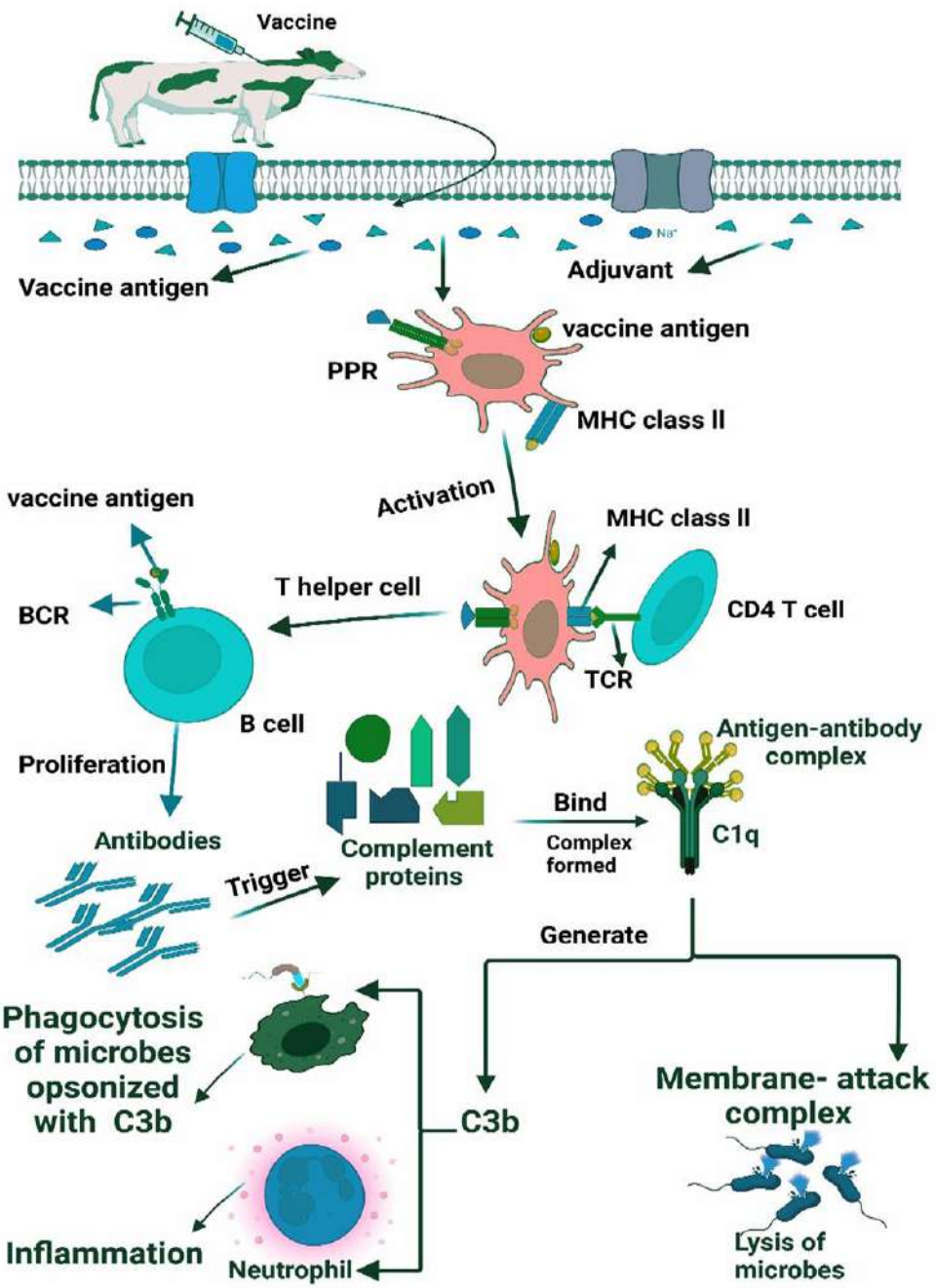

Fig. 2: This diagram which highlights, how a vaccination may help improve the immune strenght of a dairy cow. The course starts with immunizing the cow by a vaccine. These results in DCs (dendritic cells) those that present peptide fragments that are linked to MHC class II molecules on their surface. This turn on the helper T cells wherein they further cause B cell activation to produce distinct antibodies against the specific invaders. The antibodies formed against antigen gets generated and after binding to antigen complexes are formed which will, in turn, activate complementary system. Cooperation between compounds like C1q and C3b is involved in targeting pathogens during the phagocytosis process and in forming the MAC that ends up breaking down the membrane to destroy the invading pathogens. Through this specific antibodies and complement proteins binding to an invader, the pathogens are eliminated.

Altered Milk Composition

The pro-inflammatory function of the complement molecule will decrease the milk protein amount carriers. Lactic acid which are known inflammatory marker and immune cell count is a buildup that enters the milk and makes it unsuitable for humans.

Table 2: Biological Effects of Complement System in the Vaccinated Dairy Cow

Sr. No.	Pathways	Initiating Molecules	Effects	Dairy cows Benefit	Potential Drawbacks	Reference
1.	Classical	Antigen-antibody complexes.	Opsonization, Inflammation.	Reduced infection risk.	Tissue damage due to excessive inflammation.	(Ogundele, 2001)
2.	Lectin	Mannose-binding lectin (MBL).	Opsonization (effective against specific bacteria and fungi).	Improved clearance of pathogens, reducing infection risk.	Not identified.	(Sealy et al., 2008)
3.	Alternative	Microbial surfaces.	Opsonization, lysis (potential mastitis prevention).	Potential role in preventing mastitis by targeting mastitis-causing bacteria.	Not identified.	(Kabelitz et al., 2021)

Factors Influencing Complement Activation in Vaccinated Cows

The degree and type of complement activation expressed by the vaccination in dairy cows can be influenced by many factors (Table 2). These factors are essential for devising vaccination strategies as well as increasing their effectiveness.

Vaccine Type

Antigen Composition

The nature of the antigen applied in the vaccine dramatically changes the level of complement activation. The live attenuated vaccines in which the pathogens have been made weakened but still viable are non-toxic and induce a more powerful concentration of complement than the molecularly or chemically inactivated ones (Kabelitz et al., 2021).

Adjuvants

Those are any types of materials that go into the vaccines to help in improving the safety of the immune system. Specific adjuvants may show their effect by targeting complement pathways in a way that modulates the intensity and type of the activation process (Wang and Xu, 2020). Continuous research is being conducted on adjuvants that have better complement activation for increased vaccine efficacy.

Delivery Systems

The method of delivery of the vaccine was prepared also matters. Novel delivery systems, like nanoparticles, are coming out intending to target specific immune cells and activation of the complement system (Bezbaruah et al., 2022).

Strategies for Modulating Complement Activation in Vaccinated Cows

Importance of Modulation

The dual role of complement activation in vaccinated dairy cows as mentioned before is also double-edged. A moderate level of activation of the immune system can increase the efficacy of the vaccines but the excess and uncontrolled activations of the immune system can have the same opposite effect by increasing the risk of tissue damage and complement depletion (Bezbaruah et al., 2022). This means, therefore, that strategies to regulate complement activation after vaccination are very relevant to both improving the immune response and health of the animals.

Potential Strategies

Targeted Adjuvants

Adjuvants that are developed to be tailored for and modulate complement pathways is an exciting area of exploration in the research. Adjuvants can be constructed to either selectively boost particular complement pathways for a more wearable response or neutralize excessive stimulation to avoid possible adverse reactions (Bhattacharjee et al., 2022).

Vaccine Delivery Systems

Novel delivery systems like nanoparticles could be considered as a solution to this issue whereby those immune cells could be targeted and complement activation is minimized at the local level, by an extension (Zhuang et al., 2019).

Complement Component Therapeutics

Research into complement inhibitors or activators specifically may help to optimize the complement response immediately post-vaccination.

Personalized Vaccination Strategies

We may be able to study the relationship between types of vaccine, the health status of a cow, and complement activation and, subsequently, provide some specialized vaccination schedules. This might involve using biomarkers of complement activity to predict and tailor vaccination regimens for optimal response in individual cows (Zhuang et al., 2019).

Complement Activation and Dairy Cow Productivity

The functioning of dairy cows is significantly impacted by complement activation, in addition to its function in immunological responses. Milk production, reproductive efficiency, and overall cow productivity (Figure 3) are all significantly increased when complement is activated in dairy cows (Zhuang et al., 2019).

Influence on Milk Quality and Quantity

Complement activation has been associated with higher milk quantity and quality, among other things, in dairy cows. Cows with higher complement activity have a superior ability to fend off mastitis, a common udder ailment that reduces milk production. This leads, undoubtedly, to more milk. By boosting udder health and milk quality because of heightened immune defences against mastitis-causing pathogens, complement activation ultimately contributes to more milk. The potential influence of complement activation after vaccination on milk quality is a topic of emerging research with limited conclusive data (Klingler et al., 2021).

Effects on Reproductive Performance

Complement influences fertility characteristics like conception rates, calving intervals, and reproductive diseases. It also plays a critical role in the general health and performance of the reproductive system in dairy cows. Higher complement activity cows have better reproductive results because they are less likely to develop metritis, endometritis, or retain their placenta (Alhussien and Dang, 2019). It is evident from the link between immunological and metabolic condition and reproductive performance that metabolic stress impairs dairy cows' ability to reproduce). Since glucose promotes leukocyte activation and function, including the ability to kill, it is necessary for the bovine to mount an immune response that supports uterine health (LeBlanc, 2020).

Fig. 3: Complement Activation and Dairy Cows' Productivity

Impact on Metabolic Health

The response of the complement system is reported to modulate metabolic processes in dairy cows, the changes that can affect aspects of energy metabolism, nutrient utilization, and overall metabolic health. Dysregulation of complement activation has been observed in metabolic disorders including fatty liver disease and ketosis, both of which negatively correlate with milk production and reproductive performance (LeBlanc, 2020). Stressed-out cows may not be able to raise plasma glucose concentrations to maintain the immunological function that depends on glucose and Dairy cow reproductive performance is significantly impacted by metabolic stress as well (Etim et al., 2013).

Management Strategies for Complement Activation in Dairy Cows

The primary aim of the study on complement activation and the immune system is to discover ways to increase these two processes and to make the immune system as strong as possible in dairy cows (Vlasova and Saif, 2021). Complement activation, which is the immune system's support and immune response, and hence the promotion of immune system activities, is the most important aspect for the health and welfare of dairy cows, as it helps to prevent diseases and other health issues in different ways (Figure 4).

Nutritional Optimization

Nutrition is a critical management practice, which managers can use to modulate immune function and complement activation in dairy cows. Dairy cows must be fed a nutritionally balanced diet with adequate levels of key nutrients like vitamins A and E and zinc and selenium to support immune cell function and enhance complement activity (Paul and Dey, 2015).

Environmental Management

A condition of immunity, including complement activation is sustained in cows when environmental stress can be avoided. Ventilation, comfort of housing, and cleanliness for both feeding and water areas are all very important to avoid stress that can minimize the challenge of infectious diseases, as well as mammalian immunity (Sejian et al., 2021). Appropriate need for abatement strategies of thermal stress not only sustains immune function but also breaks hyperthermia-induced disorders.

Vaccination Protocols

Dairy farmers should explore strategies to boost immune responses to maintain complement activation in their dairy cows. Dairy farmers need to work with their veterinarians to develop vaccination protocols that are effective and tailored to the vaccinal needs of their herd. The use of vaccines containing adjuvants that enhance complement activation and the design of booster vaccination schedules based on the disease status of the herd can optimize immune responses and herd immunity (Stern, 2020).

Monitoring and Surveillance

Regular monitoring can provide valuable clues about herd health and aid in the recognition and control of risk in dairy herd populations. Diagnostic testing of individual animals, such as complement assays, CBC, and serological testing, provides early warning of infectious disease or immune dysregulation and should be performed in cases of acute disease (Christopher-Hennings et al., 2019)

Biosecurity Measures

Stringent biosecurity measures are necessary for protection against infectious diseases and for safeguarding complement activation in dairy herds (Renault, 2021). This requires using appropriate disinfection protocols, carefully managing herd health including monitoring of body temperature, white cell count, etc, and effective sanitation e.g. wearing gloves and using alcohol-based hand gels, which are more effective than soap and water and cleaning facilities efficiently and in a specific order such that the disinfectant is allowed to be effective in eliminating pathogens. Furthermore, herd health will be better served by severely limiting disease exposure, such as by creating multiple groups and reducing exposure to ill cattle (Gortazar et al., 2015).

Collaboration with Veterinary Professionals

Collaboration strategies with your veterinarian can be part of an effective approach to the activation of the complement in your dairy cows. This can include regular veterinarian visits, herd health assessments, disease protocols, and ongoing consultation with your veterinarian (Ritter et al., 2019). The bottom line is that veterinary and farmers strive to keep cows healthy, productive, and within their herds.

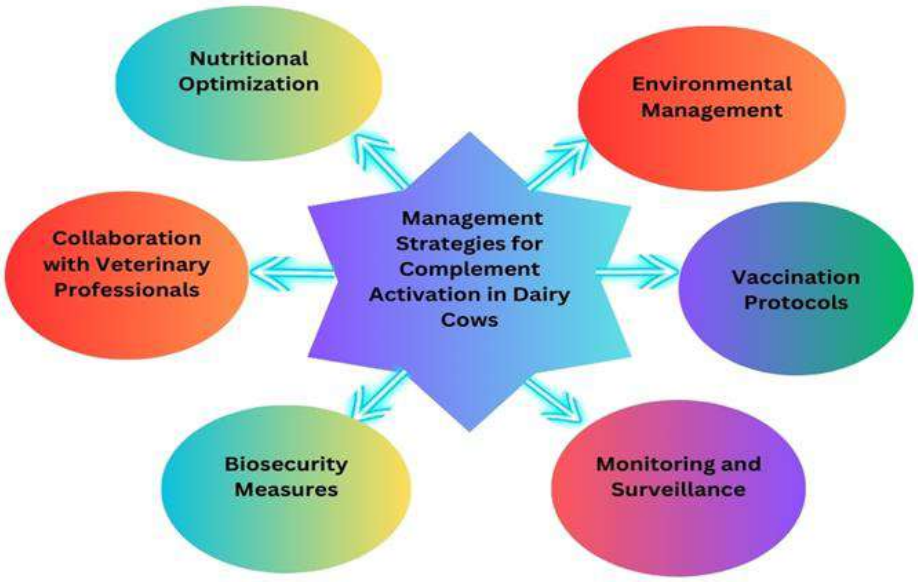

Fig. 4: Management Strategies for Complement Activation in Dairy Cows

Ethical Considerations in Complement Activation Research

Ethical considerations are vital to complement activation research, ensuring scientific inquiry is carried out responsibly with the welfare of the dairy cows, is paramount. The welfare of dairy cows is essential to completing activation research. This involves taking care of the dairy cows utilized in the complement activation research during the entire research procedure, both physically and mentally. The policies and procedures regarding animal welfare that are established by the institutional animal care and use committees (IACUCs) and other regulatory organizations must be adhered to by researchers (Hansen et al., 2017).

To ensure ethical conduct, researchers should be transparent and work collaboratively with stakeholders to clearly outline the research objectives, procedures, and potential risks. Data integrity and transparency are essential for ethical research conduct and scientific integrity.

Conclusion

Vaccination is very important to prevent dairy cows from infectious diseases. We highlighted the prospect for regulated complement activation, including heightened phagocytosis and improved antigen presentation process involved in increased adaptive immune response. On the opposite side, we mentioned the disadvantages of excessive complement activation such as inflammation and tissue damage.

Current research is investigating the specific complement activation patterns triggered by different commercially available vaccines for dairy cows. Emerging research areas were emphasized, including the need to elucidate the specific contribution of different complement pathways triggered by various vaccines and adjuvants. Additionally, the development

of novel biomarkers for real-time monitoring of complement activation and the long-term effects of repeated vaccinations on cow health were identified as crucial aspects for future investigation. Understanding complement activation in the context of dairy cow vaccination holds significant promise for: (i) Through the selection of correct complement activation patterns, vaccines can be customized to create a stronger and lengthier immune response, which can translate into less and less vaccination and cost. (ii) Strategies to prevent the excessive activation of complement can be useful in avoiding the inflammatory and tissue damage potential of vaccination. (iii) Identifying factors affecting complement response for each bovine is a step towards tailoring vaccination strategies for boosting their effectiveness and it can help achieve better health of cattle.

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Chapter 13

Guardian of Animal Health: Unveiling the Social Landscape of Veterinary Vaccination and Immunization

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ABSTRACT

The landscape of veterinary vaccination and immunization encompasses a complex interplay of socio-cultural, economic, and institutional factors that shape attitudes, behaviors, and access to veterinary services. Understanding this social landscape is essential for promoting animal health, preventing disease outbreaks, and safeguarding public health. This chapter synthesizes the multifaceted dimensions of veterinary vaccination and immunization, shedding light on the challenges, opportunities, and implications within diverse contexts. Veterinary vaccination and immunization are cornerstone practices in animal health management, aimed at preventing infectious diseases, reducing morbidity and mortality, and safeguarding animal welfare. Despite the success of veterinary vaccination in controlling and eradicating diseases, numerous challenges persist in achieving optimal vaccine coverage and effectiveness. The social landscape of veterinary vaccination is influenced by various factors such as public perception, cultural attitudes towards animals' healthcare, economic considerations, institutional dynamics, and challenges faced by the livestock keepers in the access and utilization of vaccinations. Access to vaccines, particularly in resource-limited settings, remains a barrier to disease control efforts. Furthermore, vaccine hesitancy, misinformation, socio-economic disparities and gender roles can also undermine vaccination programs and contribute to disease outbreaks. Addressing these challenges requires holistic approaches that integrate public health, veterinary medicine, and community engagement efforts.

KEYWORDS
Socio-economic, Disparities, Misconceptions, Vaccine hesitancy

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INTRODUCTION

Understanding the social landscape is paramount in various fields, including public health, education, and community development for addressing complex challenges and promoting positive change. The social landscape encompasses the myriad of interconnected social, cultural, economic, and political factors that shape human behaviors, attitudes, and interactions within a given context. It encompasses elements such as social norms, cultural beliefs, power dynamics, and institutional structures, which influence individual and collective experiences, opportunities, and outcomes (Freire and Donaldo, 2020). By comprehending the social landscape, stakeholders can gain insights into the root causes of social issues, identify leverage points for intervention, and develop contextually relevant strategies for positive change.

An understanding of the social landscape is essential for informing policy and practice in various sectors. Policies and interventions that fail to account for the social context in which they are implemented may be ineffective or inadvertently exacerbate existing inequalities. For example, in public health, interventions aimed at promoting healthy behaviors or increasing access to healthcare services must consider socio-cultural norms, beliefs, and practices that shape health-seeking behaviors (Hawkins and Tonts, 2017). Similarly, educational initiatives designed to improve learning outcomes must take into account socio-economic disparities, cultural differences, and community dynamics that affect educational access and attainment. ((Dórea, and Erdenesan, 2020).

The social landscape plays a critical role in shaping inequities and disparities in health, education, employment, and other domains. Socio-economic factors such as income inequality, poverty, and discrimination intersect with social identities such as race, ethnicity, gender, and age to produce differential access to resources, opportunities, and outcomes (Marmot, 2020). Understanding the social landscape is essential for identifying and addressing structural barriers to equity and promoting social justice. By examining the social determinants of animal health, for example, policymakers and practitioners can develop targeted interventions to address root causes of health inequities and improve animal health outcomes.

Veterinary Vaccination Program for Improved Rural Livelihoods

Disease outbreaks in animal populations have significant implications for livelihoods, impacting productivity, economic stability, and rural communities. Disease outbreaks can result in substantial economic losses for livestock producers, affecting both small-scale farmers and large-scale enterprises. Outbreaks of diseases such as foot-and-mouth disease, avian influenza, and African swine fever can lead to decreased livestock productivity, loss of animal assets, and reduced market value of animal products (Perry et al., 2020). The direct costs of disease control measures, veterinary treatments, and animal disposal further exacerbate economic losses, placing financial burdens on affected farmers and communities. Moreover, trade restrictions, import bans, and market disruptions resulting from disease outbreaks can have long-term impacts on livestock markets, supply chains, and export revenues, affecting the livelihoods of stakeholders along the entire value chain (Thompson, 2020)

One of the primary socio-economic benefits of veterinary vaccination is the enhancement of animal health and welfare. Vaccination plays a crucial role in preventing infectious diseases, reducing morbidity and mortality rates, and improving overall animal well-being (Roche et al., 2018). By reducing the incidence and severity of diseases such as foot-and-mouth disease, brucellosis, and rabies, vaccination contributes to healthier and more resilient animal populations, thereby safeguarding farmer's livelihoods and food security. (Dórea, and Erdenesan, 2020).

Disease outbreaks can also disrupt social cohesion, community resilience, and livelihood diversification strategies in rural areas. Livestock farming often forms the backbone of rural economies, providing income, employment, and social capital for communities (Grace et al., 2017). Disease outbreaks can undermine the social fabric of rural communities by causing social stigma, isolation, and mistrust among livestock owners. Moreover, livelihood diversification options, such as off-farm employment and non-agricultural activities, may be limited in rural areas heavily dependent on livestock production, exacerbating vulnerability to economic shocks and reducing adaptive capacity. Strengthening community resilience, social networks, and livelihood diversification strategies is essential for mitigating the socio-economic impacts of disease outbreaks and enhancing rural livelihoods. (Mphande, 2016).

Disease outbreaks in livestock populations can also have implications for food security and nutrition, particularly in low-income and food-insecure communities. Livestock products such as meat, milk, and eggs are important sources of protein, essential nutrients, and income for millions of people worldwide (FAO, 2020). Disease-related disruptions in livestock production can lead to food shortages, price volatility, and reduced access to nutritious foods, particularly for vulnerable populations. Moreover, the loss of livestock assets and income-generating opportunities can perpetuate cycles of poverty and food insecurity, exacerbating malnutrition and health disparities. Strengthening disease surveillance, early warning systems, and veterinary healthcare services particularly timely vaccination and immunization is critical for safeguarding food security, improving nutrition outcomes, and enhancing resilience to disease outbreaks in livestock-dependent communities. (Kappes et al., 2023).

Veterinary vaccination can also lead to increased productivity and economic gains for farmers and livestock producers. Disease outbreaks can have significant economic consequences, including loss of livestock, reduced productivity, and trade restrictions (Fasina et al., 2020). Vaccination programs that effectively control and prevent diseases can mitigate these economic losses, increase livestock productivity, and enhance market access for animal products. Furthermore, by reducing the need for costly disease treatments and veterinary interventions, vaccination can contribute to cost savings and improved profitability for farmers (Fasina et al., 2020).

The socio-economic benefits of veterinary vaccination extend beyond animal health and agricultural productivity to public health. Many zoonotic diseases, such as rabies, brucellosis, and avian influenza, pose risks to human health and can have significant socio-economic impacts on communities (Dórea and Erdensan, 2020). Vaccination programs that target zoonotic diseases not only protect animal populations but also reduce the risk of disease transmission to humans, thereby safeguarding public health, reducing healthcare costs, and preventing productivity losses associated with human illness (Carpenter et al., 2022).

One of the primary economic benefits of preventive vaccination is the reduction in disease treatment costs. Vaccination prevents the onset of infectious diseases or reduces their severity, thereby minimizing the need for expensive treatments, veterinary interventions, and supportive care (Knight-Jones et al., 2016). For example, vaccination against diseases such as foot-and-mouth disease and brucellosis can significantly reduce the incidence of clinical cases, hospitalizations, and medical expenses associated with disease treatment. By preventing disease outbreaks and reducing the burden on veterinary healthcare systems, preventive vaccination contributes to cost savings for farmers, governments, and society at large. (Kahan et al., 2002).

Preventive vaccination plays a crucial role in mitigating trade barriers and enhancing market access for animal products. Disease outbreaks can trigger trade restrictions, import bans, and quarantine measures, disrupting livestock trade and international markets (Rich and Epinosa, 2018). Vaccination programs that demonstrate disease control and surveillance can provide assurances of animal health and safety, thereby facilitating trade agreements and market access for vaccinated animals and products. By reducing the risk of disease transmission and ensuring compliance with international health and safety standards, preventive vaccination promotes stable and profitable livestock trade, benefiting exporters, importers, and consumers alike. (Chambers et al., 2016).

Investments in preventive vaccination contribute to long-term economic sustainability in animal health and agriculture. While upfront costs associated with vaccination programs may require financial resources, the long-term

benefits in terms of disease prevention, productivity gains, and market opportunities outweigh the initial investment (Knight-Jones and Didero, 2019). Moreover, preventive vaccination reduces the economic burden of disease outbreaks, emergency responses, and post-outbreak recovery efforts, thereby enhancing resilience and sustainability in agricultural systems. By prioritizing preventive vaccination as a cost-effective and proactive measure, stakeholders can promote economic stability, resilience, and prosperity in animal agriculture. (Knight-Jones and Didero, 2019).

Social Landscape of Veterinary Vaccination and Immunization

The social landscape of veterinary vaccination is multifaceted, influenced by various factors such as public perception, economic considerations, government policies, cultural attitudes towards animals and healthcare and challenges faced by the livestock keepers in the access and utilization of vaccinations.

Socio-Cultural Dynamics

Socio-cultural factors exert a significant influence on attitudes and behaviors towards veterinary vaccination and immunization. Public perceptions and attitude towards animal health, cultural beliefs and social norms about animal care, religious perspectives on animal welfare, and traditional healing practices shape perceptions of veterinary medicine and vaccination among communities. In rural areas, where traditional healing practices are prevalent, there may be skepticism or resistance towards Western veterinary medicine and vaccination (Khan et al., 2019). Understanding and addressing cultural barriers to vaccination acceptance require culturally sensitive approaches, community engagement, and collaboration with local leaders and stakeholders.

Cultural beliefs about animal health, the role of veterinarians, and the importance of vaccination may vary across different communities and populations. For example, in some cultures, animals are regarded as family members, and decisions about their healthcare may be influenced by emotional attachments and cultural values. Understanding and respecting cultural norms and beliefs are essential for effectively engaging communities and promoting vaccine acceptance. Tailoring communication strategies, providing culturally sensitive education, and addressing community concerns are key strategies for navigating socio-cultural influences on vaccination behavior (Fernández-Rivas et al., 2017). Among the most influential socio-cultural factors influencing the effective use of vaccination and immunization are discussed below:

Public Understanding of Vaccination in veterinary medicine is pivotal for the success of disease control efforts, animal welfare, and public health. The perception of vaccination benefits significantly shapes public understanding and acceptance of veterinary vaccination. Studies have shown that the public recognizes the importance of vaccination in preventing infectious diseases, reducing disease transmission, and protecting animal health (Schwarz and Hartmann, 2018). Public understanding of vaccination is influenced by awareness of disease risks and outbreaks in animal populations. High-profile disease outbreaks, such as avian influenza, foot-and-mouth disease, and rabies, raise public awareness of the importance of vaccination in controlling infectious diseases and mitigating public health threats (Conan et al., 2020). Media coverage, public health campaigns, and educational initiatives play crucial roles in disseminating information about disease risks, vaccination strategies, and the role of vaccination in disease prevention. Increasing public awareness of disease threats and the benefits of vaccination is essential for fostering informed decision-making and promoting vaccine acceptance (Robi et al., 2024).

Public perception of vaccine efficacy and safety significantly influences attitudes towards veterinary vaccination. Studies have shown that concerns about vaccine effectiveness, potential side effects, and long-term health impacts can contribute to vaccine hesitancy among animal owners (Leibler et al., 2020). Misconceptions about vaccine ingredients, administration protocols, and adverse reactions may undermine confidence in vaccination programs, leading to suboptimal vaccine coverage and increased disease risks. Addressing public concerns and providing accurate information about vaccine safety and efficacy are essential for building trust and confidence in veterinary vaccination (Day, 2017).

Trust in veterinary authorities and professionals plays a critical role in shaping public attitudes towards vaccination programs. Studies have demonstrated that perceptions of competence, transparency, and communication effectiveness influence trust in veterinary authorities and professionals (Liu and Yanyong, 2018). Positive experiences with veterinarians, clear communication about vaccination benefits, and responsiveness to public concerns can enhance trust and promote vaccine acceptance. Conversely, perceived conflicts of interest, inadequate communication, and lack of transparency may erode trust and undermine vaccination efforts (Robi et al., 2024).

Vaccination acceptance is again affected by various cultural and social factors and understanding these factors is crucial for designing effective vaccination programs, promoting public health, and controlling infectious diseases (Hopker et al., 2021).

Cultural beliefs and practices play a significant role in shaping attitudes towards vaccination acceptance. Cultural norms, values, and traditions influence perceptions of health, illness, and medical interventions within communities (Dubé et al., 2013). For example, in some cultures, there may be beliefs about the efficacy of traditional healing practices or skepticism towards modern medicine, including vaccination. Understanding and respecting cultural diversity is essential for engaging communities and promoting vaccine acceptance. Culturally sensitive communication strategies, community engagement approaches, and partnerships with local leaders are critical for addressing cultural barriers and fostering trust in vaccination programs (Dubé et al., 2013).

Religious beliefs and teachings also impact vaccination acceptance within religious communities. Religious leaders and institutions may play influential roles in shaping attitudes towards vaccination and health-seeking behaviors among their followers (Alsan et al., 2021). For example, religious objections to certain vaccine ingredients or vaccination practices may lead to vaccine hesitancy or refusal among adherents. Engaging religious leaders, providing religiously sensitive education, and addressing misconceptions about vaccination within religious contexts are essential for promoting vaccine acceptance and addressing religious barriers to vaccination (Trangerud, 2023).

Social norms and peer influences exert considerable influence on vaccination acceptance within communities. Peer networks, social circles, and community norms shape individuals' perceptions of vaccination risks and benefits (Yaqub et al., 2014). In some communities, vaccine acceptance may be influenced by social pressure to conform to prevailing norms or by the attitudes and behaviors of trusted peers and influencers. Harnessing social networks, mobilizing social support, and leveraging peer influence are strategies for promoting positive attitudes towards vaccination and fostering vaccine acceptance within communities (Acosta et al. 2022).

Trust in healthcare providers and an institution is again a critical determinant of vaccination acceptance. Patients' trust in healthcare providers, including doctors, extension officers and veterinarians, influences their willingness to accept vaccination recommendations and adhere to vaccination schedules Trust is built on factors such as perceived competence, honesty, empathy, and communication effectiveness. Positive patient-provider relationships, clear communication about vaccination benefits and risks, and transparency in vaccine policy and practice are essential for fostering trust and promoting vaccine acceptance among individuals and communities (Alsan et al., 2021).

Economic Determinants

Economic factors play a critical role in determining adoption and utilization to veterinary vaccination and immunization services. Poverty, limited access to healthcare services, and financial constraints may pose barriers in accessing veterinary care, including vaccination services, particularly among rural and marginalized communities (Rahman and Ali, 2020). Additionally, the cost of veterinary services and vaccines may be prohibitive for low-income households, leading to underutilization of vaccination services and heightened vulnerability to disease outbreaks. Strengthening veterinary infrastructure, implementing subsidized vaccination programs, and expanding access to veterinary services are essential for improving vaccination coverage and disease control efforts (Acosta et al. 2022).

Institutional Dynamics

Institutional factors, including government policies, regulatory frameworks, and veterinary healthcare systems, shape the delivery and accessibility of veterinary vaccination and immunization services. Inadequate veterinary infrastructure, weak regulatory enforcement, and limited capacity for disease surveillance and response may hinder vaccination efforts and disease control measures (Shah and Samad, 2018). Strengthening institutional capacity, investing in veterinary workforce development, and implementing evidence-based policies are critical for enhancing vaccination coverage and promoting animal health in Pakistan (Afzal, 2009).

Challenges in Veterinary Vaccination and Immunization

Despite the importance of public understanding of vaccination, challenges exist in effectively communicating vaccine-related information to diverse audiences. Misinformation, misconceptions, and vaccine hesitancy may undermine vaccination efforts and erode public trust in veterinary authorities and professionals (Boutron et al., 2018). Addressing these challenges requires tailored communication strategies, accurate information dissemination, and proactive engagement with communities.

Vaccine hesitancy and misconceptions have significant implications for animal health, disease control, and public health. Suboptimal vaccine coverage rates can lead to increased disease prevalence, transmission, and severity, posing risks to animal populations and human health (Singer et al., 2019). Disease outbreaks resulting from vaccine hesitancy can have devastating consequences for animal welfare, productivity, and economic stability. Misconceptions about vaccines and vaccination are prevalent among animal owners and stakeholders in animal health. These misconceptions may stem from various sources, including misinformation, lack of education, cultural beliefs, and previous negative experiences with vaccination (Burns et al., 2018). Common misconceptions include concerns about vaccine safety, efficacy, necessity, and perceived risks of adverse reactions. These misconceptions can contribute to vaccine hesitancy, reluctance to vaccinate animals, and suboptimal vaccine coverage rates, thereby increasing the risk of disease outbreaks and compromising animal health and welfare (Leibler et al., 2020).

Several factors contribute to vaccine hesitancy in animal health, including individual, social, and systemic factors. Individual factors may include fear of adverse reactions, distrust in vaccines and veterinary professionals, and reliance on alternative healthcare practices (Bögel et al., 2020). Social influences, such as peer pressure, social norms, and misinformation spread through social networks and online platforms, can also shape vaccine attitudes and behaviors. Systemic factors, such as limited access to veterinary services, financial constraints, and regulatory barriers, may further exacerbate vaccine hesitancy and impede vaccination efforts (Hassan et al., 2021).

Addressing vaccine hesitancy requires multifaceted strategies that target individual, social, and systemic factors. Education and communication campaigns aimed at dispelling misconceptions, providing accurate vaccine information, and

addressing public concerns are essential for promoting vaccine acceptance (Mort et al., 2021). Building trust in veterinary professionals, enhancing access to veterinary services, and addressing socio-economic barriers to vaccination can also improve vaccine uptake rates. Additionally, fostering partnerships with communities, engaging with stakeholders, and leveraging social networks are critical for addressing vaccine hesitancy and promoting positive vaccine attitudes and behaviors (Alsan et al., 2021).

Access to veterinary vaccination services is essential for promoting animal health, preventing disease outbreaks, and ensuring food security in livestock-dependent communities. Access to veterinary vaccination services can have implications for equity and livelihoods, particularly in resource-limited settings. In many low-income and rural communities, access to veterinary services, including vaccination, may be limited due to factors such as geographical remoteness, financial constraints, and inadequate infrastructure (Kitala et al., 2014).

Access to veterinary vaccination services is often influenced by social determinants such as income, education, gender, and ethnicity, leading to inequities in access within and between communities (Wieland et al., 2017). Vulnerable populations, including women, Indigenous communities, and ethnic minorities, may face additional barriers to accessing vaccination services due to social, cultural, and economic factors. Addressing inequities in access requires a holistic approach that considers the social determinants of health, promotes inclusive policies and programs, and engages with marginalized communities to understand their specific needs and priorities. By addressing systemic barriers and promoting equity in access to veterinary vaccination services, stakeholders can ensure that all livestock owners have the opportunity to protect their animals' health and livelihoods (Acosta et al. 2022; Robi et al., 2024).

One of the primary challenges to access to veterinary vaccination services is geographical barriers, particularly in remote and underserved areas. Rural communities often face limited access to veterinary clinics, extension services, and vaccination campaigns due to factors such as geographical remoteness, rugged terrain, and inadequate infrastructure (Grace et al., 2018). As a result, livestock owners in these areas may struggle to access essential veterinary services, including vaccination, leading to gaps in disease control efforts and increased risks of disease outbreaks. Addressing geographical barriers requires innovative approaches, such as mobile veterinary clinics, community-based vaccination programs, and outreach initiatives that bring vaccination services closer to remote communities (Grace et al., 2018).

Affordability issues and financial constraints also pose significant barriers to access to veterinary vaccination services, particularly for small-scale farmers and marginalized communities. The costs associated with veterinary consultations, vaccines, and transportation to veterinary clinics can be prohibitive for resource-limited households (Catley et al., 2012). To address affordability challenges, stakeholders must explore strategies such as subsidized vaccination programs, community financing mechanisms, and innovative payment models that make vaccination services more accessible and affordable for vulnerable populations.

A lack of awareness and education about the importance of vaccination and veterinary healthcare can also hinder access to vaccination services in some communities. Limited knowledge about disease prevention, vaccine efficacy, and the benefits of vaccination may contribute to low demand for veterinary services and underutilization of vaccination programs (Kitala et al., 2014). Additionally, misconceptions, cultural beliefs, and language barriers may further hinder communication and engagement with veterinary professionals. Increasing awareness and education about vaccination through targeted communication campaigns, community outreach initiatives, and capacity-building programs for livestock owners are essential for improving access to veterinary vaccination services and promoting disease control in vulnerable populations (Dubé et al., 2013; Fernández-Rivas et al., 2017; Acosta et al. 2022).

Gender Role in Veterinary Vaccination and Immunization

Women play an active role in managing and caring for livestock by participating in different activities like milking dairy animals, cutting fodder, looking after the health of the herd, marketing livestock, processing animal products, and cleaning sheds. Rural women are more involved in livestock management activities; however, women livestock keepers face many complex challenges due to limited resources. Women livestock keepers have limited access to veterinary services, education, and training about animal diseases and veterinary care due to factors such as cultural barriers (Gizaw at el., 2021). Women in rural areas have limited access to income resources for disease management practices such as vaccination, medicine, and proper housing for their animals. They have less decision-making power to manage livestock as compared to men. Cultural and traditional animal disease management practices may hinder the adoption of new veterinary techniques and technologies because women livestock keepers rely on traditional or herbal remedies. Women livestock keepers have a number of responsibilities, including childcare, household chores, and other agricultural activities, which leave them little time for animal disease management practices. The other environmental factors, such as climate change, increase the spread of certain types of diseases, such as fever, foot, and mouth diseases (Vijayalakshmy et al., 2023).

Women livestock keepers are actively engaged in managing animal’s health; and they are also more susceptible to zoonotic diseases. Every year, 2.2 million people die as a result of zoonotic diseases, with the majority of victim coming from developing countries (Amanat et al., 2015). There are a number of zoonotic diseases in the form of germs and viruses that affect the health of people associated with its management. Women play a significant role in livestock management, which can lead to the transmission of zoonotic diseases. So, it is important to implement measures to protect women involved in livestock management from these diseases (Koyun et al., 2023).

Women livestock keepers are considered as unpaid laborers with no policies addressing their needs, minimal organizations providing credit, and insufficient extension services available for their benefit. Women's empowerment in animal disease management practices demands for women livestock keepers to be involved in decision making processes regarding animal disease management. Given access to knowledge about empowerment, ensure that women have access to the information they need about animal care, disease prevention, and management (Saini and Saini., 2021).

Conclusion

In conclusion, navigating the social landscape of veterinary vaccination and immunization requires a multifaceted approach that acknowledges the diverse perspectives, beliefs, and values held by stakeholders. Effective communication, community engagement, and collaboration among veterinary professionals, policymakers, researchers, and the public are essential for promoting understanding, trust, and compliance with vaccination programs. By recognizing the interconnectedness of human, animal, and environmental health, we can work together to mitigate the spread of diseases, protect vulnerable populations, and ensure the well-being of both animals and humans in our shared ecosystems. As we move forward, let us remain vigilant in our commitment to evidence-based practices, ethical considerations, and inclusivity, striving toward a future where the benefits of vaccination are accessible and appreciated by all.

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Chapter 14

Immunization Innovation: The Future of Veterinary Vaccines

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ABSTRACT

The chapter covers animal immunization and healthcare developments. Eight new vaccine concepts and technologies should boost efficacy, safety, and advantages. From traditional to infectious disease prevention in animal conservation. Animal disease susceptibility varies. Sharing innovative vaccine approaches' molecular processes was popular. Gene and molecular changes may impact animal vaccinations. Some methods use recombinant DNA to create vaccination vectors. The evolution and distribution of genomic vaccination antigen are examined here. To improve vaccination and immunology, precision medicine could analyze these achievements. These new injection methods' practical effects. "One Health.", food safety, and infection prevention have improved animal health worldwide. Chapter covers veterinary vaccination applications, technologies, and research. Scientists, veterinarians, and policymakers use this technique to anticipate industrial futures. The rapid development of animal vaccines is described in "New Vaccines: The Future of Veterinary Vaccines". Many animal species will benefit from immunization.

KEYWORDS
Immunization, Veterinary Vaccine, Recombinants DNA, Genomic vaccination, Health care.

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INTRODUCTION

The use of vaccinations in veterinary medicine has undergone a remarkable transformation, transitioning from an experimental endeavor to a common and substantially risk-free practice. A number of infectious illnesses which have no vaccine right now which can cause many consequences through worldwide (McVey and Shi, 2010). Investment in RandD will continue to address these difficulties and bring novel vaccines to market, all with the goal of enhancing animal health and welfare (Roth and Sandbulte, 2021). Veterinary vaccines are becoming more popular nowadays because of developed analytical methodologies and cutting-edge technological tools to work in a better way (Meeusen et al., 2007). At a time when regulatory bodies are placing a premium on the accessibility of veterinary vaccinations and when new technology has the power to curb disease epidemics, the timing could not be better for the creation of improved veterinary vaccines (Pollard and Bijker, 2021).

The outcomes of the advanced technologies in veterinary vaccine development are the potential impact of messenger RNA (mRNA) vaccines on animal care and its application in immune responses (Entrican and Francis, 2022). These innovations are being developed with the hopes of bettering the lives of companion animals, making livestock production more efficient and affordable, and stopping the spread of diseases from animals to humans because of rise in medication resistance among pathogens have both played a role in propelling the veterinary vaccine business to new heights of activity (Thomas et al., 2022).

Background of Veterinary Immunization

Vaginal vaccinations, which were formerly considered a daring experiment, are now standard procedure in medicine

for animals. The development of vaccine for animal health and welfare to reduce the incidence and various illnesses (McVey and Shi, 2010).

Veterinarians administered vaccines to protect animals against a variety of infectious infections because of antibiotic-resistant microorganisms and appearance of hitherto unknown illnesses, rise the veterinary vaccines demand. Aida et al., (2021) found that vaccines made from DNA or RNA are an economical and safe option. New technological developments in vaccine development have allowed the veterinary vaccine business to continue growing despite these constraints (Thomas et al., 2022).

Importance of Immunization Innovation

Many factors demands the advancement in the field of veterinary vaccine production to improve animal health and welfare also to reduce the illnesses in animal-human transmission against many pathogens (Sander et al., 2020). Advance technologies such as recombinant viral-vector vaccines, DNA, and RNA have shown the capacity to stimulate cellular and humoral immune responses in a safe and cost-effective way (Jorge and Dellagostin, 2017).

Evolution of Veterinary Vaccines

A wide rang infectious illnesses caused by microorganism (bacteria, protozoa, viruses) have been successfully combated by vaccines originally developed for use in veterinary medicine which is promising new area of study to a common, mostly safe practice (Gutiérrez et al., 2012). Several factors including new diseases, and the rising prevalence of infections that are resistant to therapy have led to the veterinary vaccine market's consistent growth, including improvements in vaccine technology (Yıldız, 2021).

Conventional Approaches to Vaccination

In veterinary vaccinations subunit antigens, vectors, and live or inactivated viruses are commonly used to improve and prevent the transmission of illnesses from animals to people. These traditional ways of doing things first came about because of empirical trial and error methods that mimicked the immune system (Tizzard, 2021).

Shift towards Modern Vaccine Development

With an advancement in technologies thanks to genomics, which enables sequence-based immunizations via high-throughput in silico screening of the whole genome, this transformation has been expedited even further. As a result of ground-breaking efforts in vaccine research, veterinary science has given rise to third-generation vaccinations, including DNA, RNA, and recombinant viral-vector vaccines (Liljeroos et al., 2015). Vaccines are cost-effective, safe, and effective in inducing humoral and cellular immune responses, and they can differentiate between diseased and vaccinated animals. UK Centre for Veterinary Vaccine Innovation and Manufacturing (CVIM) has also achieved significant results in its first year of operation, demonstrating the value of interdisciplinary collaborations in enhancing the creation of veterinary vaccines (Entrican et al., 2020).

Disease Susceptibility Across Animal Species

Many factors like habitat, evolutionary history, and geography influence how susceptible an animal is to infectious diseases (Robles-Fernández et al., 2022). The dispersion patterns, mating systems, group sizes, sorts of physical contacts, and frequency that group-living animals exhibit may impact disease susceptibility and transmission. Animal models have shown that SARS-CoV-2 has evolved continuously and may be transmitted by a variety of animals, including cats, ferrets, hamsters, house mice, Egyptian fruit bats, deer mice, and white-tailed deer. Among domesticated animals, American minks have the highest risk of contracting and transmitting the virus from humans or other animals. Proper disposal of human waste and animals that are ill or dead should not be handled by humans at any costs (Kappeler et al., 2015).

Differences in Disease Susceptibility

In the field of veterinary medicine many factors including geographical, environmental, and phylogenetic characteristics, affect the infectious disease susceptibility of animal species. To choose the best medications to treat bacterial illnesses, availability of standardized diagnostic procedures, antimicrobial susceptibility testing is a vital technique in veterinary medicine (Larsson and Flach, 2022). There have also been notable developments in animal vaccination research and development, with novel approaches to selectively inducing effective immune responses being used. The ever-changing nature of infections, the appearance of novel ailments, and the need to enhance the well-being and output of animals have all contributed to these advancements (Watts et al., 2018).

Effects on Animal Populations

Disease outbreaks which cause quick and devastating losses in animal populations, conservationists are worried about this. In addition to reducing population levels, diseases may impede recovery attempts for populations that are at danger of extinction and, via Allee effects and other processes, increase the likelihood of species extinction. The decrease or mass death events and species extinctions occurs due to the infectious diseases (Russell et al., 2020). Certain factors including environmental, phylogenetic, and geographical have role that how animals is susceptible in diseases transmission, and disease susceptibility and transmission effects many behaviors in animals. Increased investment in personal immunity may help reduce the greater per capita risk of infectious illnesses in sociable animals (Kappeler et al., 2015).

Advances in Vaccine Models and Technologies

Novel techniques vaccine responses are affordably made, safe to use and several other technologies have made more effective veterinary vaccine. Recombinant viral vector vaccines, DNA vaccines, and RNA vaccines all fall under the category of third-generation vaccinations (Pliasas et al., 2022). These include vector and nucleic acid vaccines; antigen subunit, protein, and peptide vaccines, attenuated/live and inactivated/killed vaccination strategies. Due to resistance in pathogens and advancements in vaccine production contributed in control of illness and rise in market demand (Francis, 2018). mRNA vaccine structure and action of mechanism is shown in Fig. 1.

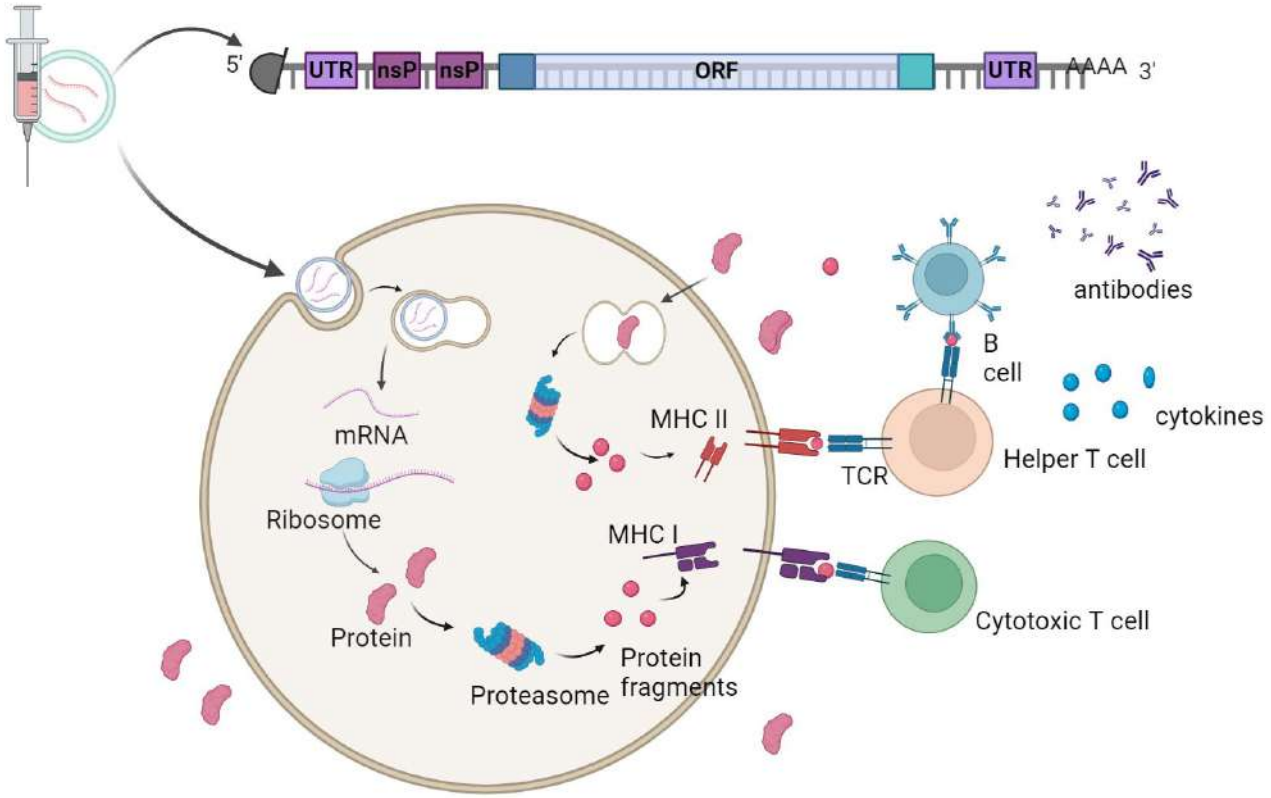

Fig. 1: mRNA vaccine structure and action of mechanism

Recombinant DNA Technology

Several animal infections have vaccines developed using recombinant DNA technology combine safety, potency, effectiveness, and purity, including rabies, canine distemper, avian influenza, Newcastle disease, Lyme disease, and pseudorabies. Recombinant vaccines have several genetic inserts works for many diseases, can even stay alive at room temperature. Recombinant DNA technology shows great promise for the treatment and prevention of animal illnesses and also in the development of effective vaccinations (Van Kampen, 2001). Recombinant DNA technology is shown in Fig. 2.

Advancement of Vaccination Vectors

The field of veterinary vaccinology has recently made strides in developing viral vector vaccine platforms for animals, including vectors that are either comparable to or identical to those used in human vaccines. Vaccines based on viral vectors have found widespread usage in the field of veterinary medicine, where they have effectively protected animals from several illnesses. The rapid use of viral vectors in animal medicine, such as adenovirus, herpesvirus, and poxviruses, rather than in human health, emphasizes the need of a 'One Health' approach to vaccine development. Due to the advanced technologies vaccine in the field of veterinary science are safe more effective (Baron et al., 2018). Vaccine vector use in Veterinary is shown in Fig. 3.

Exploring the Molecular Mechanisms of Recombinant Vaccines

Recombinant vaccinations in veterinary medicine has several advantages, like safer, more potent, and more effective than traditional methods, do not expose the vaccinated animal to the pathogen, no need for adjuvants and stay alive at room temperature (Schijns et al., 2021). Vaccines for a variety of animal illnesses have been developed using recombinant technology, avian flu, Lyme disease, pseudorabies, rabies, canine distemper, and Newcastle disease have been developed using recombinant DNA technology in veterinary medicine. Furthermore vaccine have been developed which are safer, more effective, more adaptable and enhance animal health against infections disease(Van Kampen, 2001).

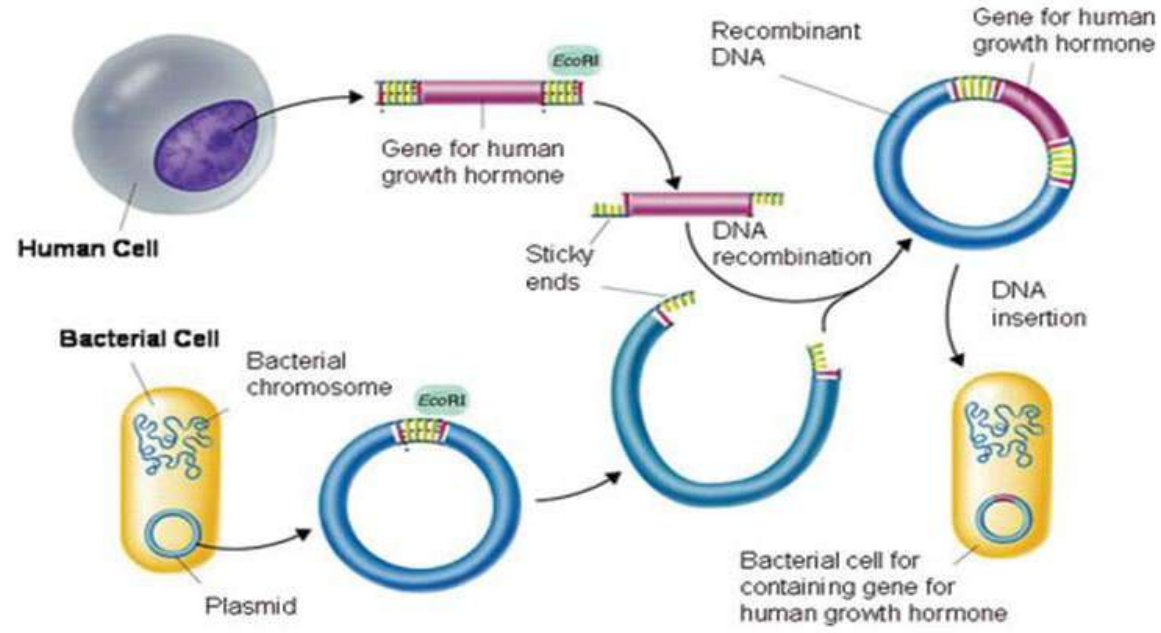

Fig. 2: Recombinant DNA technology

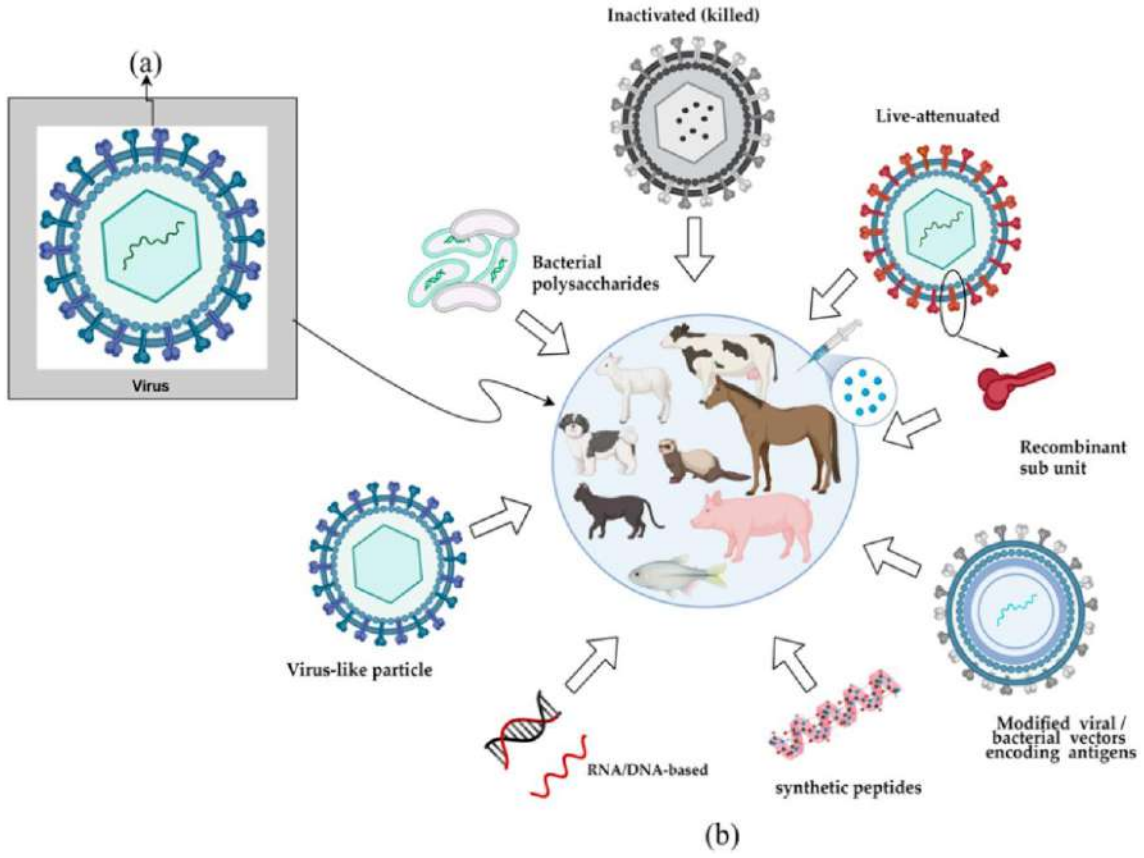

Fig. 3: Vaccine vector use in Veterinary

Genomic Vaccine Antigens

Using recombinant DNA technology animal genomic vaccine antigens were created which make novel genetic combinations by isolating and modifying DNA segments; this allows them to investigate transcripts, alter them precisely, and identify nucleotide sequences. The genomic method has opened the door for vaccinologists to the whole antigenic repertoire of infections, enabling them to create vaccines that specifically target antigens (Haynie, 2023). The development of vaccinations for many animal illnesses has made use of recombinant subunit vaccines, which use antigens to stimulate immune responses without the need of live viruses. Licensed vaccines against viruses, including avian flu, Lyme disease,

rabies, canine distemper, and pseudorabies have been developed in the field of veterinary medicine via the use of recombinant DNA technology. Furthermore vaccine have been developed which are safer, more effective, more adaptable and enhance animal health against infections disease(Francis, 2018).

Evolution and Distribution

Animal recombinant vector vaccine research and development must take evolutionary and dispersal factors into account. During the production process, the additional genes carried by recombinant vector vaccines which are live-replicating viruses may mutate to become less effective. While engineering designs can influence the development of attenuated vaccinations, the effects of evolution for recombinant vector vaccines have received less attention. Infectious diseases have restricted the capacity of many species to recover from reductions and have contributed to the quick and devastating decreases in animal populations that may occur during disease outbreaks. Both theoretical and practical research has shown that diseases, whether via Allee effects or some other mechanism, raise the danger of extinction for species. Theoretically, transmissible recombinant vector vaccines undergo ongoing evolution due to mutation and within-host evolution in large, diverse populations. Considering these factors, it is critical to track how vaccines are used and developed to make sure they are still effective in reducing the prevalence of animal illnesses (Nuismer et al., 2019).

Applications of Precision Medicine

The field of precision medicine is transforming the way animals are cared for by using genome sequencing and other cutting-edge technology to provide personalized diagnoses and treatments. With this method, vets may provide better diagnosis, tailored treatment programs, and better results for animals with the use of targeted medicines and customized tests. Precision medicine shows promise for a future when compassionate, tailored care for animals is the norm in veterinary practice by customizing therapies to each animal's own genetic and molecular composition, environment, and lifestyle characteristics. (Lloyd et al., 2016).

Novel Vaccine Formulations

Vaccine design, delivery, and administration is a part to improve novel vaccine formulations where they produced better safety, efficacy, and cost-effectiveness show better immunological activity. Methods for needle-free vaccination, controlled release profiles, and targeted delivery of antigenic material are being investigated in these formulations. These formulations play a significant role in Health approach in the veterinary setting, for the production safer and more effective alternatives, immunogenicity and protective effectiveness in people before it can be approved for use. The new technologies such as viral vector and nucleic acid vaccines, could transform the field of vaccine development by fixing the problems with older methods (Singh and Mehta, 2016).

Adjuvant Design Innovations

Nano adjuvants and new-generation adjuvant systems are the recent advancements in adjuvant design for animals that have focused on the development of novel adjuvants. Aim of these technologies to enhance the immune response to antigens, safety characteristics, enhanced immune stimulation ability and make vaccines more effective, also including designed based on existing drug delivery models and established pharmaceutical principles, are being developed to serve as immune stimulators or immune potentiators, and are under clinical trials (Moni et al., 2023).

Antigen Delivery Systems

To improve animals' immune responses, researchers around veterinary vaccine development have recently concentrated on different antigen delivery mechanisms. Vectors produced from animal adenoviruses are one example of a viral vector-based delivery method that has been tested for the administration of vaccinations to veterinary animals by intravenous injection. Furthermore, research into nanotechnology-based delivery methods, such as nanoparticles (NPs), has shown promise for improving immune response activation in farm animals when administered nasally or via the lungs. New antigen delivery technologies show potential for better animal disease prevention and more effective veterinary vaccinations (Calderon-Nieva et al., 2017).

Molecular and Genetic Influences on Animal Immunizations

Advance technologies shows a better role in the veterinary field vaccine production such as recombinant viral-vector vaccines, DNA, and RNA which should be more safer, more effective, disease preventive and more broadly applicable. DNA vaccines are appealing for cattle because they allow for the simultaneous mixing of diverse genes, which allows to produce vaccinations against numerous strains of a disease. The combination of humoral and cellular immune responses elicited by these vaccinations allows animals to mount a more effective defense against real diseases (Redding and Weiner, 2009).

Favorable Effects of Molecular and Genetic Modifications

Immunizations of animals, especially those using DNA vaccines developed for veterinary use, have benefited from molecular and genetic changes. Vaccine cocktails are a great alternative for cattle, and DNA vaccines make it possible to combine numerous genes at once, protecting them against various disease strains. After vaccinations animals fight against

diseases because they stimulate both the humoral and cellular immune systems. Furthermore, more efficient vaccinations use of genetic engineering which has enabled the production of genes from pathogens in microbial cells(Bull et al., 2019).

Prospects and Obstacles in the Future

Due to zootoinc infection veterinary vaccination industry is expected to develop significantly in the next five years. Global market size was USD million in 2022 and is predicted to reach USD million by 2028, growing at a CAGR. Precision medicine in veterinary care, which tailors' diagnostics and treatments to individual genetic and molecular makeup, is expected to save lives, reduce healthcare costs, and improve animal health (Priya and Kappalli, 2022).

To maximize its potential, precision medicine in animals may need a coordinated approach that integrates public health and wellness efforts. To adopt these animal healthcare advances, these hurdles must be carefully addressed (Matić and Šantak, 2022).

Practical Implications of Innovative Injection Methods

Innovative veterinary injectable procedures like needle-free injection have practical applications. These immunization approaches may improve animal welfare and production by reducing pain and stress. They remove unwanted needles and bits from carcasses, decreasing food safety and worker injury. Needle-free injection devices can provide more uniform vaccination and medication administration, which may improve treatment efficacy. Deleting damaged needles, improving worker safety, and avoiding inadvertent self-injections may also boost efficiency. Novel injection techniques in veterinary medicine might improve animal comfort, food safety, and vaccination and medication administration (Grubb and Lobprise, 2020).

Applications in Veterinary Practice

The production of advance vaccines through genetic engineering which should now safer, more effective and disease preventive in veterinary practice have noted recent years. There is hope that precision medicine's implementation in veterinary care, which entails customizing tests and treatments according to each patient's unique genetic and molecular composition, will help save lives, lower healthcare costs, and enhance animals' quality of life. These developments in the use of vaccines in veterinary medicine show great potential for bettering animal health, guaranteeing the safety of food, and reducing the prevalence of animal diseases (Day et al., 2016).

Factors to Consider for Successful Implementation

Several factors need to be considered for the successful implementation of vaccination in animals. These factors include:

Tailored Vaccination Programs

Considerations including age, lifestyle, and disease concerns should be part of a personalized vaccination regimen for each animal or livestock enterprise.

Standardization of Veterinary Practices

Research, development, field testing, licensing, production, quality control, regulation, purchasing, delivery, and administration must precede vaccine administration. Veterinary methods must be standardized to assure vaccination administration quality.

Comprehensive Planning

Successful immunization deployment requires thorough preparation. To manage and connect the vaccination process with the organization's objectives, it requires extensive planning, resource allocation, and risk assessment.

Adequate Resources

Successful vaccine implementation requires enough financial, human, and technical resources. This provides the assistance and infrastructure needed to implement the immunization strategy.

Effective Communication

Addressing difficulties, managing expectations, and keeping everyone informed and involved throughout the immunization process requires open and efficient communication among all stakeholders.

Continuous Monitoring and Evaluation

It is crucial to continuously monitor and evaluate the immunization process to spot problems, measure progress, and make necessary modifications as needed. This aids in keeping the immunization on track and ensuring it produces the desired results. Alignment of the vaccine with organizational strategy and structure, employee engagement, strong leadership and support from executives, and an effective plan for managing change are other essential success factors for successful vaccination implementation. Vaccination in animal healthcare has been widely used and integrated due to these causes.

Global Impact on Animal Health

Through vaccine development the animal and human health is improved. Vaccine in the livestock field reduced the losses due to illness, decrease in emissions of greenhouse gases and boost economy worldwide. 60% worldwide vaccination rate for beef cattle was connected to a 52.6% boost in production, also there is hope that this productivity boost can help end world hunger and food insecurity. Reducing antibiotic usage and preventing zoonotic illnesses are two ways in which veterinary vaccinations significantly affect public health.

Initiatives for Ensuring Food Safety

The safety of food and the well-being of animals are two areas where vaccines are vital. They help save lives by reducing antibiotic overuse and controlling zoonotic infections. Animal health and wellbeing rely heavily on vaccination, which has a long history of successfully reducing disease load in both domestic and agricultural animals. Worldwide, the use of livestock vaccinations has decreased losses, help the environment ecofriendly and also essential resource for future sustainability, food safety, and animal welfare.

Strategies for Preventing Infections- Advocating for One Health

The development of recombinant viral-vector vaccines, DNA, and RNA has allowed for more effective and flexible methods of preventing infectious illnesses in animals by inducing both humoral and cellular immune responses. (Brisse et al., 2020).

With the One Health approach highlighting the interdependence of human, animal, and environmental health, the application of these innovative vaccination technologies in veterinary medicine is in line with the goal of promoting One Health and avoiding diseases. These innovations benefit to improve animal health and welfare, prevalence of zoonotic infections and decreasing antibiotic use (Ribeiro et al., 2019).

To promote animal health and control of zootoinc infection it’s necessary to develop a novel vaccine in the field veterinary.

Applications, Technologies, and Research in Veterinary Vaccination

Alternatives to conventional vaccination methods that are less invasive, more effective, and simpler to give have emerged because of recent developments in veterinary vaccine technology. That’s include DNA, RNA, and recombinant viral-vector vaccines have also contributed to these developments, as have the tactics of inactivated/killed and attenuated/live immunization. Animals may soon have access to more effective and flexible choices for disease prevention thanks to third-generation vaccinations that may stimulate cellular as well as humoral immune responses. (Mitragotri, 2005).

As a result of these technical advancements, Vaccines that effectively combat various pathogens, including as viruses, bacteria, protozoa, and multicellular infections, have been developed. Because of platform technologies, which have helped researchers better understand vaccines production and development to control infectious diseases in animals (Victor, 2023).

Advance technologies have been developed the veterinary vaccination which are more effective, safe against the infectious diseases’ threats, which also prevent animal welfare, agricultural output, and public health from consequences.

Present State of Veterinary Vaccination

The development of more safe and more effective vaccine against a broad variety of pathogens through advanced technologies, such recombinant viral-vector vaccines, DNA, and RNA. In addition, new illnesses are constantly emerging and drug-resistant organisms are constantly evolving, which has increased the need for technical advancements in vaccine creation. In general, new vaccine technologies have emerged because of scientific and technical developments in veterinary vaccination; these alternatives for protecting animals against infectious illnesses are safer, more effective, and more flexible than before. (Le et al., 2022).

Upcoming Technologies and Continuing Research

New technologies, including DNA, RNA, and recombinant viral-vector vaccines have also contributed to these developments, as have the tactics of inactivated/killed and attenuated/live immunization. Animals may soon have access to more effective and flexible choices for disease prevention thanks to third-generation vaccinations that may stimulate cellular as well as humoral immune responses. As a result of these technical advancements, the veterinary vaccine industry has been seeing steady expansion. Vaccines that effectively combat various pathogens, including as viruses, bacteria, protozoa, and multicellular infections, have been developed. The future of animal health and disease prevention is bright, thanks to the revolutionary vaccines that will be developed because of continuing research and technology breakthroughs in veterinary vaccination (Vetter et al., 2018).

The Future Trajectory of the Veterinary Vaccination Industry

Technological developments, market tendencies, and continuing research are a few of the variables that will determine the veterinary vaccine industry's future course. There are now safer, more effective, and more adaptable ways to protect

animals from infectious illnesses because of recent developments in vaccine technology, including recombinant viral-vector vaccines, DNA, and RNA. More effective and flexible choices for disease prevention may be available with these third-generation vaccinations, which could stimulate both humoral and cellular immune responses. The worldwide Veterinary-Animal Vaccines Market is anticipated to reach USD 18.23 billion by 2030, at a CAGR of 6.9%, indicating a substantial expansion in the veterinary vaccine industry. Further, new vaccines will probably be developed in the future thanks to continuing research and technical advances in veterinary vaccination; this will help to enhance animal health and avoid diseases. In addition, a potential alternative to conventional vaccination platforms is the creation of mRNA vaccines against new zoonotic illnesses and animal infections, including foot-and-mouth disease and African swine fever. The One Health paradigm, which recognizes the interdependence of the health of humans, animals, and the environment, is consistent with these developments. An ever-increasing emphasis on new infectious disease concerns, a thriving market, and relentless innovation are the hallmarks of the veterinary vaccine industry's promising future (Danchuk et al., 2023).

Expected Innovations

Innovations and research endeavors are anticipated to influence the future of veterinary vaccinations. New features are anticipated to include:

mRNA Vaccines

In addition to finding new methods to spread current immunizations to other locations and species, these vaccines may also lead to the development of new vaccines against diseases that were previously expensive and fatal.

Custom or Autogenous Vaccines

The 'custom' or autogenous vaccine, made from a culture sample of a particular herd, is something that veterinarians may order to save healthy animals and other herds in the area.

Alternatives to Antibiotics

Researchers are trying to learn more about the immune system in cattle and come up with alternatives to antibiotics that work by targeting germs in the same manner.

Digital Technologies

The use of smart tags and other forms of digital monitoring is quickly revolutionizing the field of animal health, allowing for more efficient and faster treatment of individuals within groups.

Vaccine Platform Technologies

The use of platform technologies in veterinary vaccinology is an area of ongoing study that is vital to the production of vaccines for animals.

To guarantee the ongoing health and welfare of animals, it is crucial to address new infectious illnesses, zoonotic diseases, and other health concerns with these impending technologies.

Challenges and Opportunities

Future veterinary vaccinations bring problems and potential. New vaccination technologies, including DNA, RNA, and recombinant viral-vector vaccines are being developed to combat new infectious illnesses and speed up responses. Third-generation vaccines generate humoral and cellular immune responses, are safe, cheap, and can distinguish diseased from vaccinated animals. Vaccine advances, including mRNA vaccines, bespoke or autogenous vaccines, and antibiotic alternatives, are predicted to greatly improve animal health and disease management.

The Centre for Veterinary Vaccine Innovation and Manufacturing (CVIM) is vital to getting lab concepts into large-scale vaccine manufacturing. CVIM connects fundamental research and late-stage product development to support global goals, health security, and pandemic preparation. The Global Veterinary Vaccinology Research and Innovation Landscape Survey Report emphasizes vaccine platform research aims for vaccine development in veterinary vaccine research and innovation (Brun, 2016).

The future of veterinary vaccinations depends on new technology and overcoming problems like quick infectious disease response. This field's study and innovation might improve animal, food, and public health.

Conclusion

Challenges and possibilities are ahead for veterinary vaccinations in the future. Thrilling new developments are being funded by the animal health business, which will significantly influence economic growth, human well-being, and the status of animals. The successful development of vaccinations against many infections, including viruses, bacteria, protozoa, and multicellular organisms, has propelled the veterinary sector towards being at the forefront of technology adaptation and application. There must be a prompt response to the ever-increasing incidence of newly emerging infectious illnesses. There are several factors, including effectiveness, affordability, practicality, and licensing hurdles, that go into selecting and combining vaccine research choices. Research priorities concerning vaccine platforms for vaccine

development are highlighted in the Global Veterinary Vaccinology Research and Innovation Landscape Survey Report, which offers a foundation for future innovation and research initiatives in the veterinary vaccine sector. Animal welfare, food safety, and public wellness may all stand to benefit from the new developments and pioneering work in this area.

Summarizing Immunizations Innovations

Groundbreaking technology and a dedication to fair access will define the future of vaccination and vaccine innovation in both human and animal health. A major milestone in the field of human health is the successful drone delivery of ultra-cold mRNA COVID-19 vaccinations, demonstrating the promise of mRNA vaccines. In the field of veterinary medicine, advancements in vaccine development, such as messenger RNA (mRNA) vaccines, bespoke or autogenous vaccines, antimicrobial alternatives, and digital technologies, are anticipated to significantly influence animal welfare and illness prevention. The relevance of research objectives relating vaccine platforms for vaccine development is highlighted in the Global Veterinary Vaccinology Research and Innovation Landscape Survey Report, which offers a foundation for future research and innovation efforts around veterinary vaccines (Health et al., 2023). There are encouraging prospects to improve public health, food security, and animal health via the continuing research and innovation in this area. To combat new infectious illnesses, guarantee the health of the world's population, and reduce inequalities in the availability of vaccinations and medications, these developments are essential. There is hope for better health outcomes and disease prevention in the future of vaccination and vaccine development.

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Chapter 15

Understanding the Social and Economic Impact of Vaccines and Immunization Strategies against Emerging Diseases

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ABSTRACT

The development of secure and efficient vaccines targeting diseases that contribute to higher rates of illness and death stands out as a significant scientific progression in the 21st century. Vaccination coupled with clean drinking water and sanitation are unquestionably public health involvements which improve health outcomes worldwide. Each year, vaccines prevent the deaths of approximately 6 million people caused by preventable diseases. Nevertheless, not all newborns, kids, and adults around the world have equal access to vaccinations that protect them against potentially fatal diseases. Vaccination has a number of advantages for public health, particularly in preventing infectious disease and mortality. As doctors and biomedical scientists, we stress these advantages frequently. In 2055, there are expected to be 10 billion people on the planet, thanks in part to vaccines which help prevent diseases and make life expectancy rise. This book chapter focuses heavily on the advantages of vaccinations for society from the angles of health, social cohesion, economic importance, vaccine restrictions, as well as present issues and future directions for immunization.

KEYWORDS
Vaccine, Immunization, Human vaccine, Animals vaccine

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INTRODUCTION

Investing in health interventions, whether for non-human animals or humans, at the individual or collective level (such as a farm, region, or country), necessitates resource allocation decisions within a limited budget. In addition to directly affecting human health and welfare, vaccination against infectious diseases has improved animal health and reduced zoonotic disease outbreaks, thereby protecting food supplies. For example, childhood vaccinations avert over two million deaths annually, and vaccination rates are a reliable predictor of the prevalence of vaccine-preventable human diseases (such as polio, yellow fever, and measles) (Greenwood, 2014). Safe and effective immunizations against hazardous illnesses are one of the greatest achievements of the twenty-first century. Three public health initiatives that enhance health outcomes include vaccinations, sanitation, and safe drinking water. Furthermore, veterinary vaccinations can enhance the survival and productivity of food-producing animals, such as cattle and poultry, which will lead to a net increase in disposable household income and an increase in the quantity of protein-rich animal source foods available to the public (Roth, 2011; Marsh et al., 2016;). Despite these and other examples, it is rare, if ever, for vaccine developers to interact with policymakers about human or animal vaccines (Ehreth, 2003). It is predicted that there will be nearly 10 billion people on earth by 2055, according to the United Nations Department of Economic and Social Affairs (2019). A significant part of these achievements can be attributed to the development of highly effective vaccines that have reduced the risk of disease and increased the life expectancy across all continents. This chapter will concentrate on the advantages of vaccination to society from the viewpoints of health, economy, and social fabric for the sake of the overall assessment of impact. These

factors must be taken into account by those making financial decisions to make vaccinations a priority.

History of Vaccine Development

Vaccination has proven effective in treating both infectious and non-infectious disorders, significantly reducing fatalities. By the 20th century, numerous novel vaccinations have been developed for therapeutic and preventative purposes in medicine, demonstrating the effectiveness of vaccination in treating both infectious and non-infectious disorders (Charostad, et al., 2017). Among the largest advances throughout medical history, the application of vaccinations for the prevention and management of disease (Hilleman, 2000). Since vaccinations were developed, they have been viewed as an effective way to lower health and treatment expenses, and scientists are working to expand the range of illnesses that may be avoided by immunization (Ehreth, 2003) Individual immunization history began with the observations as well as experiences of people who lived in China and the Middle East throughout the 12th and 15th centuries (Hilleman 2000; Leung 2011). Patients diagnosed with smallpox were immunized using skin or pustule fluids to prevent recurrence. Although this method may protect against the disease, there is a high risk of disease manifestation and death, as the smallpox virus is used in the immunization procedure (Riedel, 2005). Edward Jenner's groundbreaking research on using cowpox virus for human injection marked the beginning of vaccine development. However, new vaccines were not released until the next century due to insufficient data and basic knowledge of microorganisms and microbiology. A new age in vaccination research began at the end of the 19th century with the rise of scientists like Louis Pasteur, Robert Koch, and Paul Ehrlich (Plotkin, 2011). During this period, numerous new vaccinations were developed against various infections, including tetanus, rabies, typhoid, diphtheria, tuberculosis, and pertussis, with extensive worldwide research conducted, resulting in their distribution until 1930 (Wever and Van Bergen, 2012; Rappuoli and Malito, 2014).

Health Benefits of Vaccines

Many illnesses that were formerly widespread have been virtually eliminated or much reduced in frequency because of vaccinations. But today, the public's attention is being drawn to the possible side effects of vaccinations (Kimmel and Wolfe, 2005). The global health organization (WHO) reported on the significant drop in mortality and illness complications following the introduction of vaccinations against infectious diseases, which are brought on by the stability of vaccines (Greenwood, 2014). According to records, vaccinations have been used in medical interventions to avoid 25 million fatalities between 2011 and 2020, 5 deaths each minute (Miller, 2021). When compared to ordinary chemical medications, vaccinations have several benefits in terms of long-term immunity, their ability to prevent and cure illnesses, and the absence of drug resistance (Kennedy and Read, 2017). Researchers are working to develop vaccines for various cancer kinds. Currently, vaccinations not only prevent and treat infectious diseases but also non-infectious diseases like cancer. Although there are some misunderstandings, the majority of parents favor immunizing their children (Hilton, 2005). Some parents think that receiving too many vaccinations—including those for varicella, pneumococcal conjugate, hepatitis B, inactivated poliovirus, measles, mumps, and rubella (MMR), and Haemophilus influenza Type B (Hib)—will impair immunity (Kimmel et al., 2007). Vaccines have the potential to prevent infections that put people at risk for severe illnesses, rather than impairing immunity. For instance, necrotizing group A b-hemolytic streptococcal fasciitis in children or pneumonia in adults are common complications of varicella (Gandhi, 2004). Regaining this lost faith may require better communication about the immediate advantages of vaccination as well as its benefits beyond avoiding infectious illnesses (Organization, 2017). Hepatitis B vaccination: Research indicates that this vaccine may lower the incidence of liver cancer, a serious worldwide health problem, even if its main benefit is protection against liver infections (Chang 2014).

Eradication of Infectious Diseases

Pathogens that are limited to human reservoirs can be eradicated globally. In order to avoid the spread of infectious illnesses in a globally interconnected society, high levels of population immunity are necessary for their eradication (Andre et al., 2008). Furthermore, reliable diagnostic tests for identifying current cases and surveillance techniques for monitoring the disease's decline must be implemented.

As of this writing, smallpox is the single contagious illness which has been completely eliminated in humans by vaccination. Humans have suffered from this illness for millennia; the first records of it date back to 1000 BC in Egyptian mummies (Geddes 2006). A historic triumph for public health, the smallpox epidemic was finally eradicated by ring vaccination in 1980, as declared by World Health Assembly (Strassburg, 1982), thanks to Jenner's effective creation of the vaccine utilizing the vaccinia virus (Jenner 1799). The second case of eradication included the render pest virus in cattle, a disease that killed people indirectly by hindering agriculture, which in turn led to food and poverty-related humanitarian disasters. In the 19th century, the Rinder Pest Virus expanded widely over most of Africa and Europe, infecting cattle, buffalo, and many other domestic species (Roeder et al., 2013). Developed in the 1950s, the Plowright tissue culture rinder pest vaccine was utilized in mass vaccination campaigns together with other public health initiatives, ultimately resulting in elimination in 2011 (Morens and Fauci 2007). The wild poliovirus is the next pathogen to be eradicated. Before vaccinations were developed, this debilitating paralytic disease often affected adults and children in both industrialized and undeveloped countries. Both the live-attenuated oral polio vaccine (OPV) and the inactivated polio vaccine (IPV) were able to protect against all three wild strains of the polio virus when they were first made available in 1955 and 1963, correspondingly (Stanley and Plotkin 2014). There was a 99% decrease in the prevalence of polio by the year 2000, despite the fact that this goal was not reached because of a lack of financing, political will, in competing health efforts (Lien and Heymann 2013).

Only six prevalent countries (Egypt, Niger, India, Nigeria, Afghanistan, along with Pakistan) had new cases by 2003; by 2005, only the last four had new cases. Due to increased birth rates, inadequate sanitation, marginalized people, and rapid population movement in densely populated areas, eradication in India presented challenges (Thacker et al., 2016).

Reduction in Mortality

According to study, vaccination is recognized as a social value and a right for everyone, regardless of age (adults and children). Thirty-six distinct vaccines were created in 2017 throughout the past thirty years. Several vaccines have emerged employing diverse technologies, such as protein or polysaccharide subunits, dead entire organisms, and first live-attenuated strains (Seib et al., 2017). These advancements have made it possible to compare VPD (Vaccine Preventable Disease) morbidity and mortality data from the pre-vaccine era with data from the current age in a number of different global areas, including Australia, Canada, the UK, and the USA. According to these comparisons, the prevalence of infectious illnesses that may be prevented, including smallpox, congenital rubella, diphtheria, hemophilus influenza A, measles, mumps, pertussis, tetanus, and rubella, has decreased by 68 to 100% (Seib et al., 2017). These results provide an explanation for the observation that, globally, the proportion of mortality caused by communicable illnesses fell from 33% in 1990 to 25% in 2010 (Lozano et al., 2012), therefore averting almost 2.5 million deaths per year. Related to this is the topic of whether vaccinations improve health in ways other than only reducing infectious illnesses (Organization 2019).

Global adoption of the first dose of the measles-containing vaccine (MCV1) increased from 72 to 85% between 2000 and 2017, and measles mortality dropped by a calculated 80% (from 545, 174 to 109, 638) through the same period (Dabbagh et al., 2018). These two factors have contributed to significant progress in the past 20 years towards measles control. Even in a healthy infant, measles infection is linked to a brief (about one week) lymphopenia and a longer duration of immunological suppression, despite the positive decrease in mortality (De Vries et al., 2012, Laksono et al., 2016).

Development of Herd Immunity

For the majority of vaccination recipients, personal, direct protection is the primary health benefit. The additional benefit for vaccination from the standpoint of community health is the capacity to establish herd immunity. The spread of the infectious agent is halted when a sizable enough portion of the population is immunized, protecting others who have not, such as those who are considered too young, too frail, or immune-compromised to receive benefits from immunizations. As part of the standard EPI, very effective immunization programs have been put in place versus encapsulated bacteria which persist asymptomatically in the oropharynx yet can penetrate and cause meningitis and septicemia in people of all ages. The goal of these vaccinations was to induce protective immunity or direct protection. When these polysaccharides were coupled to carrier proteins in the 1990s, they became more effective because they ensured an immune memory and T cell response, and they also reduced the acquisition of pharyngeal carriage from these organisms, which provided indirect protection and thereby prevented further transmission (Pollard et al., 2009). This was initially noted in 1999–2001 during a nationwide immunization effort against serogroup C (Neisseria meningitides) *N. meningitidis* in the United Kingdom (Maiden et al., 2008), and it had a significant role in the disease's subsequent decline. Since high vaccination rates lower the number of unvaccinated people and the risk of disease transmission between them, they are essential for achieving herd immunity, also known as population immunity. As bacterial infections with conjugate polysaccharide vaccines have fewer basic reproductive units than viral diseases such as measles, chickenpox, or polio, a smaller percentage of a given population must build herd immunity by immunization against these infections. For high-risk individuals who are vaccinated against certain diseases, herd immunity provides a potentially life-saving shield against disease. With its emphasis on the individual, human vaccination research appears to be very different from veterinary medicine, which is more concerned with the well-being of the herd.

Prevention of Cancer

Infectious agents are thought to be the cause of 16% of human malignancies worldwide, while evidence from trustworthy registries in Africa indicates that they may be responsible for 30 to 50% of cancer cases. Human papillomavirus (HPV), Human T-lymphotropic virus Type 1 (HTLV-1), Epstein-Barr virus (EBV), human herpesvirus-8 (HHV-8) along with Merkle cell polyomavirus (MCV) are among the viruses that cause hepatitis B and C (HBV and HCV) are among the several viruses that have been identified as carcinogenic (Gaglia and Munger 2018, Krump and You 2018). Replicating viruses are not present in the tumours, and cancer is not one of the main characteristics of the Illness process. Oncogenic virus-induced cancer incidence can be decreased by preventing the first infection, which also prevents chronic infection (De Flora and Bonanni 2011). This is because cancer can arise in persons who have a history of infection. It has been demonstrated that HPV vaccinations lower the risk of precancerous lesions and prevent HPV infections. While not all oncogenic viruses presently have vaccines available, there is optimism that when they do, then they are proven to prevent both initial and chronic infection, they will also reduce the incidence of cancer (Chevalier-Cottin et al., 2020). Prevention of cancer is shown in Fig. 1.

Prevention against Antibiotic Diseases

Vaccines have recently been shown to be highly effective in preventing cardiovascular and neurological problems. Few experts are aware of these advantages, while they are aware of the anticipated advantages of vaccinations in the battle

against antibiotic resistance, another significant health concern (Micoli et al., 2021).

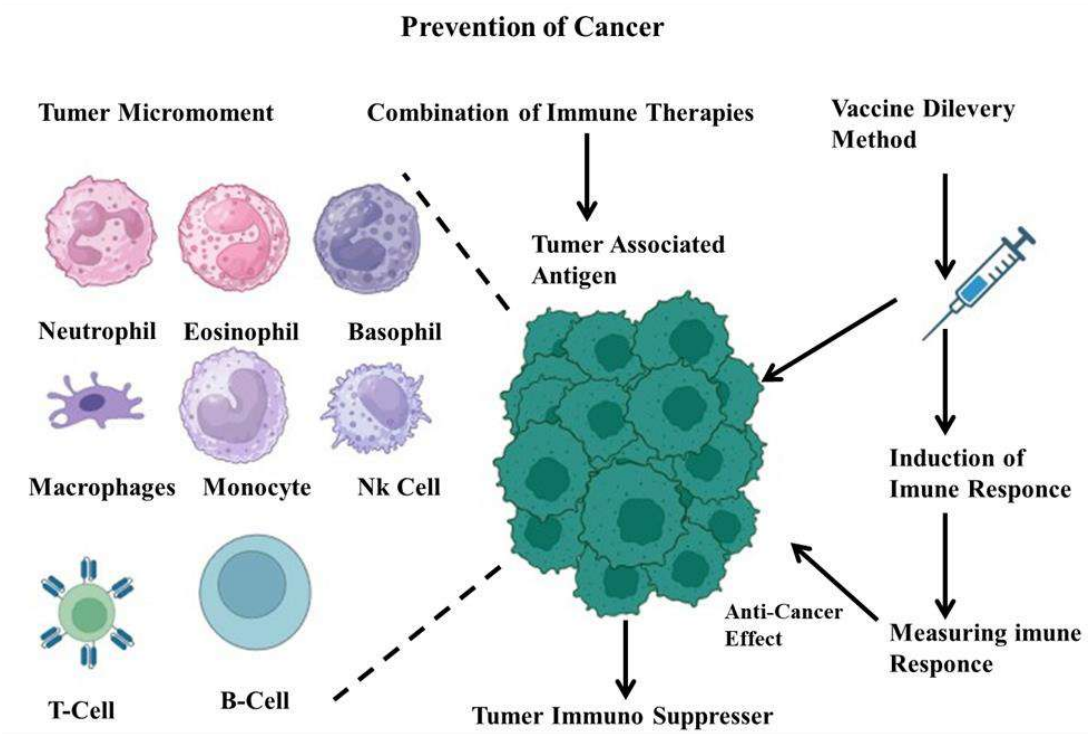

Fig. 1: Prevention of Cancer

An estimated 25,000 individuals in the European Union pass away from resistant illnesses every year, resulting in an annual cost burden of around 1.5 billion euros (van den Brink 2021). Together with the many well-known strategies that can be used to control antimicrobial resistance—such as improving sanitation and hygiene, developing rapid diagnostic tests, educating people about the proper use of antibiotics, encouraging antibiotic stewardship, and doing away with routine antibiotic use in livestock production—more avenues to eradicate resistance include the development of novel antimicrobial agents and vaccines (Jansen and Anderson 2018). Between 2000 and 2014, the USA had levels of antibiotic resistance associated with *Staphylococcus aureus, Escherichia coli, Enterobacter* spp., and *pneumococcal pneumonia* that were 34, 45, 55, and 88%, respectively. This figure does not take into consideration the proportion of strains that have grown resistant to multiple antibiotic classes. (Jansen and Anderson 2018). Quite the reverse—vaccines do not lead to a major increase in resistance. This makes them a desirable option in the battle against antibiotic resistance since they shield patients from serious infectious illnesses like the flu or pneumococcal pneumonia, which lowers the need for antibiotics and the spread of illness. Furthermore, those who are immune-compromised or who are not able to receive vaccinations are also protected by herd immunity. Benefits of vaccines are shown in Fig. 2.

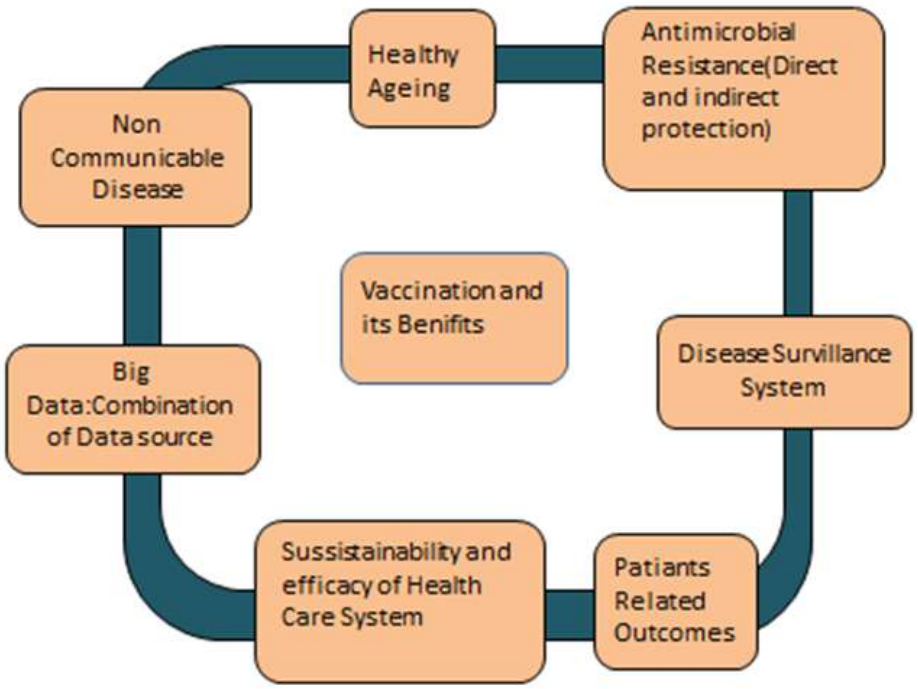

Fig. 1: Benefits of Vaccines

Social and Economic Benefits of Vaccines

Especially in low- and middle-income countries (LMICs), vaccination is an essential and successful strategy against disease-related mortality and morbidity, averting an estimated 5.1 million deaths from vaccine-preventable illnesses each year (Carte et al., 2023). It is challenging to exaggerate the impact of immunizations on the health of the world's population. The development of safe and efficient vaccines against illnesses that cause major morbidity and death has been one of the most important scientific breakthroughs of the twenty-first century (Rodrigues and Plotkin 2020).

A growing number of people worldwide are studying health economics, which is the study of how limited healthcare resources are used and should be distributed in healthcare systems, in response to the declining western economy and ongoing resource shortages in low-income nations. Donors are evaluating more closely how funds are used to help developing nations combat illness, while governments in low-income nations are analyzing more closely how much they spend on healthcare (Babigumira et al., 2013). Hence, investing in vaccinations offers a variety of tangible as well as intangible benefits which can enhance benefits for both the individual and society as a whole.

Many financial advantages can come from vaccinations. Avoided medical costs are one of the most obvious benefits. The financial burden of medical treatment, including doctor visits, prescription medications, hospital stays, related travel expenses, and caregivers' lost wages, could be avoided by preventing an outbreak of the disease via vaccination. In low- and middle-income countries (LMICs), where a significant portion of medical expenses is paid for out of pocket, this is especially crucial (Nandi and Shet 2020). The impact of vaccines with regard to health, economy or society is shown in Fig. 3.

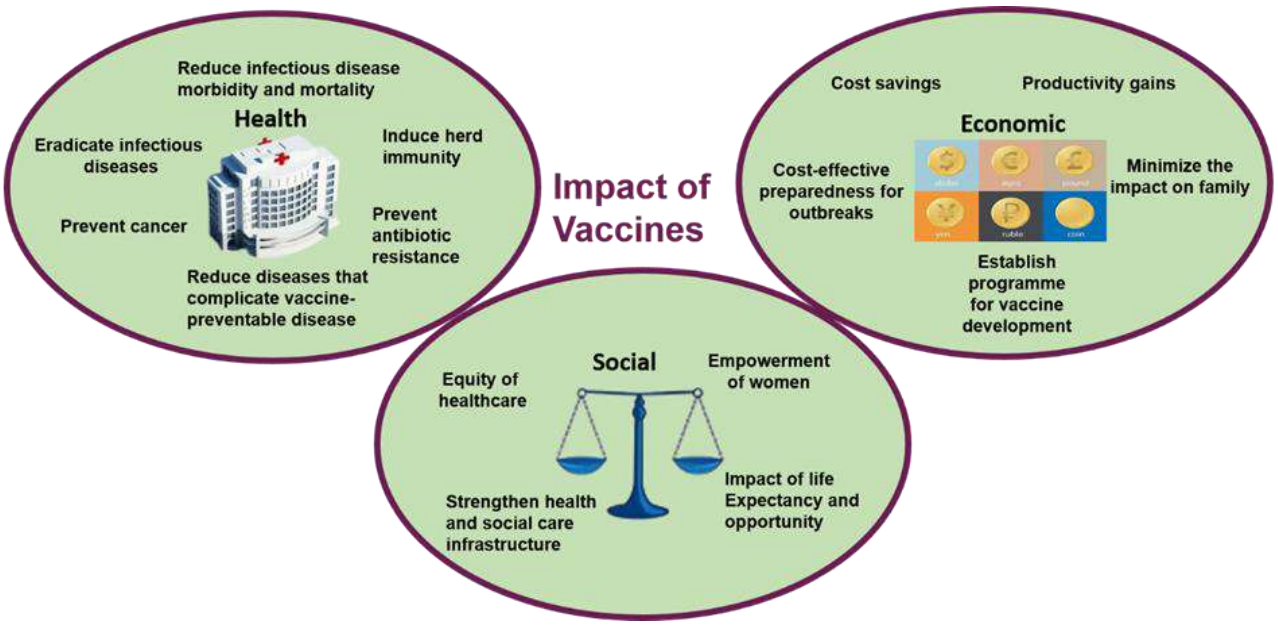

Fig. 3: The impact of vaccines with regard to health, economy or society

Equity of Healthcare

Over the course of the 20th century, access to reliable vaccines has evolved from a humanitarian concern to a moral one. Regardless of various drawbacks, the national and international EPI's immunization program served as a great leveler to begin minimizing the effects of infectious diseases on everyone scale (World Health Assembly, 1974). The argument for vaccination equality is merely the most recent manifestation of a social, historical, and philosophical tradition to examine the structural causes of variations in health. "The lack of unfair and preventable or remediable variations in health across populations as characterized socially, commercially, on a demographic basis or geographically" is how the World Health Organization defines health equity.In instances of social or global injustice, health equity can be stated as an empirical assertion about the state of observed disparities or as an ethical claim (i.e., an expectation that specifies what ought to be done) for enhancing or restoring health or health outcomes(van der Graaf et al., 2022).

Strengthen Health Care and Social Care

These two vaccine equity strategies are crucial elements in discussions of politics and governance pertaining to global health. The Mission Indradhanush in India, which aimed to provide life-saving vaccines to all children as well as pregnant women in 2020 by means of programs alongside (i) national, (ii) state, (iii) district, as well as (iv) block/urban level input, provided an example of the various components of an effective vaccine program (Hinman and McKinlay, 2015).

Governments must fund national projects and establish the policies that will be followed. States had to acquire the vaccines and properly store them while identifying children who qualified for them through outreach and public health campaigns. Districts and urban regions hired personnel with training in vaccination administration and communication to give shots and, if necessary, provide follow-up care. The foundation for providing additional health or social care services for all community members is laid by the establishment of this level of infrastructure across the country to reach people in both urban and rural areas, with a focus on reducing maternal as well as infant mortality in developing countries and among the elderly in industrialized countries (Shearley, 1999). Infrastructure and staff in the field of public health could be utilized to spread more critical messages along with health education on malnutrition, sanitation and hygiene, and diseases like human immunodeficiency viruses (HIV) and malaria that can be prevented. Global factors also play a significant role, as evidenced by the creation of the EPI in 1974, as all nations were required to supply these vaccinations, strengthening their primary and public health care systems in the process, which had benefits that extended beyond the vaccination program (Shearley, 1999).

Efficacy, Effectiveness, and Availability

Vaccine efficacy pertains to the level of protection that a specific demographic receives from vaccination. It assesses protection from vaccinations both directly and indirectly (by population growth). A vaccine's effectiveness is directly correlated with its efficacy, but other factors such as vaccination coverage, cost, and accessibility to healthcare facilities can also have an impact (oherty and Buchy, et al., 2016).

The degree to which a vaccination shields recipients against negative health outcomes like infection, symptomatic disease, hospitalization, and mortality is known as vaccine efficacy. When comparing the prevalence of health consequences between vaccinated and unvaccinated individuals, vaccination efficacy is typically determined. When a study contrasts vaccinated and unvaccinated participants, the phrase "absolute vaccine effectiveness" may be used. A comparison of the occurrence of health outcomes between recipients of one vaccine type and recipients of a different vaccine, or between recipients of more doses of a vaccine and recipients of fewer doses, can be used to assess the effectiveness of vaccines.

Cost Saving

When compared with other public health treatments, vaccinations are both very cost-effective and helpful for the public (Bloom et al., 2005). Government departments must do methodical economic studies of vaccinations and vaccination programs in order to support their procurement, given the strain that the global financial crisis has placed on public while private funds. Purchasing vaccines, setting up the program's infrastructure and keeping the cold chain intact, and hiring administrative and medical staff are all obvious, direct expenditures for a vaccination program. Occasionally aided by nonprofits and non-governmental organizations, governments make investments in these with the goal of enhancing health. Through a mix of direct as indirect protection, the reduction of morbidity as well as mortality linked to effective vaccination programs has decreased the occurrence of diseases, the expenses of treating them, and the need for medical care (Deogaonkar et al., 2012).

As a result of fewer medical tests, surgeries, treatments, and time away from work for patients and parents, less money may be spent, which could spur economic growth. Furthermore, using combination vaccinations, such as DTaP/IPV/Hib/HepB, protects against a greater number of diseases while requiring the same number of shots per kid as now offered in immunization programs, and does so without adding to infrastructure expenditures.

Minimizing Impact on Family

Early-life infections that are persistent or recurring can cause stunting and poor growth, which can have a negative impact on adult health, cognitive function, and economic productivity. The "fetal origins" theory, which connects conditions in gestation and early childhood with results in later life, provides the theoretical foundation for the long-term advantages of vaccines. Long into old age, malnourishment, infection, difficult pregnancies and deliveries, and under-stimulation in the first 1000 days of life can have a lasting effect on health, cognitive abilities, and financial results. Health measures like frequent immunizations could lessen the burden of infectious diseases in early childhood in addition to proper nutrition and caring, helping to break the intergenerational cycle of poverty, ill health, and low income (Nandi and Shet, 2020).

Limitations of Vaccines

One of the most significant initiatives in the struggle to prevent and manage infectious diseases has been the creation of vaccinations. Immunogenic antigens from the pathogenic microbe trigger a preventive immune response against the infectious agent, which is the basis for vaccination. Although the majority of microbes are not harmful, they can nonetheless improve our quality of life. Nonetheless, certain strains or species of the microorganisms within the categories of bacteria, fungus, viruses, and protozoa have the ability to cause illness. In many situations, the mechanisms by which these microbes cause infectious diseases remain poorly understood. These organisms usually need to be able to infiltrate or colonize the tissues in order to harm. In this regard, toxins—poisonous byproducts of their metabolism—are produced by a variety of pathogenic microbes (Nixdorff, 2001).

Coordination between scientists, doctors, health department officials, industry or vaccine developers, and society is necessary for the slow, methodical, costly, and tedious process of developing new vaccinations. To successfully create safe and effective vaccinations that are used widely, these shareholders must cooperate in order to solve the hurdles stated.

- High (and rising) development costs for vaccines
- Reluctance to receive vaccinations
- Increased standards for safety
- 100% efficacy expectations in society
- Cold chain for vaccinations must be maintained.
- Raising the bar for effectiveness of a single dosage
- Rapid reaction to worldwide epidemics is required
- Product development duration
- The immune response's insufficient resilience
- Failure to effectively suppress infections OR possibility of reverting to a wild-type organism
- Lack of knowledge about the biology, pathophysiology, and/or immunology of newly developing diseases
- A small number of adjuvants that are appropriate and authorized

Complex immunology can also be a hindrance; the development and application of the recently approved Dengue vaccine has been hampered by the recent discovery of antibody-dependent disease enhancement (Kennedy et al., 2020).

Immunization Strategies for Diseases

Up to three million lives are saved annually through vaccination, making it one of the most effective and economical public health interventions (UNICEF, 2019). The Expanded Program on Immunization (EPI) was started in 1974 to provide essential vaccines for immunization. The first six vaccine-preventable diseases (VPDs) that were targeted were tuberculosis, poliomyelitis, measles, tetanus, pertussis, and diphtheria. During the program's early years, a significant decrease in the number of avoidable childhood illnesses and deaths was accomplished (Basu, 1982). National immunization programs (NIPs) have become significantly more complex over the past few decades because of the availability of vaccines to protect against over 20 infectious diseases. Additionally, changes in politics, society, and health have added to the volatility and ambiguity of uncertain environments, as seen by the emergence of conflicts, epidemics, and rising vaccine hesitancy, among other events. The Vaccine Alliance, or Gavi, was founded in 2000, with the primary goal of enabling children in the world's poorest nations to receive new vaccines (Bland and Clements 1998). The Global Vaccine Action Plan (GVAP) was launched in 2012 by global partners of the Decade of Vaccines in response to stagnating rates of immunization coverage in numerous countries (Gavi the Vaccine Alliance 2019). "To extend, by 2020 and beyond, the full benefits of immunization to all people, regardless of where they are born, who they are, or where they live," is how this plan describes its mission. The current Gavi strategy, 2016–2020, the upcoming Gavi strategy, Gavi the Vaccine Alliance 2019, and the new Immunization Agenda 2030, both likely maintain the crucial focus on equitable immunization uptake and coverage by 2025 (IA2030) (WHO, 2020).

During the past decade, significant accomplishments have been made. In just seven years, from 2010 to 2017, the mortality rate for children under 5 years of age decreased from 1/19 to 1/26 (WHO 2019). 116.3 million Children, or 4.9 million more than in 2010, are receiving three doses of DTP before turning one-year-old. At least 90% of the 129 countries had received a third dose of the DTP vaccine by 2018 (WHO 2019). Strengthened national decision-making capacities have led to the introduction of numerous life-saving vaccines, mostly in the world's poorest nations (Loharikar et al., 2016). A global framework for immunization monitoring and evaluation was established, regional vaccine action plans were created,

and measures were taken to improve vaccine price transparency and shape the vaccine markets. Overall, the GVAP kept vaccination visible on the international agenda and contributed to the development of the political (SAGoEo, 2018).

Nonetheless, issues remain, and NIPs worldwide must adjust by updating and changing their vaccination plans and policies on a regular basis. There are still outbreaks of measles and vaccine-derived poliovirus, and not all areas have reached elimination and eradication goals for polio, measles, rubella, and neonatal tetanus. Crucially, not all eligible individuals or children are reached everywhere (Loharikar et al., 2016). Variations in routine immunization coverage can exacerbate the persistence of vaccine-preventable diseases (VPDs) and their outbreaks. Vaccine uptake disparities and disease persistence have been observed across national borders. Since 2010, there has been little movement in the 86% global coverage of the third dose of the diphtheria, pertussis, tetanus, and pertussis (DPT) vaccine. In certain nations, the advancements have been reversed. As evidenced by first-dose DPT coverage, one in ten infants globally remained unvaccinated, and an estimated 19.4 million infants were not provided routine immunization services, including three primary doses of the DPT vaccine (WHO 2019).

Current Challenges and Future Direction of Immunization

In fact, the addition of several new vaccines that will be available by 2012, along with the expansion of coverage to include those who would not have been reached by the traditional Expanded Program on Immunization, are both essential components of the Global Immunization Vision and Strategy (GIVS). Despite these advancements, there is still much to be done to fully utilize vaccinations in order to meet health-related Millennium Development Goals (MDG). By immunizing children under five years of age against rotavirus diarrhea and pneumococcal disease, an estimated 1.1 million child deaths could be avoided. Furthermore, vaccinations against human papillomavirus infection may stop approximately 250,000 women who die of cervical cancer each year. Large-scale access to additional vaccinations against typhoid, Japanese encephalitis, and meningococcal infections soon occur (World Health Organization, 2009). Furthermore, a number of organizations, including governments, foundations, multilateral agencies, and research centers, have significantly increased their investments in the creation of new vaccines. As a result, over the next ten years, a number of new vaccinations should be available for use. Vaccines against malaria, TB, and dengue are among them. However, nations are having to make more decisions about which of these life-saving instruments to fund and employ regularly. Current logistics and cold chain requirements are challenged by the introduction of new vaccines. Specifically, the 7-valent pneumococcal conjugate vaccine's high-volume prefilled glass syringe presentation surpasses some countries' central cold chain storage capacity, and the safe use and disposal of used glass syringes and needles present a waste management challenge. Assistance to nations to enhance vaccine and waste management, as well as collaboration with industry to find better vaccine formulations and delivery systems, are ways to address these concerns. Additionally, there are many ongoing initiatives in the field of monitoring diseases targeted by new vaccines, such as improved laboratory networks and centers of excellence (Bicaba et al., 2009). A series of initiatives have been identified by the WHO and its vaccination partners to expedite the introduction of new life-saving vaccines. The World Health Organization (WHO) manages an international action plan for new and underutilized vaccines, which serves as a platform for coordinating global partners' efforts to introduce vaccines in the neediest countries. Scientific data and evidence, a consistent supply of reasonably priced vaccines that adhere to the nation's immunization schedule, and an integrated disease monitoring and surveillance system are all necessary to make decisions about the introduction of new and underutilized vaccines. The execution of this action plan has commenced, encompassing the creation of tactical alternatives to facilitate the introduction of costlier vaccines in low-income and middle-income nations.

Disease Surveillance

The global poliomyelitis eradication initiative has shown that effective surveillance systems can be set up for relatively little money compared to the cost of the intervention itself, even in settings with limited resources. The surveillance network for poliomyelitis offers a framework for quickly identifying and addressing illnesses of domestic and global significance. When applicable, this network should act as the foundation for an integrated disease surveillance system that offers epidemiological information on various infectious diseases as well as for the identification and management of new threats to infectious diseases. The typically, funding for disease surveillance is time and disease specific. When weak national systems exist, parallel systems are typically set up to produce data tailored to the requirements of particular programmes. While these disorganized efforts might meet immediate needs, they are not viable long-term. Immunization partners have the chance to coordinate their efforts to secure long-term funding for surveillance and program monitoring of the global framework. We have a great opportunity to influence the health of our communities, especially the poor and marginalized groups that bear a disproportionate burden of disease, thanks to advancements in vaccines and immunizations. This opportunity presents significant obstacles to underdeveloped health systems. GIVS offers a framework for optimizing immunization benefits while also generating efficiencies through a coordinated and cooperative approach to the provision of healthcare (Burton et al., 2009; Lim et al., 2008).

Conclusion

Vaccines have a broad and lasting impact, although their effects aren't always measurable, understood, or shared. Vaccination has always been used to reduce the risk of illness and infection-related death. The development of new

vaccines is increasingly motivated by this idea, especially in order to prevent diseases that are not curable or to prepare for outbreaks. A growing understanding of the social and economic impacts of vaccinations and immunizations, however, is being incorporated into vaccine programs, which could lead to a wider adoption of the program and more benefit to society. Stakeholders in global health have gained insight into the efficient development of vaccines and immunizations, but there is still much to learn about their production and application, with due consideration for equity and access.

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Chapter 16

Diversity of Vaccines and Strategies of Immunization Process

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ABSTRACT

Vaccines have been the most effective public health intervention in human history, yet their potential remains untapped. This journey began with Edward Jenner's 1796 experiment, laying the foundation for modern vaccinology. The first golden era, led by pioneers like Pasteur and Koch, introduced vaccines for diphtheria, tetanus, rabies, pertussis, and tuberculosis in children. The second golden age, in the late 20th century, advanced cell culture technologies, producing vaccines for polio, hepatitis A, mumps, rubella, measles, rotavirus, and varicella. Vaccines are classified by formulation, which dictates their nature and function in the host. The main types are live attenuated, inactivated, subunit, polysaccharide, combination, recombinant DNA, and toxoid vaccines, each with distinct advantages and limitations. The choice depends on factors like the pathogen, target population, and desired immune response. Recombinant DNA technology has enabled safer vaccines against challenging pathogens. This review explores vaccine types, formulations, and their mechanisms in stimulating the immune system. It also discusses strategies to enhance immunization and community immunity. Vaccines primarily target B-cells or T-cells to initiate immune responses, fostering acquired immunity. The interconnection between vaccines and immunity highlights the need for strategic immunization efforts, demonstrating the importance of various factors in achieving effective immunization.

KEYWORDS
Inactivated, Subunit, Toxoid, Conjugate, Vaccines, Immunization, Attenuated, Pathogens, Virulence, Immunity

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INTRODUCTION

Vaccines have been the most effective public health intervention in human history. However, their complete potential remains unknown. The topic of vaccines started when Edward Jenner, the scientist, performed an experiment in 1796, which was the first ever step towards vaccine production. He took pus from the lesions on the milkmaid's hands. The pus into an eight year old boy by variolation (intentional inoculation of an infection, particularly with a small pox into an individual) method. After six weeks, Jenner variolated the boy with smallpox again, and the boy did not develop any disease (Chavda et al., 2024). He performed 12 such experiments and studied 16 additional cases, after which he published on his own expense a volume that turned classical text in medicine called Inquiry into the causes and effects of various vaccines. Jenner's statement about that "the cowpox protects the human constitution from the infection of smallpox." This has become the foundation of modern vaccinology (Alexandra Minna Stern 2005).

After establishing germ theory and creating a vaccine based on live-attenuated or inactivated pathogens and inactivated toxins, Pasture, Koch, Ramon, and Meraux initiated the first golden era of vaccines. They developed a vaccine against diphtheria, tetanus, rabies, pertussis, and tuberculosis in children. The development of cell culture technologies during the second half of the 20th century marked the beginning of the second golden age of vaccine production. Vaccines for polio (IPV), hepatitis A, and live-attenuated vaccines against polio (OPV), mumps, rubella, measles (MMR), rotavirus, and varicella (Stern and Markel, 2005).

Vaccines were proven to be the strongest and most crucial weapon against the disease in the early 19th century. Vaccines prevented many deadly diseases, saved countless lives, contributed to drastically decreased mortality rates, particularly in children, and helped to eradicate smallpox from the world (Lahariya, 2014). An additional aspect of the vaccine that opened the treatment of disease by introduction of vaccine is the therapeutic nature of the vaccine. In addition to preventing the disease, the vaccine can also be used as a therapeutic agent (Hunter, 2014).

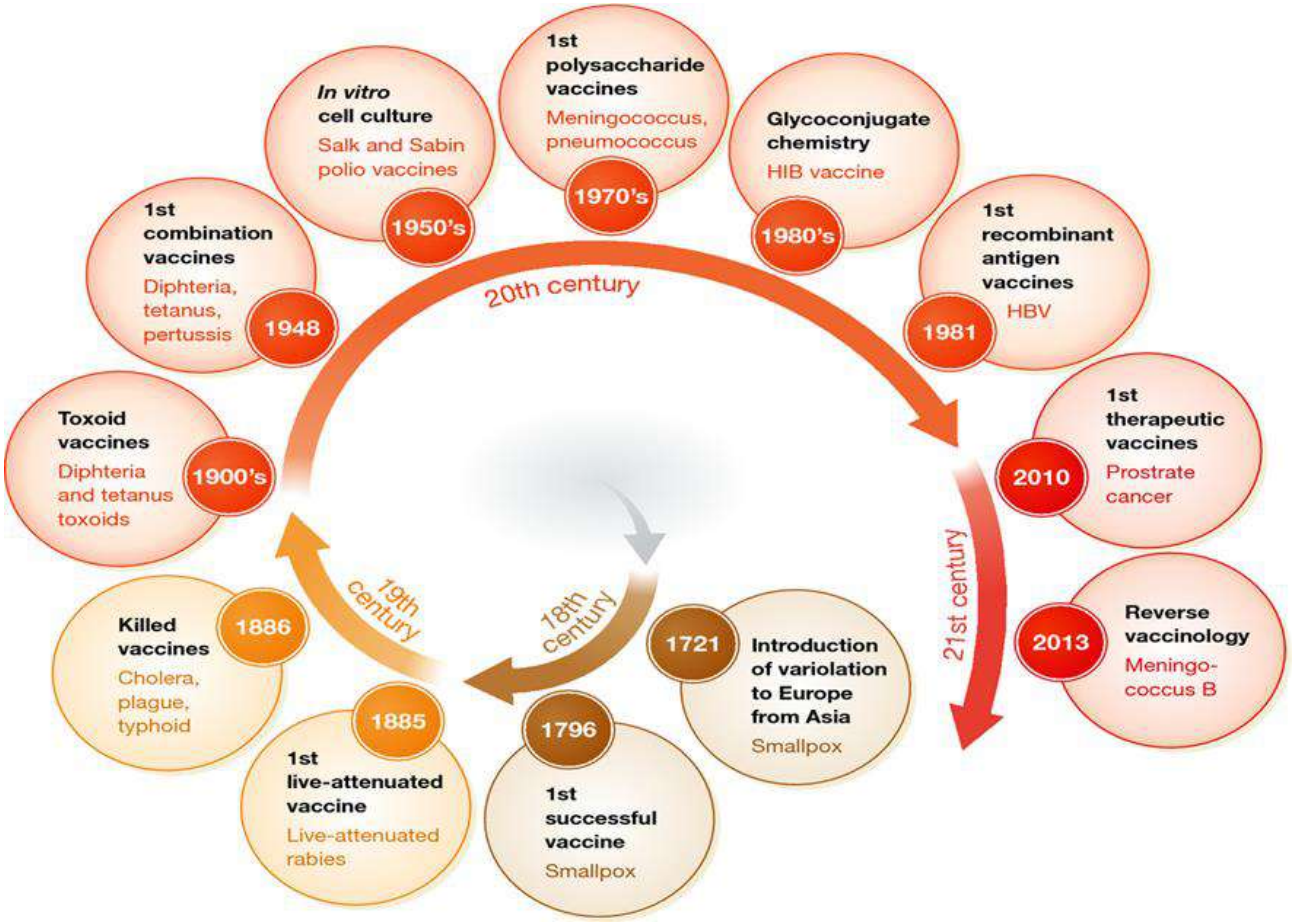

Fig. 1: Evolution of vaccines (Hilleman, 2000)

Vaccines and its Types

Vaccines are biologics responsible for active adaptive immunity against a specific disease in humans (Organization, 2009). Usually, vaccines comprise drugs that resemble the causative microorganisms for the disease or are sometimes made out of killed, weakened, or attenuated microorganisms, their toxins, or their surface proteins introduced by ingestion, injections, or nasal pathways by means of nasal spray to give a threshold to the immune system to start the process of recognizing the foreign agents and kill them (Mettu et al., 2020). Various types of vaccines are currently available. All these vaccines have been developed specifically to teach the immune system to fight certain kinds of infections (Deslauriers and Boulianne, 2023). Vaccines are classified based on their formulations. The method we used to produce a vaccine well describes the nature of the vaccine and the area of action in the host body. All the available vaccines are described below (Kallerup and Foged, 2014).

In live attenuated vaccines, an infectious agent with the ability to produce disease has been limited and has become less virulent. Microorganisms may be attenuated in several ways, such as chemically and genetically, by in vitro passage, or other means (Plotkin, 2014).Inactivation was first applied to pathogens, such as typhoid, plague, and cholera bacilli. Inactivated vaccines contain completely killed microorganisms. In the formulation of this vaccine, the infectious agent is treated with chemical, thermal, or other methods to kill the agent. Inactivated vaccines fail to provide longer immunity compared to live vaccines, so several doses over time are required for ongoing immunity (Plotkin, 2014). Examples of inactivated Hepatitis A rabies vaccines.

These vaccines contain subunits or components of the causative agent such as surface proteins, polysaccharides, or specific proteins. As these vaccines use only specific pieces of the germ, they provide a very strong immune response that is targeted to key parts of the germ. They can also be used by almost everyone who needs them, including those with weakened immune systems and long-term health problems (Maurice and Davey, 2009). The only drawback of these vaccines is that individuals may need booster shots to acquire immunity for a longer period of time. *The Haemphilus*

influenza type B disease vaccine and whooping cough are examples. Morphological studies and chemical analysis showed that many pathogens were surrounded by a polysaccharide capsule and that antibodies against the capsule could promote phagocytosis. The first use of this information to develop a vaccine was the meningococcal polysaccharide vaccine (Plotkin, 2014). Polysaccharide vaccines are made from complex sugar capsular polysaccharides that overlie the surface of capsulated bacteria (Rohokale and Guo, 2023). For example, Conjugated H. influenzae vaccine

Vaccines that are formed by combining different vaccines that work for different pathogenic organisms result in the formulation of a unique immunization. The development of combination vaccines for protection against multiple diseases began with the combination of individual diphtheria, tetanus, and pertussis (DTP) vaccines into a single product that was first used to vaccinate infants and children (Skibinski et al., 2011). For example, MMR vaccines for measles, mumps, and rubella are provided in one shot. These vaccines are derived from recombinant DNA technology. This is an updated method for manufacturing vaccines. This process comprises recombinant expression of proteins and a viral vector (I. P. Nascimento and L. C. Leite, 2012). This updated technology enables scientists to develop vaccines against challenging pathogens, such as difficult-to-culture or non-culture-able viruses and minimizes safety risks by using bioprocesses and shorter production processes. A perfect example of a recombinant vaccine is Recombivax, a vaccine against Hepatitis B that was the first to be licensed (Hudu et al., 2016). Toxoid vaccines use a toxin (harmful product) produced by the germ that causes a disease. They create immunity to the parts of the germ that cause disease, instead of the germ itself. This means that the immune response is targeted by the toxin instead of the whole germ (Yadav et al., 2014).

Method of production	Licensed vaccines
Live attenuated	Smallpox, rabies, tuberculosis (BCG), yellow fever, polio (OPV), measles, mumps, rubella, typhoid, rotavirus, influenza (cold adapted), zoster (Delany et al., 2014)
Killed whole organism	Typhoid, cholera, plague, pertussis, influenza, typhus, polio (IPV), rabies, Japanese encephalitis, tick-born encephalitis, hepatitis A (Delany et al., 2014)
Toxoid/protein	Diphtheria, tetanus, acellular pertussis, anthrax, influenza subunit (Delany et al., 2014)
Polysaccharide	Pneumococcus, meningococcus, Haemophilus influenzae B, typhoid (Vi) (Delany et al., 2014)
Glycoconjugate	*Haemophilus influenzae* B; pneumococcus, meningococcus C (Delany et al., 2014)
Recombinant	Hepatitis B, cholera toxin B, human papillomavirus (Delany et al., 2014)
Blood cell infusion	Prostate cancer (Delany et al., 2014)

Immunization and Components of Immune System

The immunization process is defined as the whole procedure of inducing immunity in an individual by injecting a vaccine against specific pathogens (Schunk and Macallum, 2005). The immunization of large populations through vaccination is an extremely important issue with obvious implications for public health. This vaccination process holds an imperative position in the betterment of a population (Mantel and Cherian, 2020). To develop effective immunity, we first need to examine the components of the immune system to completely understand how the immune system works.

The immune system has two main subunits: the innate/general resistance system and the adaptive immune system. Both the systems are interlinked to work more efficiently. The general mechanism of the innate immune system works continually and serves as the first line of defense against all pathogens (Aristizábal and González, 2013). However, these responses are not specific to a specific pathogenic agent. Instead, innate immune cells are specific to the conserved molecular patterns found in all microorganisms. This prevents the innate immune system from attacking and killing host cells and kills them. However, this prevents the innate immune responses from becoming more efficient, which means that innate immunity is not specific for any germ, and the innate immune system lacks the feature of memory (Clem, 2011).

Anatomic barriers, such as intact skin and mucous membrane membranes, prevent the entry of many microorganisms and toxic agents, which is the protective defense of the innate immune system. The skin is acidic in nature because it has a pH of 3-5 which does not favor the growth of microorganisms. Furthermore, mucus and cilia on the mucous membranes aid in trapping microorganisms and propelling them out of the body (Elias, 2007). Some more components of innate immunity are physiological barriers, such as normal body temperature, fever, gastric acidity, lysozymes, interferon, and collectins (collectins are an important part of the innate immune system and are soluble pattern recognition receptors). The normal body temperature range prevents a large variety of microorganisms from surviving in the human body (Clem 2011).

The inflammatory response is another crucial component of the innate immune response. The body's response to any kind of physical harm, antigenic challenge, or invasion by an infectious agent is known as the inflammatory response. The inflammatory response allows products of the immune system to enter areas of infection or damage and is characterized by cardinal signs of redness, heat, pain, swelling, and loss of function (Kim et al., 2019). Unlike the innate immune system, the adaptive immune system targets a specific pathogenic agent. This response takes longer than the innate response. However, with each subsequent encounter, the adaptive immune system reacts to that specific pathogen more quickly because it possesses memory (Iwasaki and Medzhitov, 2015). The main components of the adaptive immune system are b-cells/antibodies and T-cells. These are two arms of the adaptive immune system. B cells, antibodies, and T-cells create humoral immunity and cell-mediated immunity, respectively. The natural killer cells share the same lineage as B-cells and T-cells, but they are part of the innate immune system (Albeert B, 2022).

The first and main part of the adaptive immune system is humoral immunity, which functions against extracellular pathogenic agents and toxins. The major cells involved in humoral immunity are B cells. They are produced in the bone marrow and mature in lymph nodes. Unlike T-cells, B-cells have the ability to identify antigens in their original state, which implies that B-cells do not need the antigen to be processed by an antigen-presenting cell before being presented by a T-helper cell (Chan et al., 2014). These antigens are called T-independent antigens because T-cell activation is not required to activate Bcells. These antigens can also induce the activation of numerous B cells. Compared to T-helper cell activation, there is less immune response and memory formation (Albeert B, 2022).

In contrast, a far stronger immune response and more efficient memory are produced when B cells are activated in conjunction with T helper cell activation. The intended outcome of immunization is a long-lasting and efficient immune response. This process then stimulates the B–cell(s) to mature into a plasma cell(s), which then begins the production of the particular antibody with the best corresponding fit to the antigen (Buisman et al., 2009). Any further exposure to the antigen triggers a more potent and swifter secondary immune response because of the memory cells generated during the first immune response. This secondary immune response results in a more rapid reaction, which is mostly made up of IgG (Kindt, 2006).

The primary target of cell-mediated immunity, another branch of adaptive immunity, which is intracellular pathogens. After reaching adulthood in the thymus, T-cells are discharged into the circulation. T cells have two primary varieties: CD4 and CD8 cells. Having the CD4 co-receptor, CD4 cells, also known as T-helper cells, are only able to detect the major histocompatibility complex (MHC) II protein (Sauls et al., 2018). Every immune cell contains MHC II protein, which serves as an identification tool for immune cells. In contrast to B cells, T cells are limited in their ability to identify antigens that have been prepared and shown by antigen-presenting cells. There are two categories of antigen processing (Owen et al. 2013). Upon activation by antigen-presenting cells, T cells perform their specific duties based on whether they are CD4 or CD8 cells. Similar to B-cells, activated T-cells also go through colonel expansion, which generates memory T-cells for this antigen's upcoming infections as well as more effectors T-cells for the ongoing infection (Esser et al., 2003)

Types of Immunity on the Basis of Source

Immunization can be achieved using either active or passive methods. These resources may come from man-made or natural sources. Environmental, human, and animal exposures are the cause of natural resources. Artificial sources, on the other hand, result from medical procedures (Marshall et al., 2018). Transferring produced antibodies to a person who is not immune results in passive immunity. The existence of these produced antibodies would then cause this person to temporarily become immune to a certain organism or toxin (Vickers et al., 2017). The person would no longer be immune to this poison or microbe after these produced antibodies were eliminated (Marshall et al., 2018).

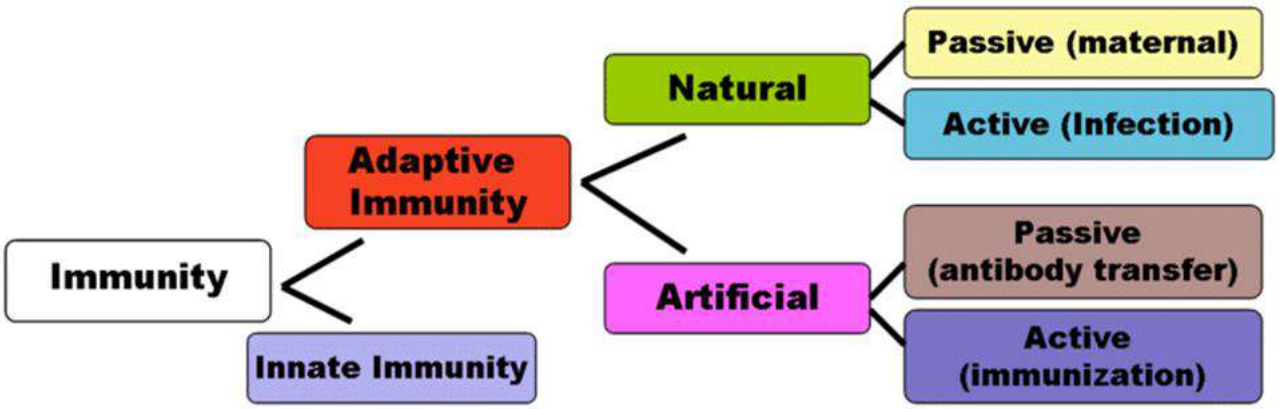

Fig. 2: Flowchart of immunity and its types

Passive immunization can occur either naturally or artificially the transfer of maternal antibodies to the fetus through the placenta and the transfer of these antibodies to the child through colostrum and milk are two excellent instances of spontaneous passive immunity. The infusion of pooled human immunological gamma globulin and antivenin are two excellent instances of artificial passive immunization (Tizard, 2021). These antivenins and gamma globulins offer transient immunity to venom or a specific disease. In addition to these transient immunity-producing effects from N to the substance and facilitate antigen-presenting cells like macrophages or monocytes in absorbing it. The pathogenic agent's antigens are processed by antigen-presenting cells, which attach the processed antigen and MHC protein to their surface (Kotsias et al., 2019).

If the antigen is viral, it will attach itself to the MHC I protein and be delivered to a CD8 cell by the antigen-presenting cell, which will probably start cell-mediated immunity. When an antigen from a bacterium or parasite is attached to the MHC II protein and delivered to a CD4 cell by an antigen-presenting cell, antibody-mediated immunity is most likely to be triggered (Lillehoj, 2018). First, laboratory-weakened forms of the original pathogenic agent are present in live, attenuated

vaccinations, such as those for chickenpox, mumps, and measles. First, laboratory-weakened forms of the original pathogenic agent are present in live, attenuated vaccinations, such as those for chickenpox, mumps, and measles (Pişkinpaşa and Karasakal, 2021). Viruses have fewer genes than bacteria, making it easier to manage their properties. Live, attenuated vaccines are created with viruses, but refrigeration is needed to maintain efficacy due to potential pathogenic reversion. Furthermore, because live vaccinations induce genuine illness, they cannot be administered to those with compromised immune systems. As the inactivated influenza vaccine serves as an example, inactivated vaccines are created by using heat, radiation, or chemicals to eliminate a pathogenic agent. The microbe is rendered inactive, increasing the stability of the vaccine (Sandle, 2023). These vaccinations can be freeze-dried for transportation and don't need to be refrigerated. Nevertheless, because these vaccinations result in weakened immune responses, booster doses to maintain protection.

A vaccine derived from irradiated Listeria monocytogenes bacteria, as opposed to bacteria that had been heat-killed, protected mice against a challenge with live Listeria in trials. The irradiation vaccine triggered a protective response from T-cells, a unique feature only found in live, attenuated Listeria bacteria-derived vaccinations (Zhang et al., 2023). Subunit vaccines, such as the recombinant hepatitis B vaccine, only include the sections of antigens known as epitopes that are most easily recognised and bound by antibodies or T cells, hence stimulating the immune system (Rammensee et al., 2002). The limited number of specific antigens used in vaccinations reduces adverse response risk, but this specificity makes it challenging to choose which antigens to include in the shot.

Toxoid vaccines, such as the tetanus and diphtheria vaccines, are made by formalin-inactivating bacterial toxins. In opposition to the bacterial toxins, these toxoids elicit an immunological response (Kit, 2015). A unique kind of subunit vaccination is conjugate vaccines, such as the *Haemophilus influenzae* type B (Hib) vaccine. A conjugate vaccination works by attaching a microbe's antigens or toxoids to polysaccharides from its outer coating in order to boost immunity, particularly in young children. The development of naked DNA vaccines is still in its early phases. To boost protection, these vaccines would employ DNA unique to microbial antigens. An injection would be used to deliver this DNA, which would then be absorbed by bodily cells (Lai and Bennett, 1998). The immune system would then be stimulated by these bodily cells when they began to produce the antigen and exhibit it on their surfaces. These vaccinations would result in a robust immune system reaction to the free antigen as well as a robust cell-surface response to the microbial antigens (Maurice and Davey, 2009). Additionally, the creation and production of these vaccines are thought to be reasonably simple and affordable. Herpes and influenza are still in the early phases of development for naked DNA vaccines.

Recombinant vector vaccines are experimental vaccinations that deliver microbial DNA into body cells using a microbe or an attenuated virus. These viral vaccines would imitate a real infection with ease, therefore boosting immunity. It is also possible to introduce genetic material for harmful microbe antigens into attenuated bacteria (I. Nascimento and L. Leite, 2012). The innocuous microorganism would then exhibit these pathogen-derived antigens, imitating the infection and boosting the immune system. Measles, rabies, and HIV vaccines using bacterial and viral recombinant vectors are currently in the experimental stages of development.

Vaccine Adjuvants

Studies have looked at the idea of developing vaccine adjuvants that target the innate immune system in addition to these vaccines. Adjuvants can be categorised into two groups: immunological potentiators, such cytokines or PRRs, or delivery mechanisms, like cationic microparticles. Antigens and immune potentiators may be localised with the use of the delivery systems, which may also be employed to concentrate and show antigens in repetitive patterns, and to direct the vaccine's antigens towards the cells that present antigens (Kit, 2015). On the other hand, the innate immune system is directly activated by the immunological potentiators.

Strategies of Immunization

The routine vaccination must be included in all national immunization programs. Usually, mobile teams and outreach sites as well as permanent locations perform routine vaccinations. The staff of the medical institution administers vaccinations on a regular basis and is equipped with a refrigerator. This is supposed to cover everyone living five km around the fixed location (Organization, 2015). Personnel working in healthcare facilities provide vaccinations on a regular and planned basis. They move in a passive cold chain, carrying vaccine carriers. The outreach strategy can cover populations living from 5 to 15 km from the health care facility. Mobile teams that follow a predetermined itinerary go around to carry out scheduled immunization operations. A significant amount of resources are provided for the teams' training, transportation, and vaccine storage (cold boxes and carriers) (Danet and Fermon, 2013). The far people (> 15 km) that one wishes to reach in advance must be informed and careful planning is needed for this method. Campaigns for vaccination are one-time events that set up several sites for vaccinations, enabling a large number of individuals to receive vaccinations in a short amount of time. When there is a high danger of an epidemic (such as among displaced persons), they are carried out as a preventative step as part of catch-up programmes, or as a reaction once an outbreak has been identified. The campaigns demand excellent partner coordination and the mobilisation of large numbers of people and resources (Corinne Danet, 2013). Campaigns to "catch up with" kids who missed their regular vaccinations are meant to provide a second dose of the vaccine to kids who missed it. They are planned and carried out as part of the global measles control effort every two to four years. One element of epidemic control is the outbreak reaction campaign (Organization,

2023). Its goal is to immunize every member of the at-risk community as soon as feasible in order to stop the epidemic from spreading.

Conclusion

The process of vaccination and development of immunity and the strategies opted for better results all these aspects are intermixed with each other. The vaccination method proved to be the best blessing of the science over the time. As it prevents the hazardous out comings of the deadly infections and this was acquired by using the deadly infectious agent. How weird it sounds. These are only possible in the area of research. Above studies shows that how we can manage the disease before its spread, we can produce immunity in order to enable the human body to fight against the strong pathogen when it get exposed to it. All these phenomena can happen only when we monitor the whole procedure from start to end. It was the effect strategies what made it possible to eradicate the smallpox from the world. It is a product of efficient working. In order to continue such brilliant workings, scientists need to create more powerful vaccines, study the human immune system more closely and make a layout which would allow eradicating more and more deadly microorganisms from the world.

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Chapter 17

Harmony of Health; Vaccination and Immunization in the Realm of Complementary and Alternative Medicine

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ABSTRACT

Vaccination is a simple, safe, and artificial method to protect us against harmful diseases, whereas immunization is a procedure through which individuals become resistant to infectious diseases. Vaccinating animals is vital for public health, disease management, prevention of zoonosis, and higher production. Zoonotic diseases (e.g., rabies, anthrax, brucellosis, etc.) are a great matter of concern. Today, many certified veterinary vaccines are live attenuated, killed/inactivated pathogens or plasma membrane components. Sometimes, some abnormal physiological response to the introduced antigen, such as rash, etc., is termed hypersensitivity. The public is genuinely concerned about the side effects of vaccinations. Nutrition is capable of improving immune function. The exact relationship between health and disease and the connection between nutrition and immunology proved to be significant. Phytogenic substances such as cinnamon, turmeric, or oregano used in poultry feed have been proven to enhance the immune response. Diet's fiber content dramatically affects the microbial community, and the products from the microbes have unavoidable effects on the immune system and immune responses. Acupuncture is also closely related to immunity in defense mechanisms, homeostasis, and surveillance. PNI can also have effects on the immune system.

KEYWORDS
Vaccination; Immune system; Nutrition; Acupuncture; Immunity

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INTRODUCTION

Vaccination is a simple, safe, and artificial method to protect us against harmful diseases. Inoculating an antigen for priming our immune system to enhance the immunity against a disease is termed vaccination. When given as an element of extensive prevention programs, vaccination is economical, can benefit animal effects and health, and may prove growth and production on animals (Roth, Jof et al., 2011). Microorganisms or viruses can be inoculated in a weakened, live, or killed state to develop an immune response. Immunogenic part of the virus genome proteins or toxins from the organism can also be used for vaccination. Immunization is a procedure through which individuals are made resistant to infectious diseases. Immunization is attained by either active or passive immunity. Active immunity is achieved through vaccination, which induces humoral (antibodies generated by B-cells to fight invading organisms) and cell-mediated immune responses (T-helper and CD4 cells work against the pathogens) to give protection, similar to that derived from natural infection (Galiza, E and Heath, P 2021). The immune system responds to that stimulus once the person is vaccinated for a specific disease. It generates antibodies that create immunity against that antigen and make us immune against a specific disease. In contrast, by transferring antibodies, passive immunity provides short-lived protection. This procedure occurs naturally when IgG is passed to the fetus through the placenta during pregnancy (Galiza, E and Heath, P 2021).

Role of Vaccines in Controlling Infectious Diseases

One of the most vital public health achievements of the 20th century is vaccination. Vaccinating animals is essential for public health, disease management, prevention of zoonosis, and higher production. Zoonotic diseases (e.g., rabies, anthrax, brucellosis, etc.) are a great matter of concern. Almost 60% of diseases in humans are of zoonotic origins and cause high mortality rates, causing epidemics and pandemics (Taylor et al., 2018). Vaccinating domestic animals to prevent

their zoonotic effects and vaccinating wildlife to tackle disease transmission to humans and animals are the main strategies of vaccination programs (Monath T.P et al., 2013). Vaccines not only prevent the disease but also contribute to the notion of herd immunity, which protects those who cannot be vaccinated for medical or age reasons, such as newborns and those with weakened immune systems. Vaccinating animals control diseases in companion and livestock animals, making food supplies secure by keeping healthy livestock populations, and proves to be a protection in preventing the transmission of zoonotic diseases to humans. (Gutiérrez et al., 2012). Numerous formerly prevalent and harmful diseases have been considerably declined or eradicated due to mass vaccination campaigns, improving worldwide public health outcomes. It is estimated that there will be a 69% increase in bovine meat production and an 181% increase in poultry meat to meet the population's needs by 2050 (Alexandratos et al., 2012). Immunizing animals against transmissible diseases aids in avoiding outbreaks that can devastate the farming sector, resulting in significant economic losses and food supply disruptions, as well as increasing animal productivity and growth rates, which leads to better productivity in farming and supply chain management.

Conventional Vaccination Methods

The research field of vaccinology has developed numerous successful vaccinations that have significantly decreased the occurrence of highly fatal diseases in companion animals and livestock. Today, many certified veterinary vaccines are live attenuated, killed/inactivated pathogens or plasma membrane components. Historically, trial-and-error methods have been used to develop veterinary vaccines to mimic the immunity produced by natural infection through vaccination (Doolan et al., 2014). The conventional technique that "isolate, inactivate, or kill and inject" tends to offer protection against various bacterial and viral diseases. Traditional vaccinations, on the other hand, are often more expensive to make and must be delivered numerous times in order to provide sufficient immunity.

Infecting an artificial host or cell with viruses or bacteria used to make live attenuated vaccines. After repeated passages of the viral or bacterial strain in various mediums, the strain is supplied to the natural host with an anticipation that random mutation has produced a non-virulent and replicative infectious agent (Meeusen et al., 2007). Live attenuated vaccines (LAVs) can be extraordinarily successful since they initiate cellular and humoral immune responses (de Costa, Walker, and Bonavia, 2015).

Pathogen genome sequencing and a better knowledge of pathogenesis mechanisms have led to the discovery of unique antigens and the creation of recombinant veterinary vaccines. Thus, genome breakthroughs have given rise to a "third generation" of vaccines created using novel approaches, such as reverse vaccinology (Dellagostin et al., 2011; Rappuoli et al., 2014). This method has been proven to help discover an increasing number of vaccine candidates, including recently discovered numerous proteins. Next-generation vaccines are multivalent, highly purified, deliver a secure profile, and offer a viable alternative to the more reactogenic whole-cell vaccines (Oliveira et al., 2015).

Adverse Immunological Effect of Vaccine

The public is overly concerned about the side effects of vaccinations. Events reported after vaccination are known as vaccine-adverse events, but a convincing connection between them and the vaccine has not yet been established via science. Incidental events are included in the category of vaccination adverse events, while reactions are those for which a scientific explanation exists. In Japan, the Health, Labor, and Welfare ministries report adverse reactions to vaccines without any causality assessment. As a result, all reported vaccine-adverse events are vaccine-adverse reactions (Testuo Nakayama, 2019).

MMR (measles, mumps, and rubella) combined vaccination was introduced in 1989; however, it was withdrawn from use in 1993 because of an unanticipatedly high incidence of aseptic meningitis caused by the mumps vaccine components (Testuo Nakayama, 2019). Reports of anaphylactic reactions in 1994, following live vaccine shots, were made. Serum samples from patients suffering from allergic illnesses were collected, and post-marketing activity was improved. Acute disseminated encephalomyelitis (ADEM) was linked to a possible risk of allergic encephalitis and was observed following immunization with the Japanese encephalitis vaccine made from mouse brains. This led to the creation of a tissue culture-based vaccination unveiled in 2009 (Testuo Nakayama, 2019).

Sometimes, some abnormal physiological response to the introduced antigen, such as rash, etc., is termed hypersensitivity. Ig E is responsible for Type I hypersensitivity, or immediate hypersensitivity to vaccine in the patients. It triggers mast cell degranulation and histamine release in response to a vaccine antigen, even though it is currently understood that mast cell degranulation can happen without Ig-E. The most significant and severe adverse event following immunization is IgE-mediated anaphylaxis.

Unlike medications, excipients significantly affect the particular Ig E and acute reactions linked to vaccinations. Though they happen in less than one instance per million doses given, acute hypersensitivity reactions to vaccinations fit the criteria for Ig E-mediated reactions. Egg, gelatine, or, more recently, galactose-α, 1,3, galactose, often known as alpha-gal, are the vaccine vehicle; an allergy to them is the main predictor and mechanism of acute hypersensitivity after immunization. This is a significant departure from medication-related anaphylaxis since the excipient is typically unrelated to the active drug component (Cosby et al., 2019).

In people with a genetic predisposition, autoimmune disease may be induced by a wild superimposed infectious agent or a vaccine component (an inactive viral or bacterial agent or attenuated living microorganism). The causes,

mechanisms, and pathways of autoimmune disease remain unclear. For example, rheumatic heart disease and persistent arthritis can be caused by group A-hemolytic streptococcus and Borrelia burgdorferi, which are present in the S. pyogenes and Lyme disease vaccines, respectively. The immune response to certain self-antigens can be systemic, affecting several tissues and targeting a range of expressed auto-antigens, or tissue-specific, affecting only one or a few (thyroid, pancreatic β-cells) (Maria Vadala, 2017).

Among other side effects, vaccines can cause skin rashes and ulcers, miscarriage, and fetal death, even while they help animals become more resistant to natural infections. Due to the discovery that some vaccinal strains retain their virulence, Russia has outlawed LAVs for LSD, which are derived from homologous viruses. Limited attenuation of the live LSD vaccine, elevated (A. Selim et al., 2021)

An epidemiological study using a questionnaire to ask pet owners about canine vaccine reactions was conducted in 2004 by the NOAH (National Office for Animal Health), an industrial body. The study specifically addressed whether there was an increased prevalence of illness in dogs in three months after vaccination, a question that had been brought up by one of the public interest groups (Edwards et al., 2004).

CAVM: The Immune System

In complementary and alternative medicine (CAVM), the immune system is essential to one's general health. Botanical medicine, acupuncture, and homeopathy are a few practices that aim to promote the immune system's function organically. The techniques above aim to boost immunological responses for overall wellness by resolving underlying imbalances and promoting harmony within the body.

The immunity in the body is established and regulated by two different cellular systems. These include lymphocytes, which are made by bone marrow and thymus. The body's lymphatic organs, such as the spleen and lymph nodes, also aid in maintaining immunity. They are derivatives of the bone marrow's reserve of stem cells and create a circulating or humoral immune system formed by B cells (bursa-dependent or bone marrow-derived), as well as a cellular or cell-mediated defense system produced by T cells (thymus-dependent) (Tizard and Schubot, 2000).

Circulating antibodies or immunoglobulins such as IgG, IgM, IgA, Ig D, and Ig E make up the body's B-cells (humoral immunity). They are a vital defense pathway against pathogens in healthy individuals.

T cells function as coordinators and effectors of the immune system, making cellular-mediated immunity. Immunity mediated by cells includes lymph nodes, thymus, spleen, intestine (gut-associated lymphoid tissue), tonsils, and IgA-mediated mucosal secretory immunity (Dodds W.J et al., 1992). Helper T-cells, cytotoxic T-cells, and suppressor T-cells are the three main types of T-cells. The helper T-cells "aid" in coordinating immune response activity, while the cytotoxic T-cells constitute the network of effectors that helps to eliminate virus-infected cells from the body. The third type of suppressor, T-cells, plays a vital part in diminishing the immune response when the immune system becomes hyperactive or loses control. Lastly, coordination between the different T-cell classes and between B and T cells is crucial to the effective humoral and cellular immune response, respectively (Dodds W.J et al., 1992).

Nutritional Effects on the Immune System

Nutrition is capable of improving immune function. The exact relationship between health and disease and the connection between nutrition and immunology proved to be significant. (Di Cerbo et al., 2017). Diets and feeds are formulated for companion animals and livestock to attain optimal growth, disease management, and reproduction. Sound health is vital for required production across all livestock species. A fully functional immune system is necessary for animals to fight against environmental and health challenges. Both innate and adaptive immunity and the neuroendocrine system maintain homeostasis and keep the production at elevated levels. The development of these systems requires appropriate nutrition. The prenatal diet also has a significant role in developing the immune system; according to the research done by Moriel et al. (2016), a reduction of 70% in energy requirements of the dam (beef cows) for the last forty days of gestation caused impaired humoral response to vaccination against bovine respiratory vaccine. In another research by Marques et al. (2017), supplementing beef cows with omega-3 and omega-6 for the last trimester of gestation positively affects immune-modulated development. These studies showed a complex relationship between immunity and diet, and an appropriate diet can positively affect the immune system.

Regarding nutrition, meals are carefully planned for companion animals and cattle, and feed is ingested to meet the requirements of growth, maintenance, and reproduction. Furthermore, from the standpoint of resource allocation, it has been highlighted that selection for efficient growth and reduction of unnecessary immune reactivity in favor of growth are essential for livestock species. From an immunological perspective alone, the tools for studying cattle and companion immunity are less sophisticated than those for human and mouse models. Some vitamins and minerals may be added that are better for your health than what is recommended. The adaptive and innate immune system segments mediate the immune system's short-lived and long-lived pathogen response capabilities (Domínguez-Andrés et al., 2019).

Role of Vitamins

Vitamin E is a fat-soluble antioxidant with the ability to modify signal transmission and regulate the production of ROS (reactive oxygen species) and RNS (reactive negative species). It also prevents the oxidation of polyunsaturated fats (PUFAs) in cellular membranes. In both healthy and diseased animal and human models, vitamin E has immunomodulatory

effects. Deficit in vitamin E is associated with decreased function and proliferation of lymphocytes. Older mice given a diet containing 500 mg of vitamin E instead of 30 mg/kg showed better lymphocyte proliferation, IL-2 production, T cell-mediated immunity, and reduced prostaglandin E2 (PGE2) production, inhibiting T cells.

Vitamin D receptors and hydroxylases, essential for the development of bones, are found in tissues and cells unrelated to the metabolism of minerals and bones. At physiological doses, vitamin D stimulates innate immunity, promoting monocyte proliferation and macrophage chemotactic and phagocytic activity. In order to strengthen the innate antimicrobial response, vitamin D is essential. Increased vitamin D receptor and 1-α-hydroxylase expression results from binding to toll-like receptors. In addition to being an overall inhibitor of B and T cell development as well as the synthesis of IF-gamma and IL-12, two crucial T cell cytokines, vitamin D also regulates monocytes, neutrophils, and epithelial cells that controls the production of endogenous antimicrobial peptides (Grimble, 2001; Aranow, 2011).

Critical biological processes that impact healthy cell integrity and function, development, growth, metabolism, and immunological resistance in both adaptive and innate immune systems depend on zinc. Zinc deficiency causes thymus involution and functional compromise in almost all immune cell classes. Therefore, premature involution compromises immunosurveillance, increasing the risk of disease. Thymic involution is reduced in thymic bulk, which lowers function and is usually correlated with age.

One of the best-known supplements for immunomodulation is omega-3 fatty acids. The source is crucial when selecting an omega-3 PUFA supplement to boost immunity. Plant sources provide alpha-linolenic acid (ALA), which is frequently added to animal diets in the form of flaxseed. Fish and algae are examples of marine sources, including the compounds EPA (eicosatetraenoic) and DHA (docosahexaenoic acid). Non-ruminant animals lack enzymes that convert all ALA to bioactive EPA and DHA. Therefore, non-ruminant animals need to be fed to obtain immunological benefits. Omega-3 polyunsaturated fatty acids (PUFAs) have strong anti-inflammatory qualities. These include their capacity to prevent the production of inflammatory mediators such as eicosanoids (PGE2, 4-series leukotrienes), pro-inflammatory cytokines (IL-1ß, TNF-α, IL-6), chemokines (IL-8, intercellular adhesion molecule-1 [MCP-1], adhesion molecules (intercellular adhesion molecule-1 [ICAM-1], VCAM-1 vascular cell adhesion molecule-1 [VCAM-1], selectins, platelet-activating factor, ROS, and RNS. (Calder, 2007).

Phytogenic

Phytogenic are the plant-derived substances used in animal nutrition to promote animal growth and improve immunity. Phytogenic includes naturally occurring herbs, plants, spices, and their derivatives. Phytogenic substances such as cinnamon, turmeric, or oregano used in poultry feed have been proven to enhance the immune response. These improvements include high FCR (feed conversion rate) and lymphocyte count (Lee, 2018). The photogenic mechanism increases the stress level by inhibiting the mitogen-activated protein kinase and nuclear factor kappa-light-chain-enhancer of activated B cells. It also increases the anti-inflammatory cytokines. Due to this mechanism, the heterophils (nonspecific immune cells) are reduced, and antibodies are increased to fight against the pathogen entered into the body (Zhai et al., 2018).

Green Tea is also an essential photogenic. Epigallocatechin-3-gallate (EGCG) is an important part of green tea that increases the IL-10 and Treg differentiation and promotes expert regulators in T-cell development. EGCG also decreases neutrophil migration and D.C. maturation. As a result of these changes, the immune system reduces inflammation (Grimble, 2001; Wu et al., 2018a).

Probiotics

Recent developments in immunology and microbiology showed us that these fields are closely related (Maslowski and Mackay, 2011; Hooper et al., 2012). Diet's fiber content dramatically affects the microbial community, and the products from the microbes have unavoidable effects on the immune system and immune responses (Makki et al., 2018). Butyrate is essential as it increases barrier function and IgA production and promotes an anaerobic environment. However, due to the use of antibiotics, there is a reduction in the anaerobic bacteria, and hence, it decreases the butyrate concentration, which promotes an aerobic environment that promotes the growth of aerobic bacteria such as Salmonella (Parada Venegas et al., 2019). It also has effects on the respiratory immune response as well.

Supplements with probiotics interact with gut mucosa, M cells, Peyer's patch, and D.C.s by affecting mucosal respiratory and immune system response and reducing pro-inflammatory cytokines. It also has effects on the respiratory immune response as well. Regulating the role of T and B cells by using probiotics is strain dependent. Long-duration continuous supplementation is essential to see the effect of probiotics (Ganguly, 2013; Baffoni, 2018; Ma et al., 2018; Li et al., 2019)

Mind Body Practices for Immune Support

Psychoneuroimmunology (PNI) is a field that investigates the relationship between psychological factors and the neuroendocrine and immune response (Dantzer, 2010). CNS and the immune system interact; negative emotions generated by the stressful environment can dysregulate immunity by changing the coordination level between both systems (Glaser and Kiecolt-Glaser, 2005). Despite that, the immune system can be divided into two branches: adaptive immunity (repetitive, specific) and innate immunity (nonspecific and natural). Through the presentation of antigens, innate

immune cells aid in the facilitation of specific (memory) immune responses. In contrast, adaptive immune cells can release cytokines and other messenger molecules that control the activity of innate immune cells. Exercise significantly impacts the quantity and make-up of leukocytes in circulation in a single session. Even short dynamic exercise (minutes or less) can frequently result in a two- to three-fold increase in the total leukocyte count.

In contrast, more extended endurance exercise (three to five hours) might result in a five-fold increase in leukocyte count. (J.P. Campbell et al., 2009). The immune system's innate component consists of both soluble substances and cells. Using chemotaxis, innate cells—like neutrophils—are drawn to areas of infection or inflammation, where they eat and eliminate microorganisms by phagocytosis.

Hetts et al. (1992) investigated the impact of additional exercise (either alone or in conjunction with a conspecific) on immunological, endocrine, and behavioral responses on dogs' physical and mental well-being. Four treatments were given; exercised alone (E.I.), exercised with a conspecific (E.C.), nonexercised (N.E.), or cage control (CC)—and applied to 40 mature male beagles that were purpose-bred. The following parameters were regularly measured: body weight, peripheral blood mononuclear cell subsets, WBC count, humoral immune responses to the antigen keyhole-limpet hemocyanin, and plasma cortisol concentration. E.C. dogs exhibited smaller percentages of B lymphocytes. Nonetheless, independent of the type of treatment, some physiological and behavioral variables altered with time.

Immunology and Acupuncture

Acupuncture

Acupuncture is Traditional Chinese Medicine (TCM) (Zhang and Li, 2021) (Zhu et al., 2021); it is a practice that involves inserting skinny needles at specific points in different locations of the body at different depths in specific conditions like Pain, Stress, Nausea, Headaches, Neurogenic bladder dysfunction, postoperative pain, Stroke, Insomnia, Nervousness, Neurosis, etc. (Lin et al., 2022). In TCM, it is believed that these specific points are interconnected by pathways that together form a flow of energy, which in TCM was referred to as "Qi," pronounced as "chi." According to TCM philosophy, this energy flow is essential for sound health. Acupuncture is also important in veterinary medicine (Dewey and Xie, 2021); most veterinarians use this practice in their treatment plans for relieving pain, stress, etc., in animals.

Acupuncture in Veterinary Medicine

Acupuncture is now also used for animals in various conditions, such as pain relief, neurological impairments, GIT issues, behavioral issues, respiratory issues, and immune responses (Huntingford and Petty, 2022). The technique is the same as used in humans: thin needles are inserted at specific points in the body that are interconnected and form a flow of energy, which is beneficial for good health (Mangan, 2023) (Medina and Goldberg, 2024).

One of the main reasons for using this technique is to manage pain, as it is beneficial in conditions like arthritis, skeletal issues, postoperative or postsurgical pain, etc. Acupuncture is also used in neurological issues like IVDD or neurologic injuries. Similarly, some veterinarians use this technique in GIT issues to relieve pain. The primary mechanism behind this is still under consideration and unclear; however, this practice helps minimize GIT (Gastrointestinal Tract) problems (Kim et al., 2023). In the same way, respiratory problems like asthma in felines and bronchitis at a chronic level in dogs can be treated to some extent with acupuncture. It is also helpful in managing behavioral issues like stress, anxiety, and aggression, and it also supports the immune system and regulates it by enhancing anti-cancer and anti-stress, immune function, overall providing well-being and improving the welfare of animals (Kim and Bae, 2010).

Acupuncture and Immunity

Acupuncture is closely related to immunity (M. Wang et al., 2023) in defense mechanisms, homeostasis, and surveillance. Some research has shown that this technique is helpful in regulating immune response, for example, by enhancing anti-cancer and anti-stress factors and exerting an anti-inflammatory response (Li et al., 2021).

The one acupuncture point known as ST36, also called "Zusalni," is now widely applied in immunosuppressive diseases (J. Wang et al., 2023). It was found that electro-acupuncture (E.A.) at multiple ST36 points protects the intestinal mucosal immune barrier in various conditions, including sepsis and G.I. problems. E.A. at ST36 is known to mitigate intestinal injuries. E.A. exerts an immune response to the intestinal mucosal immune barrier by raising the concentration of Ig A, CD3+, γ/δ, CD4+, and T-cells. This also increases the ratio of CD4+/CD8+ T-cells, which was found to decrease the risk of mortality by sepsis. The main link between acupuncture and immunity is still debatable.

Risks and Benefits

The possible risks of acupuncture include:

●Bleeding, injury, cramps, discomfort, etc.

●Unsterilized needles may lead to infection, which may be fatal in severe cases.

●If the therapist or veterinarian is not skilled, the wrong or in-depth insertion of the needle may damage internal organs or nerves.

The benefits of this technique include:

●Remedy for various diseases and conditions like Pain, Stress, Nausea, Migraines, Neurogenic bladder dysfunction, Postoperative pain, Stroke, Nervousness, Neurosis, etc.

●It is an adjustable form of treatment that can target multiple conditions at a time.
●It possesses a minimal risk of side effects.
●Immune system function and metabolism can be accurately assessed based on the preference in the fuel section of immune cells (Elizabeth, 2020).

Vaccines are a Vital Tool against AMR

Antimicrobial resistance (AMR) is an emerging threat worldwide, and AMR happens when anti-microbials become ineffective against microbes as they change or find a way to escape from antimicrobials. Resistance to pathogens is a naturally occurring, rapid phenomenon. Vaccines are the prophylactic measure and prevent the disease from happening. When there are infectious diseases, the use of antimicrobials will reduce, hence reducing the AMR (Micoli et al., 2023). For example, Haemophilus influenzae type B (Hib) and pneumococcal conjugate vaccines are success stories as they have reduced the use of antimicrobials and ultimately reduced the AMR (Peltola et al., 1990). Vaccine can also save non-vaccinated individuals by the phenomenon of "herd immunity."

Bacteria is having q negative impact on the development of vaccines as they have a great genetic diversity, and they can cause multiple infections depending on the host and environment conditions. This occurs due to the heterogeneity of genes of many pathogens that leads to change in structure of proteins and polysaccharide antigens, challenging vaccine development (Bekeredjian-Ding, 2020). It is the need of the hour to tackle AMR and to control bacterial adverse effects on vaccines, to control morbidity, mortality and for the animal welfare.

Conclusion

The public is genuinely concerned about the side effects of vaccinations. The conventional technique that "isolate, inactivate, or kill and inject" tends to offer protection against various bacterial and viral diseases. Traditional vaccinations, on the other hand, are often more expensive to make and must be delivered numerous times in order to provide sufficient immunity. Different alternative techniques can be fruitful in immunizing animals against various fatal diseases. More research should be done to check the procedure and quality of alternative medicine immunization.

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Chapter 18

mRNA Vaccine Mechanisms: Unraveling the Biological Wonders

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ABSTRACT

Over the past few years, messenger RNA (mRNA) vaccines have evolved from an idea that generated doubt to a practical reality in clinical practice. The Coronavirus Disease-19 (COVID-19) pandemic in 2020 resulted in the most rapid development of vaccinations ever recorded, with mRNA vaccines taking the lead in these efforts. When it comes to overcoming the fundamental limitations of unprotected mRNA molecules, nanoparticles have proven to be an efficient technique for delivering mRNA vaccinations. The nanoscale carriers offer protective and delivery functions, enabling effective absorption by cells, safeguarding against enzymatic breakdown, and allowing controlled release of mRNA payloads. Although mRNA vaccines have demonstrated their efficacy and safety in protecting patients from infectious diseases, additional research is required to improve the design of mRNA and its intracellular distribution, and its potential applications beyond preventing SARS-CoV-2 (severe acute respiratory syndrome–coronavirus 2). The COVID-19 pandemic has led to an increased support and interest in mRNA vaccinations. It is essential to acknowledge that mRNA vaccines represent a relatively novel technology, and ongoing research is being conducted to determine their long-term effects on different diseases and regenerative medicine.

KEYWORDS
mRNA; Vaccine; Immunization; COVID-19; Lipid-based nanoparticles

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INTRODUCTION

Proteins are essential for the execution of almost all bodily functions. Although deoxyribonucleic acid (DNA) carries the necessary information for protein synthesis, it is actually mRNA that performs the task of protein creation. Vaccines are a highly effective weapon that humans employ to treat infectious diseases caused by bacteria or viruses. Traditional immunizations are often manufactured using live-attenuated or inactivated germs, or subunit antigens derived from pathogenic microorganisms (Francis, 2018). Traditional immunizations have successfully prevented the transmission of contagious illnesses such as smallpox and polio. However, the process of developing and producing them on a large scale can be time-consuming, making them impractical for quickly spreading infectious diseases such as Severe Acute Respiratory Syndrome (SARS) and Coronavirus disease-19 (COVID-19) (Li et al., 2022).

The concept of nucleic acid vaccines utilizing mRNA was developed over thirty years ago with the aim of creating vaccinations that are both safe and adaptable, while also being simple to manufacture. mRNA vaccines possess numerous advantages compared to traditional immunizations (Aleem et al., 2022). Despite certain viral immunizations, mRNA does not integrate into the DNA, therefore avoiding worries about insertional mutations. mRNA vaccines may be manufactured rapidly, efficiently, and at a large scale without the requirement of cells, resulting in cost-effective production. mRNA vaccines are composed of mRNA strands enclosed in ~~a~~ lipid-based nanoparticles (LNPs) with a neutral charge (Pardi et al., 2020).

A Short Overview of Vaccines

Ever since Edward Jenner conducted his revolutionary vaccination research in the late 1700s, the discovery of vaccines and the implementation of widespread immunization programs have been the global community's way of fighting infectious disease epidemics. The COVID-19 pandemic is not an exception in this regard. However, it is noteworthy that the worldwide effort to find a vaccine for SARS-CoV-2 has led to the emergence of a novel category of vaccine products. The leading candidates in the race to develop a vaccine are mRNA nanoparticles. This is a significant breakthrough in the field

of vaccinology. For example, the initial vaccines approved by the World Health Organization (WHO) induced immunity by introducing weakened or fully inactivated viruses through injection. Newer vaccines, such as those for human papillomavirus (HPV), Hepatitis B, or seasonal flu, no longer contain whole virus particles. Instead, they contain purified or genetically engineered viral proteins. These proteins are delivered along with immune adjuvants to enhance the vaccine's ability to stimulate an immune response (Verbeke et al., 2021).

mRNA vaccines offer many significant benefits in comparison to traditional vaccinations, such as those based on live and weakened pathogens, subunit-based vaccines, and DNA-based vaccines. One of the advantages of mRNA is its safety, as it does not integrate with the host DNA. The mRNA vaccine is non-infectious and does not interact with the host DNA. It can be made more stable and effective by modifying the mRNA structure, resulting in reduced immunogenicity. Additionally, mRNA vaccines can be produced rapidly, at a large scale, and in a cost-effective manner due to their manufacturing process in a cell-free environment (Gote et al., 2023).

mRNA Structure

The eukaryotic mRNA contains several essential structural components that are necessary for its functionality. These include the five-prime cap (5′ cap), the five-prime untranslated region (5′ UTR), the open reading frame (ORF) region or coding region, the three-prime untranslated region (3′ UTR), and the poly A tail structure. Keeping the structure of mRNA intact is good for both its safety and its ability to be expressed. Changing the mRNA code based on its full structure is another way to improve the effectiveness of an mRNA vaccine. In any case, mRNA in vitro transcription produces a mix of targeted mRNA, untargeted RNA, nucleotides, oligodeoxynucleotides, and proteins. To clean up the mRNA by getting rid of common impurities, precipitation and extraction methods are used. Chromatographic methods are usually employed in this method to separate the target mRNA from other ~~m~~RNA particles (Xu et al., 2020).

Five-Prime Cap (5′ Cap) Modification

Understanding the importance of the 5' cap structure is essential for enabling most effective protein synthesis, as it plays an important part in stabilizing the mRNA molecule. The natural process involves the addition of a 7-methylguanosine cap (m7G) to the initial nucleotide of an mRNA chain, forming a 5' to 5' connection. Adding the 5' cap can be achieved in two ways: either through a post-transcriptional enzymatic reaction with the help of the vaccinia virus capping enzyme, or through a co-transcriptional response by incorporating synthetic cap. Improvements in translational efficiency and the prevention of unwanted immune responses have been observed through the addition of changes after translation. One such modification is the addition of the cap1 structure, also referred to as 7GpppNm, to an mRNA that is synthesized in a laboratory. The cap of 7GpppNm consists of a methylated guanosine connected to three phosphate groups, followed by the other nucleotide (Le et al., 2022).

Optimization of Untranslated Regions (UTRs)

Considering the selection of untranslated regions (UTRs) is crucial, as they greatly influence mRNA degradation and translation efficiency. Having initiation codons and tertiary forms in the 5' untranslated region can make it harder for ribosomes to join, for the scanning process to work, and for initiation codons to be identified. Hence, it is recommended to refrain from using such characteristics. The 5' untranslated regions (UTRs) usually play a big role in making proteins, while the 3' UTRs are more likely to have an impact on the stability of mRNA. For instance, the 3' untranslated region (UTR) of the β-globin gene and a form of the β-globin 3' UTR that has been doubled are often used to make mRNA last longer (Kon et al., 2022).

Poly A Tail

A poly A tail, which is made up of many adenosine nucleotides, is added to the 3' end of mRNA through a process called polyadenylation. It is composed of 10–250 nucleotides, and when combined with the poly A binding protein, it forms a complex that enhances translation, mRNA stability, and resistance to nuclease degradation. Protein expression levels and translation efficiency can be affected by the length of the poly A tail. Adenosine chains are often found in the poly A tail of mRNA. However, an additional study showed that mRNA tails having cytosine (C) make mRNA expression last longer and be stronger. The cytosine (C) change can also extend the half-life of mRNA by making it less likely to be broken down (Cheng et al., 2023).

Protein Coding Region

The coding region of mRNA, also known as the open reading frame (ORF), contains the sequence of nucleotides that specifies the amino acid sequence of the protein to be synthesized. The design of the ORF sequence also has a significant influence on the efficiency of translation and the immunogenicity of the mRNA, as it is recognized by cellular sensors. Furthermore, it has been shown that codon optimization can greatly improve protein expression by adding commonly used codons that are more abundant in tRNA (Kon et al., 2022b). Fig. 1 shows the mRNA vaccine consists of 5′ Cap, a 5′ UTR, the gene of interest encoding region, a 3′ UTR, and poly A tails.

Delivery Carriers for mRNA Vaccines

Researchers have examined a variety of approaches for administering mRNA vaccines. For instance, in recent years, delivery vehicles, including those derived from lipids and polymers, have significantly enhanced the cellular assimilation of

RNAs, thus garnering considerable interest. Free mRNA vaccines were also administered (Zeng et al., 2022).

This section is devoted to the technologies utilized in the formulation and delivery of mRNA vaccines, specifically lipid nanoparticles and bare mRNA.

Fig. 1: mRNA vaccine consists of 5′ Cap, a 5′ UTR, the gene of interest encoding region, a 3′ UTR, and poly A tails.

Injection of Naked mRNA as Delivery System

Naked mRNA injection is a method of delivering messenger RNA without the use of carrier molecules. The main benefits of this strategy include the simplicity of preparation, the ease of storage, and the cost efficiency. The freeze-dried naked mRNA can be stored in a suitable buffer, such as one containing 10% trehalose, for a period of 10 months at a temperature of 4 °C, while maintaining its stability. Prior to delivery, the unencapsulated mRNA requires simple recovery and dilution in a suitable buffer, such as Ringer's solution or Lactated Ringers solution. The injection of mRNA without any protective covering is vulnerable to breakdown by RNase enzymes. Additionally, since these mRNA molecules cannot pass through the lipid bilayer, their transport into the cells is constantly a subject of debate (Ramachandran et al., 2022).

Lipid Nanoparticles as Delivery System

Lipid nanoparticles (LNPs) are now the most common way to deliver genes without viruses. mRNA is moved into the cytoplasm by lipid nanoparticles, which are tiny vehicles made up of lipids. The study of mRNA-LNP structures shows that the LNP core contains mRNA, the cationic lipid that can be charged with ions, and water. The neutral helper lipids are mostly found in the membrane that surrounds the cell. The main thing that affects how unstable mRNA-LNP is ~~is~~ how mRNA breaks down. It is still unclear how mRNA interacts with water in the LNP core and how much protection easily broken-down portions of mRNA receive from a coating of ionizable cationic lipids. Prioritizing the optimization of the mRNA nucleotide composition is essential for extending the half-life of mRNA-LNP vaccines (Brader et al., 2021).

Protective delivery techniques have been developed to tackle the challenges of transferring naked mRNA. Currently, all the latest COVID-19 vaccines utilize LNP technology, which is commonly used in mRNA-based vaccines. This showcases the achievements obtained by utilizing this specific nanoparticle to stabilize mRNA and effectively deliver it into cells. The mRNA COVID-19 vaccines consist of four main components referred to as LNPs. These components consist of a phospholipid that is neither acidic nor basic, cholesterol, a lipid with polyethylene-glycol (PEG), and a lipid that can be ionized and carries a positive charge. Similar to a biologist, the molecule contains amine groups that can become positively charged at low pH (Shakoor et al., 2021). These charged groups help in the fusing of membranes during incorporation and enable the molecule to bind with the negatively charged mRNA during particle production. PEG-lipid also acts as a protective barrier to stop clumping during storage and is utilized to regulate the size of the particles. Through the use of a rapid mixing manufacturing technique, the mRNA and other elements come together to form particles that measure around 60-100 nm in size (Schoenmaker et al., 2021).

How mRNA Vaccines Work

Genes that have been transcribed from the genomic DNA found in the nucleus are typically referred to as mRNA in cells. From there, it can be translated by the ribosome located in the cytoplasm to produce the necessary protein. The first step involves using RNA polymerase to convert the genomic DNA into a primary mRNA molecule. The primary mRNA molecule is composed of exons and introns. Further modifications are then performed on the initial mRNA to convert it into mature mRNA. These modifications involve the removal of non-coding introns through splicing, the addition of a cap to the 5′ UTR, and the incorporation of a poly-A sequence located at the 3' terminal (Desterro et al., 2020).

Injecting the vaccines intramuscularly (IM) is a commonly used technique that enables them to penetrate tissues that are below the skin's surface. Following injection, muscle cells come into contact with the LNP-mRNA objects through endocytosis. The mRNA is then translated to generate the metastable trimeric prefusion Spike protein. Furthermore, the activation of invading antigen-presenting cells (APCs) can be facilitated by a complex network of blood arteries adjacent to the muscles (Zeng et al., 2022).

The introduced mRNA undergoes folding to produce a functioning protein. mRNA vaccines that utilize the whole Spike protein generate a signal peptide consisting of amino acids 1 to 15. This signal peptide transports the Spike protein to the plasma membranes or allows it to be released into the cytoplasm. One advantage of mRNA vaccines is that they utilize the cellular translational machinery and other cytosolic components to generate a functional product (Verbeke et al., 2021b). The proteasome produced from endosomes is responsible for the degradation of the majority of proteins. After that, it will be presented to CD8+ and CD4+ T cells as a histocompatibility complex (MHC) class I protein. Dendritic cells undergo assembly and transfer of a histocompatibility complex (MHC) class II protein when they are transfected with an mRNA vaccine or absorbing immunogens. Then, they present the MHC class II protein to immune cells. Nevertheless, mRNA vaccines produce immunization by activating B cells, so triggering a humoral immune response. Following the

contact with specific CD4+ T cells and CD40 binding, new B cells undergo proliferation and differentiation inside lymphoid organs, resulting in the formation of memory B cells or plasma cells that secrete antibodies. B cells that have been produced recently undergo differentiation into plasma cells with short lives and memory B cells that are not now active. Subsequent exposure to the antigen stimulates the production of antibodies by plasma cells, which then work to neutralize the antigen. The antigen prevents the virus from infiltrating target cells. Insufficient availability of antibodies triggers the activation of memory B cells, resulting in the initiation of further immunological reactions (Park et al., 2021). Fig. 2 shows the mechanism of action of mRNA vaccine.

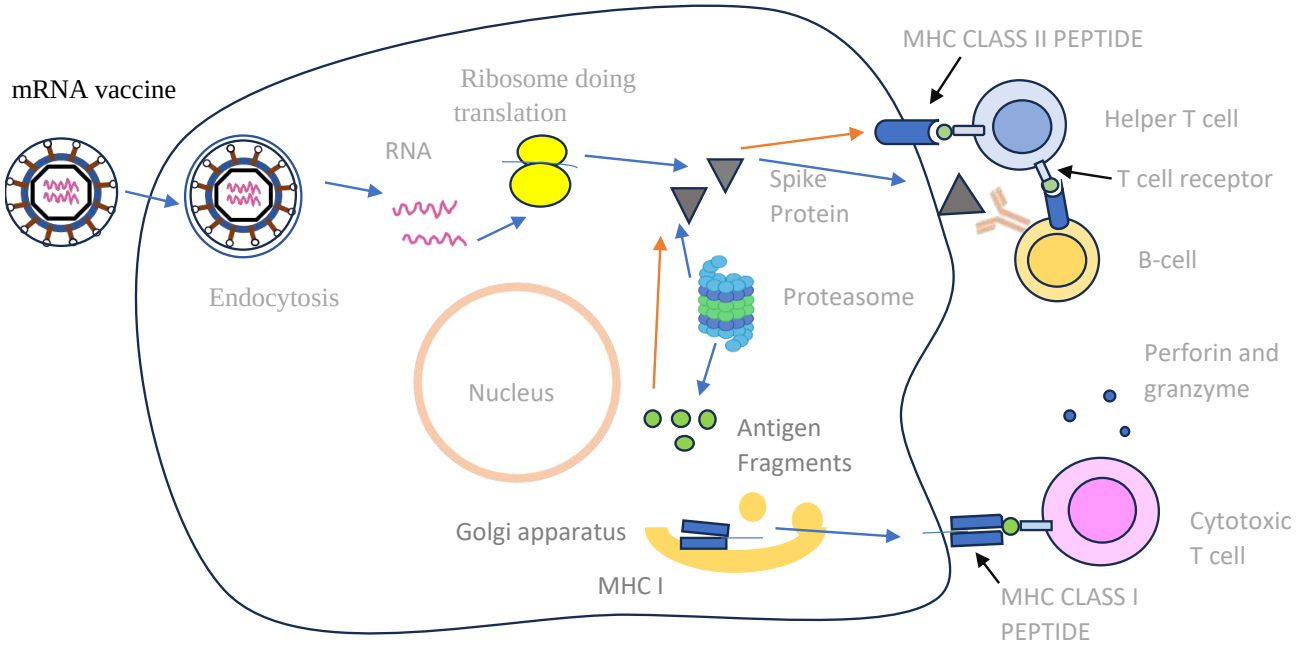

Fig. 2: The mechanism of action of mRNA vaccines

It involves the delivery of genetic material to antigen-presenting cells, which in turn produces immunity. Antigen-presenting cells absorb mRNA vaccines by endocytosis. After bypassing the endosome and entering the cytoplasm, the ribosome converts mRNA into spike protein through translation. Once translated, the antigenic peptide has the ability to stimulate the immune system through many methods. The proteasome complex degrades intracellular antigens into smaller fragments, which are then displayed on the cell surface to cytotoxic T lymphocytes via major histocompatibility complex (MHC) class I protein. Cytotoxic T cells eradicate infected cells through the release of cytolytic molecules, including perforin and granzyme. In addition, fragments of released antigens can be taken up by cells and undergo breakdown within endosomes. Subsequently, they are loaded onto major histocompatibility complex (MHC) class II molecules for identification by T cells. MHC class II proteins display antigens on the cell surface for recognition by helper T cells. Helper T cells assist in eradicating infections in the circulation by stimulating the generation of neutralizing antibodies by B cells and by activating phagocytes, such as macrophages, through inflammatory cytokines. (Chaudhary et al., 2021).

Applications of mRNA Vaccine

mRNA vaccines have been extensively studied and developed for several diseases, such as infectious diseases and personalized cancer vaccines. We believe that mRNA technology will expand in the future with the development of mRNA modifications and delivery systems. Here are some applications of mRNA vaccine.

mRNA Vaccines against Infectious Diseases

The SARS-CoV-2 (severe acute respiratory syndrome–coronavirus), which causes COVID-19, causes respiratory illnesses and, in severe cases, can be fatal. This virus primarily infects bats as its primary host, and it primarily transfers to people through direct contact. The spike protein (S), membrane protein (M), nucleocapsid protein (N), envelope protein (E), and hemagglutinin esterase dimer protein (H) attached to negative-sense RNA make up the virus. COVID-19 appeared in December 2019 and quickly spread over the world, emphasizing the urgent need for a vaccination. Numerous vaccinations have been developed since the beginning of the pandemic (Nitika et al., 2021).

The most advanced approach in the creation of infectious disease vaccines at the moment is mRNA therapies. In preclinical and clinical trials, bolus injections into the skin, muscle, or subcutaneous area are used to administer the majority of mRNA vaccines. Immune or non-immune cells absorb them, convert them into antigens that T and B cells can see. The delivery method and the mRNA increase the efficacy of mRNA vaccinations (Pal et al., 2020).

mRNA vaccines have demonstrated exceptional efficacy and safety against COVID-19 and are currently being

developed for other illnesses such as influenza, Zika, rabies, HIV, and tuberculosis. By introducing mRNA encoding specific antigens to the recipient's cells, which later synthesis the antigens and deliver them to the immune system, mRNA vaccines can create protective immunity. Additionally, they can be made to target different pathogen strains or antigens, which could improve their effectiveness and resistance to co-infections and newly emerging forms of the infection (Qin et al., 2022).

In general, advancements in mRNA platforms, design, and delivery have improved our ability to stop bacterial infections. To fully realize the potential of mRNA and determine how well it can be delivered when used with protein and polysaccharide antigens to treat bacterial illnesses, more research is required (Maruggi et al. 2017).

mRNA Vaccines against Cancers

One of the biggest threats to worldwide human health and the primary cause of death is cancer. The hunt for anticancer treatments has received enormous attention due to the high rates of disease and death. Traditional therapeutic approaches, like as radiation, chemotherapy, and surgery, have advanced, but there are still few effective treatments available. Immunotherapy has emerged as an essential field of study for cancer treatment in the past few years, and several immune checkpoint inhibitors (ICIs) have been granted approval as cancer treatments, but it is expensive. The aim of immunotherapy is to train the immune system to recognize and eradicate tumors (Shekarian et al., 2017). COVID-19 significantly accelerated the development of mRNA vaccines, which are now helping with cancer treatment. Therapeutic mRNA cancer vaccines have attracted a lot of attention as a modern immunotherapeutic approach that seeks to destroy tumor cells by activating antitumor adaptive immune responses. Particularly, tumor-specific antigens (TSAs), tumor-associated antigens (TAAs), and immune modulatory factors are among the essential elements of the immune response that are encoded by therapeutic mRNA cancer vaccines. This promotes immune activation to carry out antitumor tasks. Its benefits also include low cost, quick development, safety and flexibility. Many therapeutic mRNA cancer vaccines are currently being tested in phase I/II clinical trials, and the early results look promising. This field of mRNA Vaccine is still in early stages and enquires a lot of attention (Wang et al., 2023).

mRNA Vaccines against Regenerative Medicine

In regenerative medicine, mRNA treatment can cause pluripotency, differentiation, or reprogramming of cells for organ transplantation or tissue repair. Recently, a reprogramming technique based on mRNA has been devised. Like mRNA vaccinations, mRNA reprogramming generates induced pluripotent stem cells (iPSCs) by introducing genetically encoded mRNA that instructs cells to make target proteins and cell reprogramming factors (Khan et al., 2023). mRNA doesn't remain in the iPSCs since it slowly degrades within cells, in contrast to the retrovirus technique that uses DNA. This indicates that mRNA does not increase the likelihood of tumor growth or result in mutations in genomic DNA. Furthermore, mRNA reprogramming is typically more effective than current viral vector-based approaches. It is advised to add chemical alterations while reprogramming mRNA, such as in mRNA vaccines (Inagaki, 2024).

Conclusions

The development of mRNA vaccines was accelerated by the COVID-19 pandemic. The mRNA vaccine offers enormous potential for treating and preventing infectious diseases because of its quick research and development and large-scale production capability. A new and potentially challenging approach to anticancer medicines is the use of mRNA cancer vaccines. To properly evaluate the safety and effectiveness of mRNA cancer vaccines, more preclinical research is required, and more clinical trials are required. These investigations will examine the potential combination of mRNA cancer vaccine and other anticancer medicines. mRNA cancer vaccines have enormous potential as a game-changing treatment for cancer patients with further study and funding. However, the production of mRNA vaccines requires challenging conditions, along with the need for lower temperatures during transportation and storage, due to the essential fragility of RNA. This reduces the vaccine's lifespan and restricts its global availability. Enhancing the mRNA vaccine's stability is therefore a difficulty in this field.

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Chapter 19

Harmony in Health: Unveiling the one Health Approach Via Vaccine

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ABSTRACT

The One Health concept has gained traction as an integrative approach to tackle intricate health issues at the intersection of environmental, animal, and human health. This chapter explores vaccines' central place in the One Health paradigm and emphasizes how important they are for fostering peace in these interrelated fields. The chapter demonstrates how vaccinations are effective instruments for preventing and reducing infectious diseases, protecting human populations, animal populations, and ecosystems through a thorough analysis of literature and case studies.The concept of One Health acknowledges the complex interdependencies that exist between people, animals, and the environment and the fact that disturbances in one area can lead to health crises in other areas. Historical triumphs like the elimination of smallpox and the fight against rabies serve as examples of the One Health idea since they improve human immunity, lessen zoonotic spillovers, improve animal welfare, and support environmental conservation..The chapter also discusses the difficulties and possibilities associated with putting vaccination strategies into practice within One Health, such as addressing vaccine hesitancy, removing socioeconomic access barriers, and encouraging cooperation between the public health, veterinary, and environmental sectors. As a whole, the chapter emphasizes the critical role that vaccinations play as unifying factors within the One Health paradigm and promotes working together to improve the health of people, animals, and the environment in order to create a more resilient and sustainable future.

KEYWORDS
Public Health: Vaccination: Zoonotic Disease, Immunization, Disease Prevention

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INTRODUCTION

The One Health approach is a comprehensive strategy that recognizes the interconnections of environmental, animal, and human health (Mackenzie and Jeggo, 2019). In order to effectively handle intricate global health issues including food safety, antibiotic resistance, and zoonotic illnesses, this approach is essential (Danasekaran, FY2024). The need to effectively prevent and control health concerns through the integration of environmental science with veterinary and human medicine is highlighted by the rising incidence of zoonotic illnesses such as COVID-19 (Elsohaby and Villa, 2023).

The foundation of the One Health concept is the idea that animal and environmental health are inextricably related to human health (Garg and Banerjee, 2021).One Health seeks to develop a unified approach to health risks that arise at the intersection of various fields by encouraging interdisciplinary collaboration. Pin et al., 2024. To monitor, prevent, and control health concerns, this strategy brings together experts from a variety of professions, including ecologists, veterinarians, epidemiologists, public health specialists, and policymakers. (Chaddock, 2012). One essential element of the One Health approach is vaccination (Calistri et al., 2013). In order to stop infectious diseases from spreading across species boundaries, vaccinations are essential (Fries et al., 2021).Vaccinating cattle, for example, can lower the risk of zoonotic diseases that might otherwise spread to human populations. Additionally, by lowering the reservoir of infection in animal hosts, vaccinations can aid in the management of diseases carried by vectors (Layton et al., 2017).

Recent developments in vaccine technology, such mRNA vaccines, have demonstrated great potential for use in

veterinary and human health. Within the context of One Health, these innovations not only increase the effectiveness of illness prevention but also create new opportunities for cooperative research and development (Fayez et al., 2023). Recent developments in vaccine technology, such mRNA vaccines, have demonstrated great potential for use in veterinary and human health. Within the context of One Health, these innovations not only increase the effectiveness of illness prevention but also create new opportunities for cooperative research and development (Fayez et al., 2023).The fight against emerging infectious diseases is one area where the synergy between vaccination tactics and One Health is very apparent (Ghai et al., 2022). Vaccines designed to combat particular zoonotic viruses can stop the spread of these illnesses at the source (Nuismer and Bull, 2020). One notable example of the significant influence of animal vaccination on human health is the sharp decline in human rabies cases worldwide caused by the immunization of dogs against rabies (World Health Organization, 2018). Similarly, vaccinations against livestock diseases such as Rift Valley fever benefit communities that depend on these animals for their livelihoods and health in addition to animal populations (Gerken et al., 2020).

Moreover, the environmental dimension of One Health is addressed through vaccination efforts that help maintain ecological balance. By controlling diseases in wildlife, vaccines can prevent the disruption of ecosystems that might occur due to disease outbreaks. This, in turn, supports biodiversity and helps sustain natural resources that are vital for human well-being (Daszak et al., 2020).The One Health approach also emphasizes the importance of surveillance and monitoring in vaccine deployment. Effective vaccination programs require robust systems to track disease prevalence and vaccine coverage across different species and regions. Such surveillance enables timely responses to emerging health threats and facilitates the continuous improvement of vaccine strategies (Sharan et al,. 2023).

Harmony in Health

Harmony is the state of agreement in sentiment, viewpoint, or accord. The system's components are aesthetically pleasantly combined to create a more inclusive, engaged, and productive team. Health experts collaborate daily. Improving the health guides will greatly benefit from harmony among healthcare professionals. But collaboration is more than just working together. It is important to ensure that the tasks performed by each profession complement each other. Health professionals must cooperate, coordinate, and work together (Davies et al., 2000). The appealing arrangement of system components to create a cohesive, engaging, and more productive team is known as harmony. The purpose of this chapter is to examine the variables that work against global harmony among healthcare professionals. The research for this chapter involved looking up articles about harmony amongst health professions in the healthcare industry. Harmony among healthcare professionals is essential to raising the health indices, according to a literature search and findings from earlier studies (Osaro et al., 2014). Every infectious disease has unique ecological and biological characteristics that impact different geographic areas in distinct ways. As a result, distinct control and preventive measures are needed for each disease. Nonetheless, there exist successful “universal strategies” for the prevention and mitigation of infectious diseases in general, yielding remarkably strong outcomes, particularly when said strategies integrate aspects related to humans, the environment, animals, and pathogens from a One Health standpoint (Cunningham et al., 2017). This viewpoint holds that the nature and degree of interactions that exist between people, animals, and the environment can either help or hinder the spread of infectious illnesses and their subsequent outbreaks and epidemics. One Health-based approach is crucial, particularly in low- and middle-income nations where resources are limited, and infectious diseases are common. All around the world, scientists and health professionals need to be including these traditional activities in their discussions and actual actions. The best strategy for combating infectious diseases in a realistic manner is to increase efforts to implement and distribute these actions and to invest in technologies to improve them (Ellwanger et al., 2020).

Understanding the One Health

A developing idea called "One Health" seeks to integrate environmental, animal, and human health. It is challenging to achieve unified techniques for disease prevention and detection because conventional lines separating veterinary and medical practice must be crossed. This was not the case in the late nineteenth and early twentieth centuries, when scientists such as Louis Pasteur and Robert Koch, as well as doctors like William Osler and Rudolph Virchow, blurred the lines between the health of humans and animals. Calvin Schwabe brought the idea of One Medicine back more recently. This was essential for the development of epidemiology, particularly zoonotic illnesses (Atlas et al., 2012; Ahmad et al., 2024).

A One Health approach, which involves a collaborative, multi-sectoral, and trans-disciplinary approach working at the local, regional, national, and worldwide levels to achieve optimal health outcomes while acknowledging the interconnections between people, animals, plants, and their shared environment, can be used to address vaccine-preventable zoonotic diseases (Erkyihun et al., 2022). Rudolf Virchow (1821–1902) introduced the idea of the One Health approach in the 19th century by combining veterinary medicine with human health and claiming there was no separation between the two. The One Health idea subsequently spread around the world and signaled a paradigm change at the beginning of the twenty-first century; interdisciplinary collaboration has since grown (Monath et al., 2010). In a similar vein, veterinary vaccination against endemic illnesses improves the productivity and survival of food-producing animals like cattle and poultry, resulting in net improvements in disposable household income and improved human nutrition through increased availability to foods high in protein from animal sources (Marsh et al., 2016; Roth et al. (2011); Knueppel et al. (2010); McElwain et al. (2017).

Importance of Vaccines

One of the best defenses against infectious diseases is vaccination. Sadly, vaccinations against the illnesses that have the greatest worldwide health costs like HIV, malaria, and tuberculosis do not yet exist. Some of the greatest medical achievements in history have been made possible by vaccinations, such as the nearly complete eradication of polio, the eradication of smallpox, and the significant reduction in morbidity and death from a variety of infectious diseases each year (Koff et al., 2008). The ability of vaccination to protect against a variety of infectious diseases in both humans and animals has been demonstrated. Its ability to stop significant outbreaks of foot-and-mouth disease (FMD) in cattle is debatable, though. (Kelling et al., 2003). Unlike most medications, vaccines are given to massive, generally healthy populations, including infants and children, meaning that there is a low tolerance for any possible hazards or adverse effects. Furthermore, because there aren't many cases of an infectious disease, the long-term advantages of vaccination in lowering or curing it could lead to complacency. Vaccines are special because they are given to huge groups of people who are generally healthy often newborns and young toddlers. Consequently, even in cases when the sickness itself can result in serious or deadly side effects, it is unacceptable for vaccinations to cause a large load of adverse effects (Di Pasquale et al., 2016).

Among the greatest contributions to public health in the 20th century are vaccine. Vaccination is without a doubt one of the most effective ways to prevent, control, and even eradicate disease. This was demonstrated by Edward Jenner's use of cowpox to protect against smallpox in the 18th century, Pasteur's discovery of how to inactivate the rabies virus to save human lives through vaccination, and the necessity of developing effective vaccines quickly during the explosive SARS-CoV-2 pandemic (Lombard et al., 2007; Heaton et al., 2020). Animal vaccinations prevent the spread of some zoonotic illnesses to people, regulate diseases in companion animals, and guarantee secure food supply by keeping cattle populations healthy (Pastoret et al., 2012; Paul-Pierre et al.,2009) Vaccinating domestic animal species to stop disease transmission to humans and vaccinating wild animal species to stop disease transmission to domestic animals and humans have been two strategies for animal vaccination programs against zoonotic illnesses (Monath et al., 2010). Creating novel and enhanced vaccinations to stop the spread of difficult or newly discovered zoonotic illnesses is a significant area of future study. There have been animal vaccines for many years. These vaccines are quite affordable when included in complete preventative programs, and they have the potential to save lives, enhance animal health, and increase food and financial security (Paul-Pierre et al., 2009; Roth et al., 2011). There is, however, very little interaction between vaccines administered to humans and animals, despite these and several other evidence of vaccination impact.

The main objectives of veterinary vaccinations are to enhance companion animal health and wellbeing, boost livestock productivity in an economical way, and stop the spread of diseases from domestic animals and wildlife to humans. Different approaches have been taken in the creation of veterinary vaccinations as a result of these varied goals. These approaches range from simple yet efficient whole-pathogen preparations to molecularly specified subunit vaccines, chimeras or genetically altered organisms, vector antigen formulations, and naked DNA injections. It will be essential for researchers and medical practitioners to continue collaborating with animals to adapt new technology provide animal models of sickness, and combat newly discovered infectious diseases (Meeusen et al., 2007).

The Significance of Vaccine in One Health

Given the world's growing populations of people and animals, as well as the environment's rapid changes, the links between human, animal, and environmental health are becoming more and more obvious.To identify and address new public health risks, it makes sense to broaden the scope of public health beyond a single species, given the shared health risks that animals and humans face from shifting environments. (Mumford et al,.2023) . Global public health capabilities and resources need to be retooled across many species in order to offset the effects of emerging diseases, toxicant releases, climate change, and changes in the built environment.In addition, to execute necessary health plans, specialists in the fields of human and animal health must surmount particular obstacles to inter-professional collaboration. This overview describes the connections between the health of humans, animals, and ecosystems as well as the opportunities and difficulties these connections bring for public health. (Rabinowitz et al., 2013). One of the biggest problems facing public health is the emergence of infectious illnesses. Roughly 75 percent of these illnesses have an animal origin. Among these illnesses are traditional zoonosis that are only transmitted to humans by other vertebrates and those that are brought on by a successful one-time zoonotic event (host-switch) combined with effective human-to-human transmission (such as H1N1 influenza). Here, we offer a comprehensive assessment to determine the key features of previous animal-origin epidemics and identify regions with a high probability of disease development in the future. We also combine this with a meta-analysis and spatial risk modeling (Dharmarajan et al., 2022).

In both, people and domesticated animals, vaccinations play a critical role in the prevention and management of zoonotic infectious illnesses (Sharan et al., 2023). Nevertheless, the goal of vaccination is nearly often the directly affected species, and there aren't many examples of how this could be done in practice to avoid human disease indirectly by immunizing companion or domesticated animal sources of infection. (Monath, 2013).Even more difficult and little research has been done on the idea of immunizing wild animal reservoirs to prevent sickness in people or domestic animals. Furthermore, the biopharmaceutical industry's divisions dedicated to human and animal health are typically divided and segregated, and the development of novel vaccines recommended for the prevention of disease transmission from domestic or wild animals to humans lacks a systematic methodology (Monath et al., 2013).

Initiatives for One Health establish collaborations between doctors, veterinarians, and other health-related scientists,

acknowledging the interdependence of human health, animal health, and the environment (Monath et al., 2013). The ultimate purpose of vaccination is to significantly improve the public health of the population within the catchment area, in addition to preventing infection in individuals. To assess the impact of a vaccine, it is necessary to provide a more comprehensive measure that goes beyond efficacy and safety. This measure should consider the program's ability to decrease infection transmission, disease burden (incidence, mortality, and sequelae), the strain on health systems, and health disparities between populations. It should also measure coverage and mechanisms of action (Clemens et al., 2011; Lehtinen et al., 2015).

One of the most significant scientific developments of the twenty-first century has been the creation of safe and effective vaccinations against diseases that significantly increase morbidity and mortality. Vaccination is unquestionably one of the public health measures that has improved health outcomes worldwide, along with sanitation and good drinking water (Ehreth et al., 2003). Vaccines are thought to have saved 6 million lives each year from diseases that may have been averted by vaccination. It is difficult to overstate the influence that vaccinations have had on global health. No other method has had such a significant impact on population growth and mortality reduction as safe water (Rodrigues and Plotkin, 2020) linical trials must demonstrate the vaccine's immunogenicity and/or protective efficacy in addition to its quality and safety.

Our objective is to chapter and delineate, among the various evaluations of vaccine intervention, what applies to the effectiveness of vaccines and to the impact of vaccination programs. We propose a bridge between the effects of vaccine, as defined in previous work (Miller et al., 2011; De Serres et al., 2012) and epidemiological measures of public health impact. We describe relevant methods to measure these effects and discuss the assumptions and potential biases that are involved. The main objectives of veterinary vaccinations are to enhance companion animal health and well-being, boost livestock productivity in an economical way, and stop the spread of diseases from domestic animals and wildlife to humans (Meeusen et al., 2007). In veterinary medicine, vaccinations are frequently used to prevent a wide range of infectious diseases in pets, farm animals, and even wild animals. Thanks to the successful global rinderpest eradication in 2011 (De Swart et al., 2012), there is hope for the further eradication of horrible animal diseases, such as rabies, foot-and-mouth disease (FMD), and the plague of sheep and goats (peste des petites ruminants) (Albina et al., 2013).

Challenges Opportunities of Vaccine in one Health

The "Obstacles to vaccination: A look forward" workshop explored the global push for more and better veterinary vaccines, which would help food security and safety worldwide and encourage healthy animals as the standard. The possibility of FMDV re-introduction into mainland Europe is one example of the ongoing issues with enzootic animal diseases, zoonotic diseases, and emerging and re-emerging diseases in certain places (Holm and Kortekaas,. 2020).Furthermore, vaccinations may contribute to the answer to the developing problem of antibiotic resistance by lowering the burden of disease and, consequently, the requirement for treating sick animals with antibiotics (Gutiérrez et al., 2012). A crucial strategy in the fight against interspecies zoonotic disease transmission is immunization of both humans and animals. But rather than considering the shared burden of disease, attempts to develop and deploy vaccination interventions to lessen the impacts of zoonotic diseases are frequently restricted to the veterinary and agricultural industries (Carpenter et al., 2022).

In addition to affecting animal output and food security, zoonotic infections cause over 2.5 billion illnesses and 2.7 million loss of lives in humans each year (Grace et al., 2012). By keeping cattle populations healthy, animal vaccines prevent disease in companion animals, guarantee safe food supplies, and act as a crucial barrier to stop the spread of some zoonotic diseases to humans (Pastoret et al., 2012; Paul-Pierre et al., 2009; Roth et al., 2011; Gutiérrez et al., 2012).

The idea of "One Health," which tries to integrate environmental, animal, and human health, is new. Because the conventional lines separating veterinary and medical practice must be crossed, it is challenging to provide integrated techniques for disease diagnosis and prevention. One Health is at a turning point in its history, and it needs to show off its advantages and establish its limits more precisely. Curiously, One Health is most widely accepted in developing nations, where it is making a big difference in the prevention and treatment of infectious diseases (Atlas et al., 2012). In conclusion, it is critical to have leaders from the public health, scientific, and leadership domains provide scientific and policy-focused talks on current global initiatives pertaining to surveillance for emerging infectious diseases in the ecosystem. Although many professional organizations and their members have emphasized the idea of One Health, it is still in its infancy and has not yet gained traction with policy makers, students, or healthcare professionals that specialize in human, animal, or public health. The One Health concept of "One World, One Medicine, and One Health" needs to be heavily researched and taught, and its message needs to be understood by all (Dhama et al., 2013).

The One Health approach to vaccine development has gained increasing attention in recent years, particularly in the context of the COVID-19 pandemic, which has highlighted the interconnectedness of human, animal, and environmental health (Alam et al., 2021). This approach recognizes that the health of humans, animals, and the environment are inextricably linked, and that addressing complex health challenges, such as the emergence and spread of zoonotic diseases, requires a holistic and collaborative approach that brings together experts from various disciplines (Zinsstag et al., 2012) (Muma et al., 2014).

One of the primary challenges of the One Health approach is the need to overcome siloed thinking and traditional disciplinary boundaries, which can hinder effective collaboration and information-sharing among professionals in the human health, animal health, and environmental sectors (Griffith et al., 2020). (Zinsstag et al., 2012) Addressing this challenge requires a concerted effort to promote cross-disciplinary education, joint training programs, and the development of shared protocols and data-sharing platforms (Muma et al., 2014).

Another key challenge is the need to secure adequate and sustainable funding to support the implementation of the

One Health approach (Alam et al., 2021). Lack of financial resources can hamper the ability to conduct necessary research, implement effective surveillance and monitoring systems, and ensure the equitable distribution of vaccines and other interventions (Alam et al., 2021). Despite these challenges, the One Health approach also presents significant opportunities for advancing vaccine development and addressing global health challenges.

Conclusion

The One Health concept presents a viable route to Harmony in Health Via the lens of immunization,. Through acknowledging the interdependence of human, animal, and environmental health and capitalizing on the efficacy of vaccines, we may effectively tackle some of the most urgent health issues of our day. A collaborative approach that integrates scientific innovation, policy support, and community participation can open doors towards a more resilient and sustainable future, even in the face of persistent challenges like vaccination reluctance and access constraints. By working together, we can fully utilize the benefits of vaccinations to enhance the health of people, animals, and the environment, eventually promoting the security and well-being of global health.

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Chapter 20

Use of Recombinant Vectors as Vaccine in Dairy Animals

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ABSTRACT

Recombinant vector vaccines have emerged in the recent past to be used in preventing diseases in dairy animals as they tend to overpower conventional vaccination techniques. This chapter explains the general aspects of recombinant DNA technology, various vectors, and how these vaccines elicit adaptive immunity in dairy animals. Among the areas of focus that are discussed include vaccine production as well as its use in averting frequent illnesses in dairy animals. While emphasizing improved efficiency, increased safety, and cost-effectiveness, the chapter also presents the technical issues and limitations. It goes further to describe successful applications against illnesses including mastitis, bovine respiratory disease, and foot and mouth diseases, showing how dairy animal health can be greatly transformed by the applications. Despite these hurdles, progress in gene editing and synchronization with other limbs of veterinary science makes recombinant vector vaccines a strong prospect to guarantee a better future for the dairy industry. It would, therefore, be useful for researchers, veterinarians, and stakeholders interested in improving the health of dairy animals through novel vaccination strategies.

KEYWORDS
Vaccine, Dairy Animals, Biotechnology, Vector, DNA.

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INTRODUCTION

Vaccination is a widely used method in the dairy industry to protect animals against diseases that can cause death and severe health consequences, although it has some drawbacks. These limitations include the duration of the limited immune response, the risk of adverse immune reactions, and the general difficulty of addressing pathogenic mutations (Piñón-Hofbauer et al., 2024). The world dairy industry faces pressure to produce high-quality milk and meat without animal diseases, making recombinant vector vaccines the best solution due to factors like feed choice and animal sickness. After the general structure, the following section painstakingly examines the various aspects of Recombinant Vector vaccines, giving vaccines a new dimension. The chief difference between the recombinant vector vaccines and the conventional vaccines is that instead of using the weakened viruses or bacteria as the main component or just killing or inactivating them (Tizard, 2019), the former relies on harmless viruses or bacteria that can transport DNA genes of the particular pathogen of interest from one cell to the other within the animal. This remarkable approach elicits a vigorous and directed immune response, which provides numerous benefits to those using the method. Recombinant vector vaccines are safer than live-attenuated vaccines since there can be no danger from the vector itself (Silva et al., 2014). This is because the vectors used are often temperature-sensitive/get attenuated or non-pathogenic, meaning that the chances of the vaccine recipient developing an adverse reaction are very low. Moreover, the process of antigen presentation in recombinant vectors is quite different from that of the other standard vectors, and the immune response elicited uniformly tends to be stronger and more sustained in normal humans and animals, which means that the need to give booster vaccinations would be less frequent in populations whose immune response is boosted by such recombinant vectors (Pöri, 2018).

Recombinant vector technology offers advantages in specimen collection and clinical applications, as it can hold multiple antigens, unlike conventional vaccines which typically work with a single antigen. This can be done to target specific stages of a pathogen's life cycle or even eradicate numerous diseases using a single shot (Delves et al., 2012) supports this view and explains that using functional antigens in vaccines has several advantages (Table 1). The multiple-antigen potential

of pop video enhances vaccine effectiveness and reduces animal and farmer time pressure. Recombinant vector technology allows for easy adaptation to new diseases, making it valuable for designing and implementing vaccines promptly, as existing and emerging infectious diseases can have significant negative impacts. For these purposes, the recombinant vector vaccines have been under consideration as they have the capability of responding quickly in the event of an outbreak and might help to avoid the epidemic of the virus (Hofmeyer et al., 2022).

Table 1: Advantages and Disadvantages of Traditional vs. Recombinant Vector Vaccines (Jorge and Dellagostin, 2017)

Feature	Traditional Vaccines	Recombinant Vector Vaccines
Antigen Source	Whole pathogen (attenuated or inactivated)	Specific pathogen gene(s)
Immune Response	Primarily humoral (antibody-based)	Both humoral and cell-mediated
Safety	Risk of reversion to virulence (attenuated) or incomplete inactivation	Generally safer due to the use of non-pathogenic vectors
Efficacy	Variable may require boosters	Potentially higher and longer-lasting
Production	Relatively simple but may require large amounts of pathogen	More complex but scalable and adaptable
Cost	Can be inexpensive	Potentially higher development costs but may offset with improved efficacy

Positive results from the use of recombinant vector vaccines in reducing diseases in dairy animals have even been observed in some instances in livestock. Likewise, positive outcomes have been indicated for the recombinant vector vaccine against the bovine respiratory syncytial virus (BRSV), which is one of the potent cattle respiratory morbid and the infectious bovine rhinotracheitis (IBR), which is another common respiratory illness in cattle (Souza et al., 2005). These successes hold the possibility that recombinant vector vaccines have the potential to alter the delivery of preventive health among the dairy.

Basics of Recombinant Vector Technology

Recombinant DNA technology, which can be considered a sub-discipline of contemporary molecular biology, also holds a primary position for the formation of recombinant vector vaccines. This technology involves the recombination of DNA from the source of your choice and the rearrangement of different genes to produce a set of genes that favors some desirable traits that would have been difficult to develop through natural processes (Nicholl, 2023). Concerning the vaccine, rDNA technology helps in inserting a gene that harbors a specific organism into a non-harmful one, like viruses or bacteria. It then replaces this vector to serve as a shipment or transport system of the cDNA into the host cells to initiate an immune response (Lee et al., 2017).

Techniques Involved in Creating Recombinant Vectors

The creation of recombinant vectors involves several intricate steps, each requiring precision and expertise: The detailed process of generating recombinant vectors is as follows, which is a complex procedure that needs a professional touch.

Isolation of Target Gene

To create a pathogen-specific gamma retrovirus vector, one must first isolate the target gene from the genome of the selected pathogen. This can also be achieved in several ways such as Polymerase Chain Reaction PCR which is a technique of biochemical amplification of a specific segment of DNA and restriction enzyme digestion which is a technique in molecular biology whereby DNA is cleaved at the particular restriction site (Ota et al., 2007).

Selection of Vector

The selection of the vector is crucial because it determines the potential for gene transduction and that of gene transcription. Some of the vectors include viruses such as adenovirus and Pox viruses and bacteria like salmonella and Lactococcus antacids. These vectors are chosen according to the likelihood of infecting the targeted host cells, toxicity to the surrounding tissues, and compatibility with the targeted gene (New, 2019).

Insertion of Target Gene into Vector

The target gene and the vector are digested with the two cognate restriction enzymes, and the overhangs, which are formed, are compatible. The gene is then cut into desirable restriction sites in the vector with the help of the DNA ligase enzyme, and hence, the joining of DNA fragments occurs. This leads to a recombinant DNA molecule, aided by engineering techniques; transcribed into a host organism like bacteria or yeast (Sharma and Tiwari, 2022).

Multiplication of Recombinant Vector

The host organism is grown to optimum requirements for the replication and expansion of the recombinant gene and vector. This step is important to procure the needed quantity for immunization (Nascimento and Leite, 2012).

Purification and Formulation

The last process is to purify the recombinant vectors with the host organism and to develop the vaccine. Further, to

better deliver the antigens contained in the purified vector, they are mixed with adjuvants and other pharmaceutical accessories, called excipients, to form a content stable enough for immunization purposes (Nascimento and Leite, 2012).

Types of Recombinant Vectors

Recombinant vector technology, categorized by tissue, virus, or carrier organism, possesses unique strengths and weaknesses based on the type of vector used to transfer genetic material. The choice of vector depends on parameters such as the target host, the size of the inserted gene, the level of gene expression to be expected, and risks from the vector, if any (Schambach et al., 2013).

Viral Vectors

Recombinant vaccines use viral vectors to introduce antigens into the host organism. These vectors are derived from viruses, which are optimized organisms that can penetrate the host cell and transmit genetic information. Commonly used viral vectors include:

Adenovirus Vectors

Adenoviruses are ranked among the non-enveloped viral particles, (Figure 1) which harbor double-stranded DNA genomes (Sallard et al., 2022). Adenoviruses, which can infect both dividing and non-dividing cells, are suitable vectors for vaccine production. They have been extensively studied and proven to induce immune responses without significant adverse effects.

Lentivirus Vectors

Retroviruses are enveloped viruses with single-strand RNA genomes, capable of inserting DNA into host cells, resulting in a genetically active gene that remains active over time. Lentiviral vectors are used in Gene therapy for a patient and have been receiving more attention as vaccines (Bulcha et al., 2021).

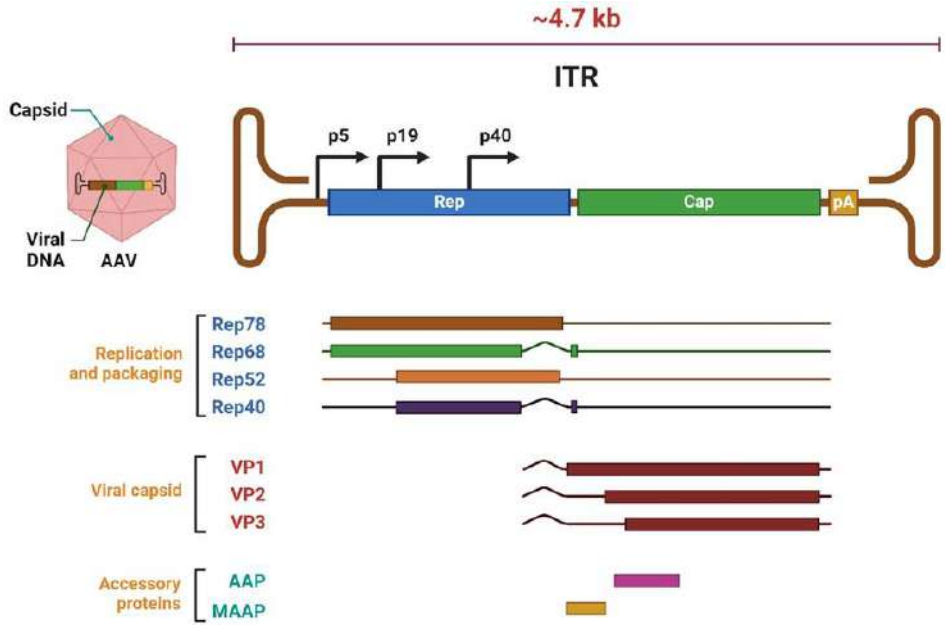

Fig. 1: Adeno Associate Virus Genome

Bacterial Vectors

Bacterial vectors, derived from bacteria, are considered safer than viral vectors for vaccine delivery due to their lower likelihood of triggering undesirable responses. Commonly used bacterial vectors include:

Salmonella Vectors

Salmonella species are medium to large Gram-negative bacilli that are facultative intracellular parasites able to invade several host cells. They have been employed in other ways including their application in the delivery of DNA vaccines and the development of immune responses against different pathogens (Gurunathan et al., 2000).

Listeria Vectors

Listeria monocytogenes is a facultative pathogen; it can actively reside intracellularly and can survive in phagosomes then escape into the cytoplasm of the host cell and replicate therein. This factor alone is a strong argument for why this is the preferred site to deliver antigens that are better presented when processed in the cytosol (Kotsias et al., 2019).

Plasmid DNA Vectors

Plasmid DNA vectors are circular molecules of single or double-stranded DNA, capable of independent replication

(Figure 2). They are widely employed in research and molecular biology for the reason that they can easily be manhandled and are diverse the most well-known non-natural oligonucleotides. The plasmid DNA vectors may be used in vaccines to transform the DNA of a host cell to express antigens from a pathogen; this makes the host immune system recognize the introduced foreign antigen and respond accordingly in the event the host is exposed to the actual pathogen (Schoen et al., 2004).

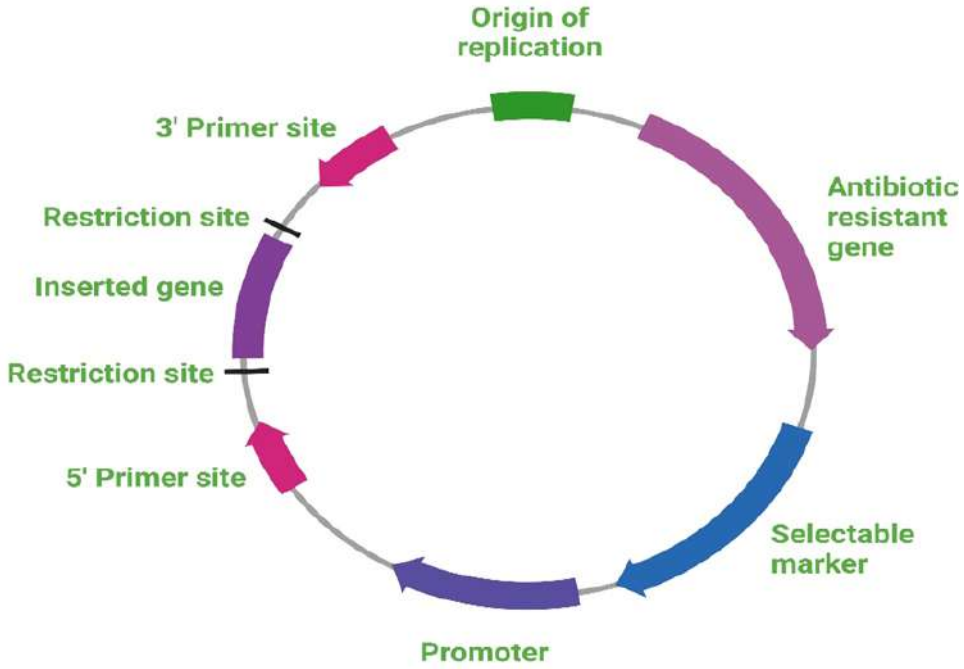

Fig. 2: Plasmid DNA Vector Sites

Mechanism of Action of Recombinant Vector Vaccines

By building on natural antigen presentation and immune system stimulation, recombinant vector vaccines promote a strong immune response. Regarding recombinant vector vaccines, when a vaccine containing the recombinant vector is used to immunize a dairy animal, the vector invades the host cells, and within the host cells, it carries the gene encoding the target antigen (Liu, 2019). Inside the cell, the genetic material of the vector provides instruction for the initiation of the release of the antigen in the cytoplasm which is then transported to the cell surface accompanied by major histocompatibility complex (MHC) molecules (Liu et al., 2021).

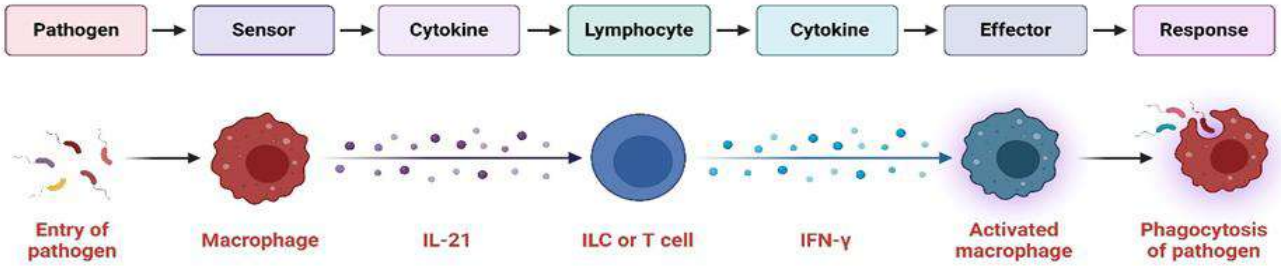

Fig. 3: General Principles of the Immune Response

This antigen presentation unleashes several immune activities including the activity of cytotoxic T cells and B cells which produce antibodies. This is through the help of the antigen-presenting cells (APCs) which include the dendritic cells and macrophages that detect the presented antigen and activate (Figure 3) both the innate and adaptive immunity (Eiz-Vesper and Schmetzer, 2020). This first immune response mechanism involves natural killer cells, neutrophils, and macrophage cells, whose role is to kill the infected cell or initiate the inflammatory process. At the same time, the innate immune response continues to enhance the fight against pathogens through the recruitment and activation of T cells and B cells (Vos et al., 2000). T cells respond to the antigens presented on MHC molecules through its T cell receptor and the T cell undergoes differentiation to form different subtypes including the cytotoxic T cells that kill the infected cells as well as helper T cells, which coordinate the entire immune response. There are two broad categories of cells involved in the immune response B cells and T cells as well as various specialized immune cells; B cells secrete antibodies that attach themselves to the antigen and neutralize it (Figure 4), thus making it easier for the immune system to destroy it (den Haan et al., 2014).

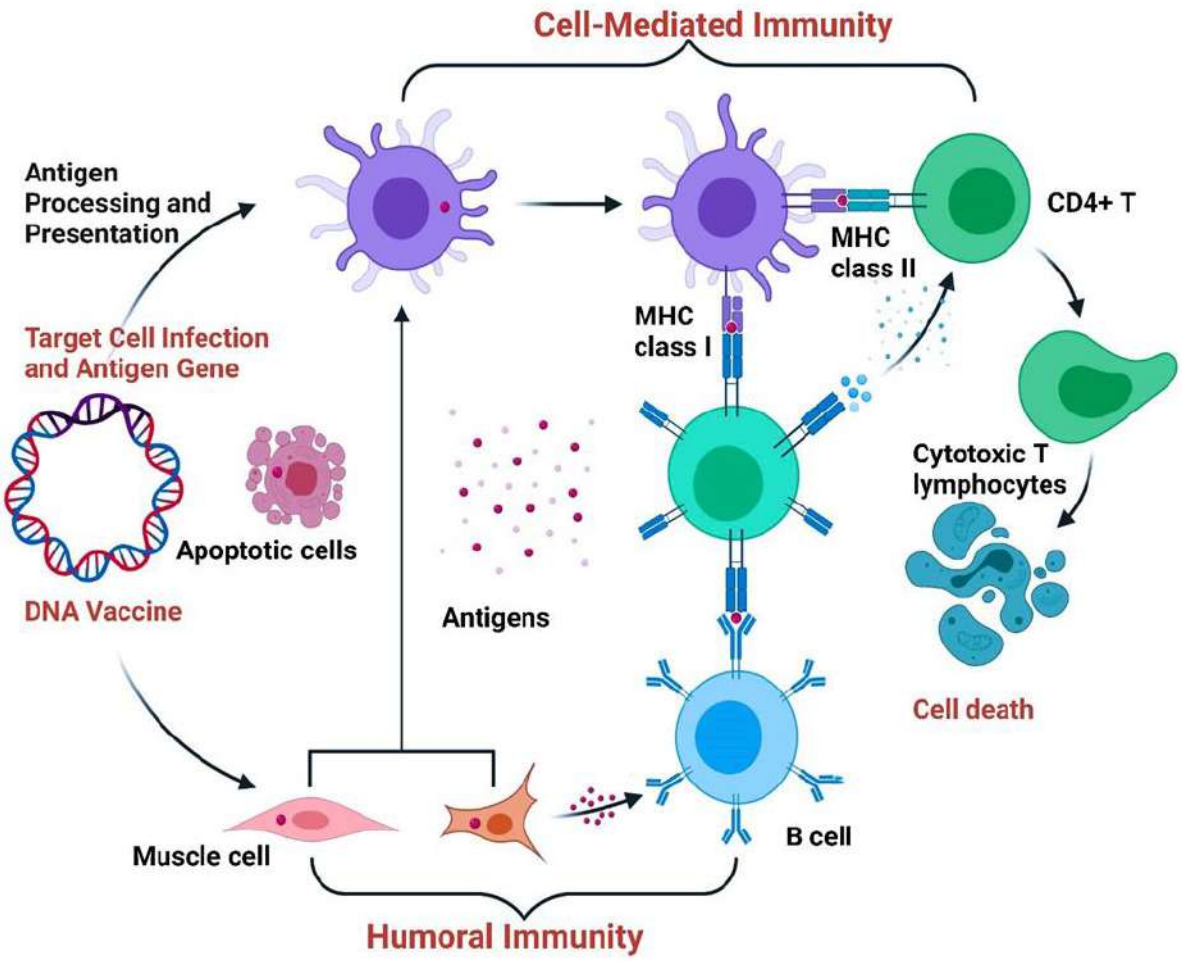

Fig. 4: Mechanism of Action of Recombinant Vector Vaccines

The integrated immune response, dependent on both T-cell and antibody-producing B-cells, is one of the major benefits of recombinant vector vaccines over traditional vaccines. Inactivated vaccines, in particular, mainly stimulate the production of antibodies within the bodily fluid, also known as the humoral effect, but this immune reaction is not always adequately accompanied by cellular immunity. They include recombinant vector vaccines, which as opposed to the inactivated/sub-unit vaccines mentioned above, allow the activation of both antibody and cell-mediated immunity hence providing broader resistance against pathogens, especially those having a cellular tropism (Gerdts et al., 2006).

Immunological Memory and Long-term Protection

Immunological memory is one of the incredible phenomena of the immune system, which is applied to remember the previous experience with pathogens. It enables the immune system to respond more vigorously and rapidly to begin combating the pathogen in question a second time around; this results in immunological memory that protects the body from disease in the long run. Recombinant vector vaccines have, however, been noted to bring about strong immunological memory, thus making vaccinations long-wearing within vaccinated animals (Berzosa et al., 2022). Immunological memory operates by the production of what is known as memory T cells and memory B cells. These cells live in lymphoid tissues and are so highly mobile that they can circulate in the body, waiting for a second chance of contact with the organism's antigen. Memory T cells, out of the two, are particularly important for lifelong immunity, as in the event of reinfection, memory T cells rapidly differentiate into effector forms, thereby eradicating the pathogen before the onset of disease (Gattinoni et al., 2017). Recombinant vaccines offer the above advantages of having a more reliable and precise vaccine vector besides being able to boot immunological memory. Similarly, with conventional vaccines, there are likely memory responses, but the intensity and longevity of protection will depend on specific aspects of the vaccines and the pathogen (Bugya et al., 2021). Recombinant vector vaccines possess immunological benefits of both humoral and cell-mediated immunity that could generate a higher and longer-lasting memory of the disease, thus eliminating the necessity to get frequent booster vaccinations.

Development and Production of Recombinant Vector Vaccines

The process of initially designing Recombinant vector vaccines starts with the selection of the vector and the specific antigen. Depending on the requirements for the administration route, cell type, toxicity, and capacity to generate the

required immune responses, the selection of a vector can be done. For example, the Adino vector may be selected due to the high tropism of the latter for various cell types in contrast to the ability of the lentiviral vectors to integrate into the host genome, and as a result, sustain long-term operation of the antigen (Zacchigna et al., 2014). Once the vector is selected, various techniques in genetic engineering are applied to initiate the gene of the chosen antigen into the vector. The process of producing the vector usually involves making an incision into the vector's genome using restriction enzymes, also known as molecular scissors, followed by the integration of the antigen gene using an enzyme known as DNA ligase that is used to join DNA strands together. Other regions like promoters and enhancers may be added within the referred genetic structure to facilitate a high expression of the antigen anticipated within the host cells (Cruz-Tapias et al., 2013). Thus, obtaining the final recombinant vector construct, which is then fully examined to determine the correct position and solidity of the antigen gene. The production of recombinant vector vaccines can be referred to laboratory scale or an industrial scale. Small-scale production is a common method in the laboratory as it is employed for the production of vaccines for research and development only to design the final vaccine construct, and test it on animals (Klimyuk et al., 2012). Once the initial steps involved in the testing of the efficacy and safety of a vaccine have been completed, mass production is carried out and the vaccine is released to the market.

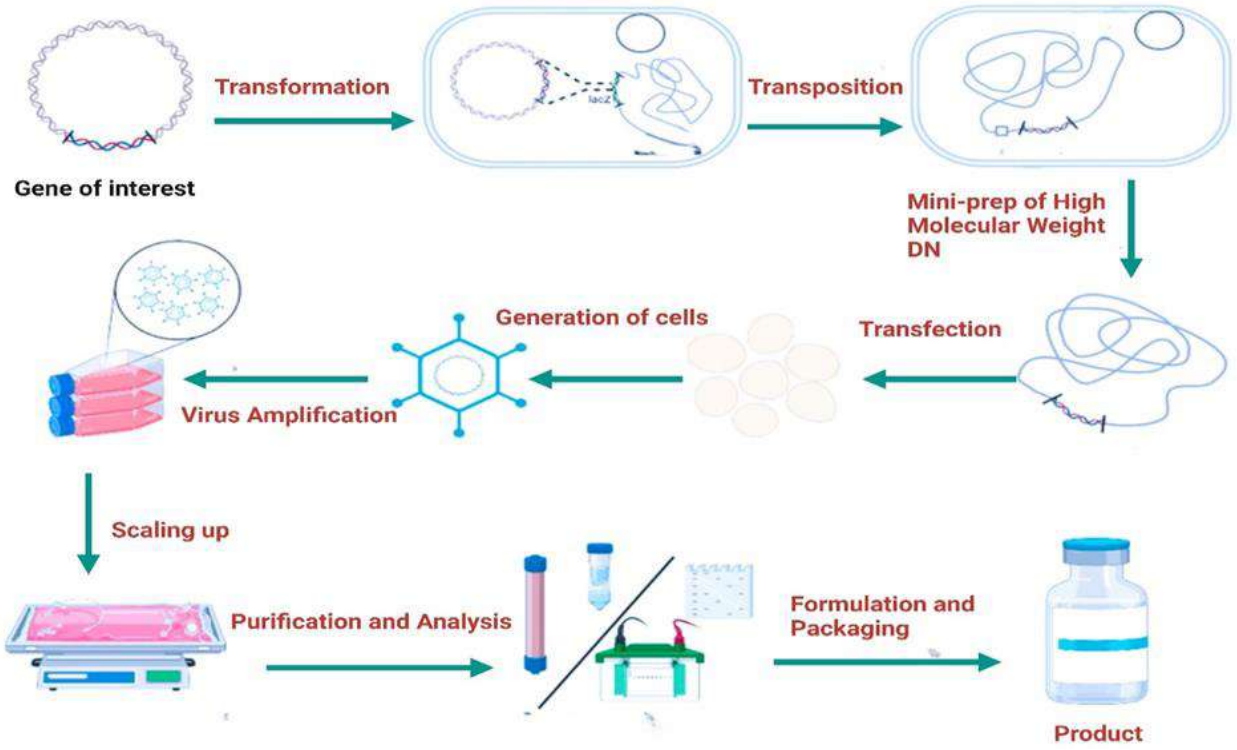

Fig. 5: Recombinant Vector Vaccine Development

After the production of the recombinant vector vaccine, unwanted components incurred from the mechanical process are precipitated out of the vaccine-containing solution and separated through a series of purification methods. Some of the Molecule purification methods that can be used include filtration, chromatography, and ultracentrifugation based on the type of vectoring and the extent of purification wanted (Segura et al., 2011).

From the production to the purification of the vaccine, there are measures taken to ensure that the vaccine, which is supposed to be taken by humans, is safe, potent, and free from other impurities (Gomez et al., 2013). This also involves checking for contamination, correctness in expression, and stability of the antigen, and finally, the immunogenicity test where the ability of the vaccine to stimulate an immune response is tested. The final product is then again mixed to form a complete pharmaceutical product as required to have the right characteristics, then packaged, labeled, and stored appropriately for its shelf life to arrive at the time when the patient is to receive it (figure 5).

Applications in Dairy Animals

Common Diseases Targeted by Recombinant Vaccines

Combinations of vaccines by utilization of recombined vectors have evidenced high capability in tackling several disease conditions commonly affecting dairy animals (Table 2). Among the most significant targets are:

Mastitis

This inflammatory disease of the mammary gland, attributable to bacterial infections, remains one of the major factors contributing to the high rate of animal mortalities in the milk production sector (Seegers et al., 2003). The vaccines produced

using recombination vectors involving Staphylococcus aureus and Escherichia coli have drawn out an optimistic response toward diminishing infection ratios and optimizing milk quality (Leitner et al., 2000).

Bovine Respiratory Disease (BRD)

Bovine Respiratory Disease (BRD) is a group of diseases caused by multifactorial respiratory infection in cattle and with more frequent in young calves, feedlot cattle, and in the cold season. Concerning vaccines involving vectors with application for BRD, it is possible to use the vaccines targeting the fundamental viral and bacterial pathogens, including BRSV, IBR, and *Mannheimia haemolytica*, which can minimize the disease occurrence and severe manifestations (Baker, 2004).

Foot-and-mouth disease (FMD)

This is a highly infectious viral disease that affects cloven-hoofed animals and its impacts are highly regrettable since it may lead to trade bans and animal mortality. Subsequently, recombined vector vaccines against FMD have been used and it has been established that they protect the immune system of genetically susceptible hosts from clinical disease and limit the spread of the virus (Balamurugan et al., 2004).

Table 2: Recombinant Vector Vaccines for Dairy Animal Diseases (Domínguez et al., 2014)

Disease	Vector	Antigen	Applications
Mastitis	Adenovirus	*Staphylococcus aureus* surface proteins	Reduced somatic cell count in milk
Mastitis	Listeria monocytogenes	*Escherichia coli* fimbrial antigens	Decreased clinical mastitis incidence
Bovine Respiratory Disease (BRD)	Bovine herpesvirus-1 (BHV-1)	BRSV, IBR, PI3 glycoproteins	Improved respiratory health in calves
Bovine Respiratory Disease (BRD)	Adenovirus	*Mannheimia haemolytica* leukotoxin	Reduced mortality and treatment costs
Foot-and-mouth disease (FMD)	Adenovirus	FMD virus capsid proteins	Effective in outbreak control
Bovine Viral Diarrhea (BVD)	Pestivirus	BVDV structural proteins	Reduced viral shedding and disease incidence

Johne's Disease

An unreported chronic bacterial disease that primarily affects the intestines, fastens weight loss and diarrhea, and is often fatal in cattle production. Since paratuberculosis (MAP), the causative agent of the disease could be a viable approach, we believe that an initial examination of attitudes (Acharya et al., 2020).

Bovine Viral Diarrhea (BVD)

This viral disease can affect the reproductive capability of the animals, can result in respiratory diseases among the cattle, and also causes immunosuppression among the cattle. Subsequently, the recombinant vector vaccines against BVDV have been proven to be more effective for disease prevention and control of viral shedding which in turn reduces the transmission of the disease within the herds (Sangewar, 2021).

Advantages and Challenges

Benefits of Using Recombinant Vector Vaccines

Recombinant vector vaccines are preferred over conventional vaccination systems due to their superior tolerability and effectiveness in treating patients. They activate both antibody and cell-mediated immune responses, ensuring higher and longer effectiveness. This robust response helps prevent sinus infections and decreases pathogen spread through herds, controlling disease spread within the herds. Moreover, employing less virulent or innocuous vectors reduces incidences of adverse outcomes when the young animals are vaccinated thus enhancing their safety (Thomas et al., 2022). A major selling point of recombinant vector vaccines is their feasibility which they are cheap to make and easy to prepare. These vaccines use established genetic engineering procedures to standardize the antigens in the vector, thus enabling the input of additional antigens in one particular vaccine (Ronsard et al., 2021). Recombinant vectors offer increased protection against multiple pathogens, simplifying vaccination processes and saving time and funds for farmers. The efficiency of producing large quantities makes vaccine production affordable and deployable in large numbers, particularly in the dairy sector.

Challenges and Limitations

Recombinant vector vaccines face challenges such as natural stability and delivery systems, vulnerability to external conditions like temperature and pH, and delicate balance in storage and transportation. The site of delivery may vary depending on the pathogen or immunity expected. Regulatory and ethical considerations are crucial for the development and deployment of these vaccines. The transgenic system using GMOs as vectors is problematic due to potential negative external effects not identified in preliminary experiments. The FDA and EMA have comprehensive rules dictating the decision-

making process on recombinant vector vaccines, considering the potential infection of humans and animals (Stevenson et al., 2018). Animal rights and potential negative effects should also be considered throughout the development process.

Future Perspectives

Advances in Recombinant Vector Technology

The advances made in the field of recombinant vector technology are quite impressive, and there appear to be even more future applications of recombinant vectors that have already been described. New technologies, including CRISPR-Cas9 gene editing technologies, also possess an opportunity to advance the design and development of recombinant vectors in constructing even more complex vectors to be used in the manipulation of genetic materials (Sandhya et al., 2020). It could also mean the delivery of new vaccines, which are more effective and safer, and after being processed, they are more stable.

Table 3: Emerging Technologies and Innovations in Recombinant Vector Vaccine

Technology/Innovation	Description	Potential Benefits
CRISPR-Cas9 Gene Editing	The goal is to precisely modify vector genomes to enhance safety, efficacy, and antigen expression.	The design of vaccines has been improved, with faster development timelines and reduced risk of unintended effects.
Novel Vector Platforms	The study explores alternative vectors such as plant viruses, insect viruses, and bacteriophages.	The production of vaccines has been enhanced in terms of safety, cost-effectiveness, and scalability.
mRNA Vaccines	Directly deliver mRNA encoding the antigen, resulting in rapid protein production in host cells.	The potential for personalized vaccines is being explored for faster response to emerging diseases.
Nanoparticle Delivery Systems	The process involves encapsulating vaccines for targeted delivery and enhanced stability.	The vaccine has been shown to improve uptake, enhance immune response, and reduce dosage requirements.
Machine Learning and AI	The goal is to predict the most effective vaccine formulations and identify potential targets for new vaccines.	The process of vaccine development has been expedited, with personalized design and enhanced efficacy being achieved.

Development (Graham et al., 2018)

However, there is another potential area that also warrants a focus and this is the area of developing new vector systems. Innovative ideas that are currently a subject of research interest include the use of plant viruses, insect viruses, and bacterial phages as vaccine delivery systems (Sokullu et al., 2019). In some cases, alternative vectors will be safer, average cost per dose, be easily scalable, and give wider working opportunities to recombinant vaccine development. The benefits of the recombinant vector technology are huge when it comes to probable new vaccines. Scientists are focusing on developing vaccines against many diseases as shown in Table 3 for which there is no prevention yet now, including BLV and TB in cattle in particular (Saied et al., 2021). Finally, the flexibility of this system, where it becomes easy to accommodate two or more antigens in a single multipath vaccine, is a great advantage since farmers can have multiple vaccines for multiple diseases in a single formulation, thus making the vaccination exercises much easier.

Relationship with Other Veterinary Activities

It is, therefore, important for one to understand that it is not the recombinant vector vaccines' intention to replace all the existing mechanisms in veterinary practices. To optimize the effectiveness of these vaccines and enhance the general health status of animals that are under the mass human populations, it's wise to incorporate these vaccines into other herd health management systems that are in place (Knight-Jones et al., 2014). For example, when vaccination is applied together with other preventive factors, including hygiene, biosecurity, and nutrition, there are chances of getting better results as a result of an accentuation effect whereby the incidence of diseases in animals will significantly reduce.

Conclusion

Recombinant vector vaccines are a perfect example of how the intervention of biotechnology in producing vaccinations that protect dairy animal health is of great importance. Having used biologically inactive viral particles to deliver the specific antigen, these vaccines have tremendous advantages over the traditional methods of vaccination. Beneficial aspects of this technique include improved safety and efficiency, decreased production cost, and ease of production making them suitable tools that veterinarians and farmers. This versatility of incorporating multiple antigens and the ability to protect against new diseases increases their role in protecting a various spread of diseases in dairy animals such as mastitis, bovine respiratory disease, and foot-and-mouth disease. The future perspective for the recombinant vector vaccines is quite positive but certain limitations exist. Challenges also remain due to concerns about the stability of the vaccines, delivery modalities, and regulatory and ethical issues are some of the challenges to ensure that COVID-19 is eradicated. As well as consumers'

attitudes towards the product and its popularity determine the main factors of their dissemination. Prospects are also impressive for further developments in the sphere of gene manipulation and, for instance, the utilization of new vector systems in vaccines. The synthesis of recombinant vector vaccines with other existing practices in veterinary medicine and innovative advancements, including the precision livestock farming system, will provide guidelines for the effective treatment of dairy animals in the future. Therefore, this progressive technology can help pave the way for a healthier future for this important facet of the economy, the dairy industry.

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Chapter 21

Challenges in Developing Effective Canine Infectious Respiratory Disease Vaccines

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ABSTRACT

Millions of people all around the world have domestic dogs. Some people keep them as a means of guarding and farming help while others have them as companion animals. Besides being loyal, dogs are easy to keep and maintain. However, there are some factors like diseases that affect or even lead to the death of dogs. One of such diseases is CIRD (canine infectious respiratory disease). It is also known as kennel cough and it affects mostly the dogs that love in kennels or re-home centers. This disorder leads to cough, dyspnea and immunosuppression in dogs making them vulnerable to other diseases as well. Currently, various types of drugs and vaccines are being used synergistically to boost innate immunity of dogs for battling this disease. Despite these efforts, the spread of CIRD suggests that presently available vaccines are insufficient for its control. Hence, there is a need for developing new types of vaccines and control measures to reduce the impact of disease, simultaneously counting its spread.

KEYWORDS
CIRD, Vaccines, Viruses, Innate immune responses, Subcutaneous route

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INTRODUCTION

Vaccines against most of the infectious diseases in canines have been used for the past 40 years except vaccines against rabies as it was developed earlier this century. For the past many years the research was focused on prevention of fatal diseases including canine distemper and parvovirus infections while later on the need of controlling nonfatal diseases like kennel cough gained the attention of the researchers (Mitchell and Brownlie, 2015). To control canine infectious respiratory diseases, both live and attenuated vaccines have been used using subcutaneous route specifically while intranasal inoculation is also performed but only in dogs with less aggressive attitude. Both live and killed vaccines have been used in dogs but killed vaccines are more commonly used in dogs with compromised immunity or pregnant animals (Pesavento and Murphy, 2014).

Canine Infectious Respiratory Disease

Canine infectious respiratory disease (CIRD) is commonly known as kennel cough and is found more prevalent in large populations of dogs for instance dogs in kennels for training or in re-homing. CIRD is a major threat for the welfare kennel facilities, veterinarians and as well as for the owners of the pet all across the world (Maboni et al., 2019). The outbreaks of CIRD results in costly treatments and also causes delays in the training and re-homing of the dogs. The clinical signs shown in the affected dogs include coughing, nasal discharge and dyspnoea and these signs may last for some weeks and the dogs are more vulnerable to many diseases in this time for example bronchopneumonia and may result in death of the dog or the veterinarian have to euthanize the affected dog (Horzinek and Appel, 1987). Besides the respiratory problems in cattle like bovine respiratory disease complex (Taylor et al., 2010) and pigs (porcine respiratory disease complex

(Opriessnig et al., 2011) the etiology of CIRD in multifactorial as in CIRD many different microorganisms work synergistically and cause infection. Other than microorganisms, environmental factors also have a vital role in causing CIRD in dogs including age of the dog, stress and other health concerns. Dogs in kennels are at high risk of CIRD because of the high population rate on kennels also in re-homing shelters the susceptibility rate of dogs getting pathogens is very high (Pesavento and Murphy, 2014). Major pathogens which are responsible for the CIRD are *canine parainfluenza virus* (CPIV or PIV5) (Appel and Percy, 1970), *canine adenovirus type 2* (CAV-2), *canine herpes virus 1* (CHV-1) (Karpas et al., 1968) and *Brodetella bronchiseptica* (Bb) (Bemis, 1992). Recent findings show that there are some newly emerging pathogens found in the outbreaks of CIRD. Recent reviews show a number of novel pathogenic agents responsible for CIRD (Priestnall et al., 2014) including *canine respiratory coronavirus* (CRCoV) (Erles et al., 2003) *canine pneumovirus* (CnPnV) (Renshaw et al., 2010) *canine influenza virus* (CIV) (Crawford et al., 2005) *pan-tropic canine coronavirus* (Buonavoglia et al., 2006) *Streptococcus zooepidemicus* (Chalker et al., 2003) and *Mycoplasma cynos* (Chalker et al., 2004). Other than CIV all above mentioned novel pathogenic agents are the main target of vaccine development in future as they are the potential source of CIRD in kennels with vaccinated dogs (Bemis et al., 1977). In USA, vaccine has been licensed against CIV recently to use in dogs with potential CIRD treat.

Mechanisms of Protection for the Respiratory Tract

Both viral and bacterial pathogenic agents responsible for the CIRD enters the body of host through the mucosal lining of the respiratory tract of the host (Mitchell et al., 2013). Other than the microorganisms causing infection in the dogs, the lower respiratory tract of the host is continuously being threatened by the microflora present in the upper respiratory tract including mouth, buccal cavity and pharynx. The respiratory tract of the host is protected through two main mechanisms which are the innate immune system and the adaptive immune system. Both of the mechanism have different roles to play and protect the respiratory tract (Damián et al., 2005).

Innate Immunity

First line of defense is provided by the innate immune system (Figure 1) of the respiratory tract of the host which can be compromised by physical, chemical and cellular components (Ganesan et al., 2013). The physical protection against the pathogenic agents provided by the mechanisms of epithelial barrier and the mucocilliary clearance. First, the epithelial barrier works to regulate the movement of matter through the barrier as it is formed of pseudo-stratified columnar epithelial cells which are joined together by tight junctions. Mucocilliary clearance is done as a result of shedding of sticky mucus continuously and the pathogenic agents trapped in this mucus. The continuous movement of this mucus due to the ciliated epithelial cells drawn away from the lower respiratory tract and into the pharynx (Parker and Prince, 2011). Chemical components of the innate immune system of the respiratory tract of the host consist of antimicrobial agents including defensins, lysozomes and proteins. These antimicrobial agents are the part of the compliment cascade which prohibit or kill the pathogenic agents (Vareille et al., 2011). These compounds are produced by the cellular components of the innate immune system and epithelial cells which modify the composition of the respiratory airways. As a defense system, innate immune responses do not require any prior exposure to the pathogen so these responses are considered very necessary when a dog is exposed to a new pathogenic agent for the very first time (Anderton et al., 2004). Most of the respiratory pathogens develop strategies to destroy the primary defense system. This is done by changes in morphology of cells due to viral replication and its cytotoxic effects, release of toxins and apoptosis, loss of ciliary function, destruction of tight junctions, production of mucus at high rate, and the uncontrolled regulation of the pro-inflammatory cytokines (Priestnall et al., 2009). The compromised immune responses result in the deeper penetration of pathogens in airways (Avadhanula et al., 2006).

Adaptive Immunity

Adaptive immune responses depend on the antigen antibody interaction in the host body which results in the immunological memory for the cells as if they encounter the same pathogenic agents in the future (Figure 2). Adaptive immune responses are able to differentiate the antigens that either they enter in the host body through the mucosal epithelium or by injection so that they act against them accordingly. In the duration of infection, the pathogens involved in developing CIRD trigger the immune responses and mucosal responses (Shaw, 2000). To prevent the disease, it is important to consider that immunity against respiratory pathogens highly depends on both the mucosal and systemic immune responses so while making vaccines against these respiratory pathogens both of these responses should be kept in mind. Mucosal immune responses are the responses which protect the host cells against adherence of pathogens, colonization and their invasion at the mucosal surfaces (Carey, 2015). On the other hand, immune responses work to contain and clear infection once the pathogen takes hold. This provides the need for vaccines which trigger mucosal immunity and give beneficial immunity against pathogens.

Immunity and Lymphoid Tissues

Mucosa-associated lymphoid tissue provides immunity at the mucosal surface and lymphoid follicles act as the prime immune induction sites (Cesta, 2006). Immunity provided by MALT is different from the systemic immune responses. The basic functions performed by MALT include protection of mucus membrane from pathogens, differentiate and tolerate

harmless antigens such as ingested food particles airborne particles and commensal microorganisms and prevention of harmful responses of immune system when an antigen cross the mucosa and enter the body of host. The palatine, lingual and nasopharyngeal tonsils are considered as the components of the respiratory MALT in dogs (Casteleyn et al., 2011).

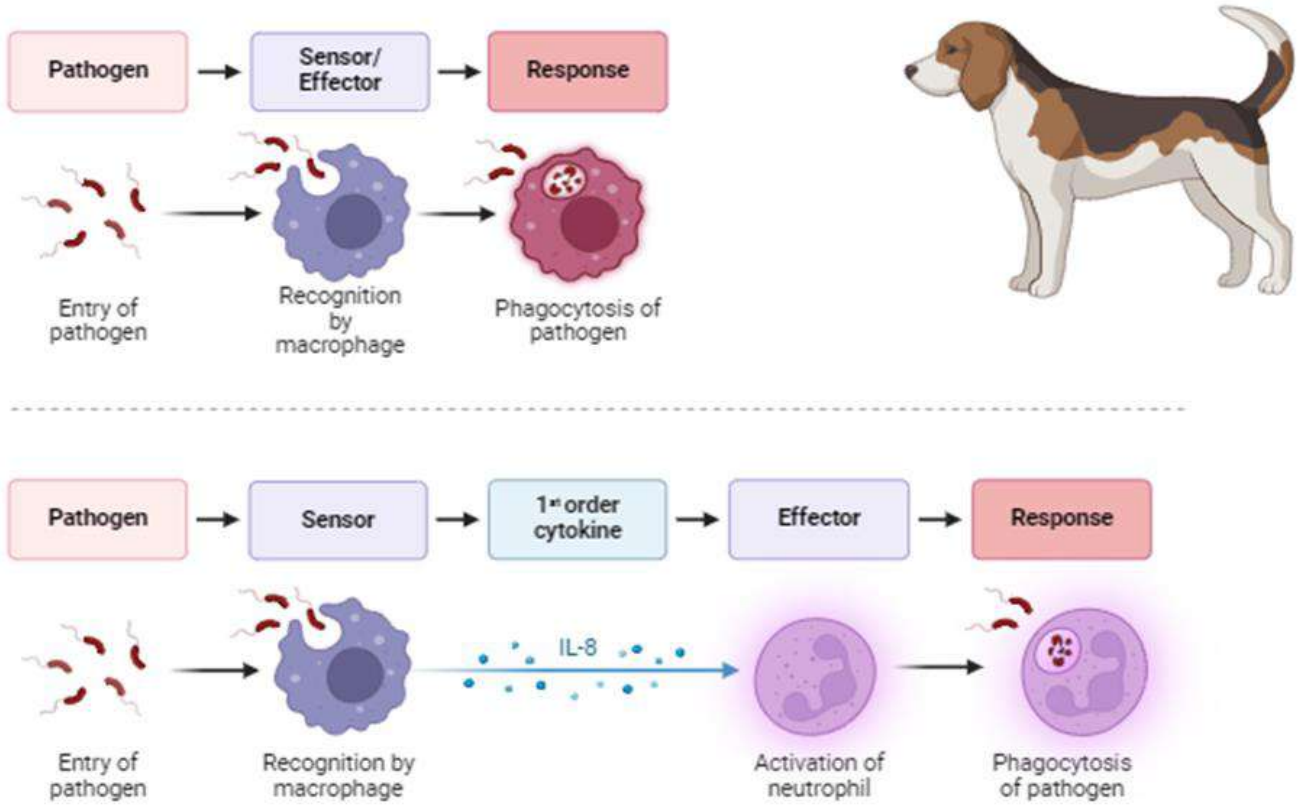

Fig. 1: Principles of innate immune responses.

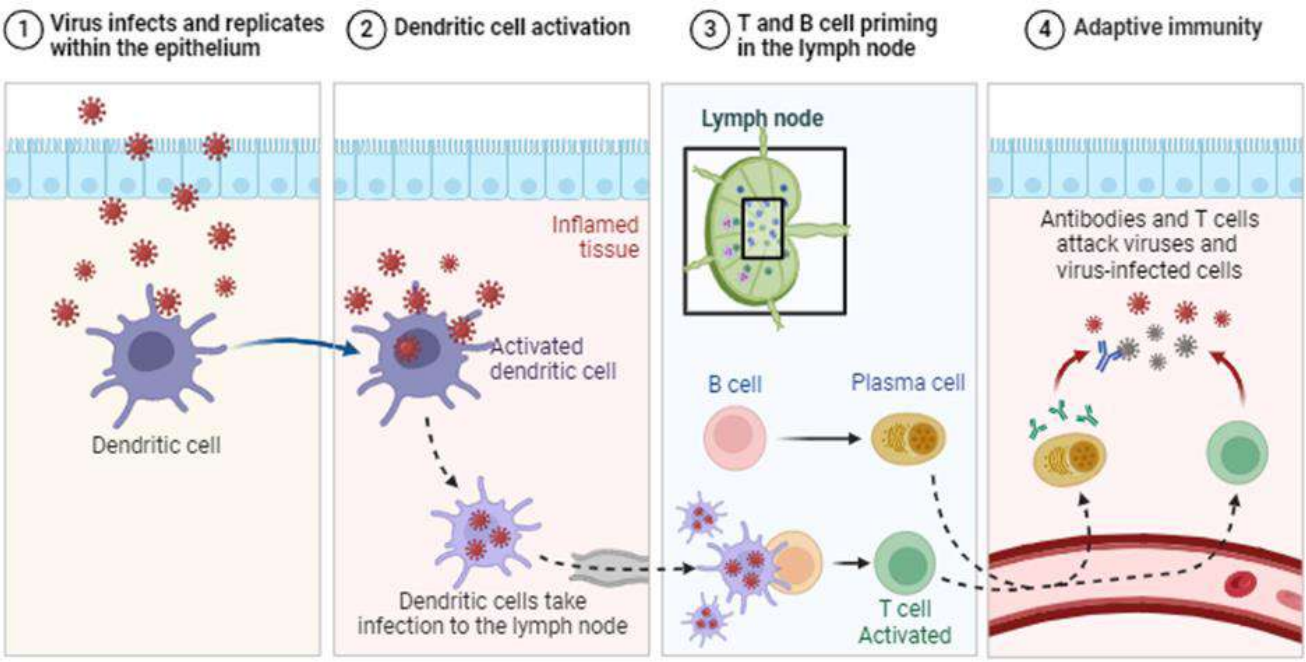

Fig. 2: Principles of adaptive immune responses.

Humoral Immunity at the Mucosa

The pathogen-specific immunity at the mucosa is provided by the humoral responses of adaptive immunity mainly by the secreted antibody named sIgA (Brandtzaeg, 2007), Th2 type cytokines induces the maturation of IgA-committed B lymphocyte which then migrates to the effector sites and differentiate into IgA-producing plasma cells. At effector sites

they performed different functions for example they trap antigen and different pathogens in the mucus, stop the adhesion of bacteria to mucus surface, neutralize both intracellular and extracellular viruses plus toxins, and improve innate immune responses. Other than sIgA, some other antibodies also take part in immune protection such as IgM and IgG present in the lower respiratory tract (Mitchell and Brownlie, 2015).

Cell-mediated Immunity at the Mucosa

Cytotoxic T-lymphocytes do not play any role in the inhibitions of pathogens to get entry in the host body but their role is to contain the viral load and clearance of the virus as they infect the cells of host. The function of cytotoxic T-lymphocytes is to differentiate between the normal body cells and the antigen presented to them attached to a specific T-lymphocytes binding proteins called as MCH class 1 molecules. Cytotoxic T-lymphocytes perform their function in the presence of Th1 Cytokines such as IFN-¥ and IL-2 by inducing the apoptotic destruction of the cells infected with the pathogens as a result of which pathogen also destroyed (McGhee and Fujihashi, 2012).

Current Canine Vaccines

Currently, there is a wide range of vaccines used in dogs, these are both single and multivalent vaccines. These vaccines are used to cover different aspects especially depending on environmental conditions and other risk factors. Vaccines used in dogs are basically classified as core vaccines and non-core vaccines (Andrukonis et al., 2021). Core vaccines are used in all dogs which covers *canine distemper virus* (CDV), canine parvovirus (CPV), canine adenovirus (CAV 1+2), *Leptospira canicola* and *Leptospira icterohaemorrhagiae*. On the other hand, non-core vaccines are used occasionally depending on the lifestyle of the dog (if the dog has to spend some time in a kennel or move to another country or state) and the risk factors (Tizard, 2020). Examples of non-core vaccines are rabies and the respiratory vaccines including CPIV and Bb).

Modified Live/Attenuated Vaccines

Modified live/attenuated vaccines are those vaccines which multiply inside the host body so usually the amount of antigen in the vaccines is not much important. These vaccines should be handled properly so that the antigen (virus) in the vaccine remains intact. As the virus is alive in the vaccine so there are chances that the virus may cause disease instead of giving protection and this may be attributed to the mutation of viruses. Live attenuated vaccines may cause disease in the host with immunosuppressed conditions or on fetuses (Rikula et al., 2000). Live or attenuated vaccines usually end up in higher titers of antibodies. As compared to killed vaccines, live or attenuated vaccines provide long lasting immunity for the same type of antigen and give better results while triggering the cell-mediated responses. In case of live or attenuated vaccines there is no need for adjuvants and only a single shot gives better results (Day et al., 2016).

Killed Vaccines

A high mass of killed vaccines is needed as they have not a chance to multiply in the host body. As the pathogens are killed so these vaccines are more stable than the live or attenuated vaccines. There is no risk of viral mutation in case of killed vaccines so these vaccines are considered safer to use in immunosuppressed and pregnant animals. The risks of contamination and transmission are very low. These vaccines result in less antibody titer as compared to live or attenuated vaccines (Böhm et al., 2004). The duration for immunity in animals is also less as compared to live or attenuated vaccines. Once they are injected in the host body, they stimulate the production of antibodies and need adjuvants to stimulate immunity. At least two doses of killed vaccines are necessary to protect animals. These vaccines need adjuvants and may cause allergic reactions, pain and sarcoma at the site of injection (Larson and Schultz, 1997).

Route of Inoculation

Only a few exceptions, the major route used for the vaccine inoculation in canines is the subcutaneous route. Research on the vaccines in both human and animal is usually based on injected vaccines which helps in monitoring the safe quantity of vaccines delivered to the patient which provides immunity for a long duration that can be checked and measured in the serum and blood samples of the patient. In the case of the intranasal route of vaccination, a number of challenges have been faced by the vaccines as they have to lower down many innate immune responses encountered by the vaccines in the upper respiratory airways (Gerdts et al., 2006). In case of live vaccines, the above mentioned situation can be overcome due to the inherent properties of pathogens which also facilitate the infection. The discoveries and advancements in the delivery vehicle for vaccines also give promising results (Heegaard et al., 2011) but still the conditions are not ideal and it is difficult to deliver vaccines accurately and give adequate quantities of antigen to the patient. In veterinary medicine, there is another major issue that is to deliver a safe and proper amount of mucosal vaccines (Davis et al., 2007). If the dog (large breed) is not very aggressive, it is somehow difficult to deliver an oral or intranasal vaccine. There is another major problem with the delivery of intranasal vaccines: the dog may snort back the vaccine immediately after delivery (Kontor et al., 1981). So in veterinary medicine it is much easier and safer to use a subcutaneous route for the administration of vaccines in dogs. To prevent respiratory pathogens from causing diseases, mucosal immunity is the best way of protection against these pathogens (Appel et al., 1975).

Future Perspectives

There are many challenges for future development of vaccines for dogs' respiratory problems. Recently it has been seen that there are a number of newly discovered pathogens that have been seen to cause multicomponent diseases also called complex infection like respiratory diseases (Parks and Alexander-Miller, 2013). This situation makes the development of vaccines in future much more difficult as it has been seen in research that some viral pathogens are capable to inhibit the innate immune responses and help other pathogens to cause infection. In case of CIRD, certain viruses such as CRCoV and CnPnV allow deeper penetration of some bacteria and mycoplasmas in respiratory airways causing several clinical disorders. In future the vaccine development should be focused to prevent these viruses as a result of which the patient should be protected from secondary infections of the opportunistic bacteria. The use of mucosal vaccines has many beneficial effects but the comparison of the efficacy between mucosal and parenteral vaccines is still debatable (Murphy, 1988). The mucosal vaccines are very efficient but they do not provide immunity for longer duration. The immediate effect of mucosal vaccines is thought to be attributed to their action of stimulating innate immune responses which provide rapid protection but the responses are nonspecific. In the future there may be a schedule to boost the mucosal vaccines which helps to utilize both the vaccination strategies including mucosal and parenteral vaccines and this can be proven with further experimental data (Mitchell and Brownlie, 2015). By improving the mucosal vaccine delivery and improved strategies of vaccination regime against CIRD there is a chance to overcome the CIRD in future. While developing intranasal vaccines, it should be considered that the delivery of these vaccines in aggressive animals is very difficult.

Conclusion

Various diseases of dogs are widespread around the world that severely affect these loyal companions causing them extreme distress. CIRD or canine infectious respiratory disease is an example of such disease. CIRD affects the respiratory tract of dogs leading to signs of dyspnea, cough and nasal discharge. CIRD can be caused due to various viral or bacterial agents. The bacterial pathogens may be controlled through antibiotics but for it is a temporary solution. Additionally, for viruses and permanently solving the bacterial invasion, the best possible solution is vaccination. That is why various types of vaccines are now being researched for use against CIRD. Although some trials have been successful but overall procedure of manufacturing good quality and effective vaccines is proving to be really challenging. Several contributing factors like innate immunity of host, type of vaccine and route of inoculation are being studied thoroughly to properly understand all the variables and then develop a vaccination with most effective vaccination strategies to counter and control this disease.

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Chapter 22

Nano Vaccines and their Role in Cancer Immunization

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ABSTRACT

Nanovaccines have been widely recognized as a major tool in medical science for the treatment of numerous diseases. They are replacing the pre-existing less efficacious therapies with new therapeutic techniques, promising a better solution with limited drawbacks. Multiple types of vaccines have been designed for cancer treatment however nano vaccines are proving the most promising ones. We have various sources (biogenic, semi-biogenic, and synthetic) for the preparation of nano vaccine which differ based on their origin. Primarily, nano vaccines consist of nanocarriers, adjuvants, and tumor-antigens. These either work as active targeting or passive targeting and cause cancerous cell death. Multiple types of anti-tumor nanovaccines are available such as STING-activating nanovaccines, multicomponent nanovaccines (MCNVs), neoantigen nanovaccines and, mRNA nanovaccines. This promising tool helps to boost innate and humoral immunity, enhances drug delivery, and reduces toxicity. Despite all the advantages, there are some limitations which need to be pondered.

KEYWORDS
Nanovaccine, Nanotechnology, Cancer, Nanocarriers, Nanoparticles

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INTRODUCTION

The concept of vaccination is ancient and so far, one of the most striking achievements in human health history to control and treat the disease. This concept was first recorded in Asia and later explained by Jenner and Pasteur during the eighteenth and nineteenth centuries. In the past few decades, cancer immunization by vaccines has been booming in the field. These cancer vaccines can be used alone or in combination with other immune therapies (Zhang et al., 2019). Immune therapies such as immune checkpoint blockade therapy or adipose cell therapy are the most attractive class. Despite these advances in the vaccination field, there is still a need for novel and effective vaccines because pre-existing vaccines seem less efficacious due to many reasons such as failure to stimulate the immune system completely, multiple administrations, and high toxicity (Gheibi Hayat et al., 2019).

Nanotechnology has been rising as an influential tool for the solution of these problems. Over the last few years, scientists have been working on nano vaccines as these are the most prominent and promising in the field of cancer immunization. Nanotechnology began earlier in 1974 and in 1985 first commercialized human nano vaccine was seen in the market (Celis-Giraldo et al., 2021). Different techniques of genetic engineering have made it possible to introduce nano vaccines. These kinds of vaccines are called new-generation vaccines, in which scientists use nanoparticles as a carrier. These small particles help to stimulate the immune system completely (Rosales-Mendoza et al., 2019). Nowadays, nano vaccines have been endorsing over the subunit and viral vaccines (Fig. 1).

Nanotechnology and Cancer

Nanotechnology is an emerging and fast-developing field. It involves both science and engineering in its design, synthesis, characterization, and application. It is implemented at the nanoscale with a length range from 1-100 nm and has numerous benefits in the real world. Gradually, it is becoming a megatrend and general-purpose technology. It is boosting medical research and procedures all across the world (Bhushan, 2017). The application of nanotechnology requires a high level of technical proficiency and precision so that the created mechanisms and devices can interact with molecules with excellent specificity (Zhao et al., 2020). It allows us to manipulate things at atomic, molecular, and macromolecular scales. This rapidly growing field provides the chance to design multifunctional nanoparticles that help to target, diagnose, and treat multiple fatal diseases such as cancer (McNeil and S.E, 2005).

Scientists have been designing highly specific nano-based materials and devices for the interaction within the body at the molecular level, so they can achieve high therapeutic effects with minimal side effects (Silva and G. A, 2004). The

growth of nanotechnology will have a significant impact on the medical industry. These nanomaterials have been employed in a variety of biomedical applications, including cancer therapy, MRI, optical imaging, wound healing, and targeted drug administration (Kayser et al., 2005). By 2020, 19.1 million new cancer-reported cases and 10 million deaths were seen worldwide. Mostly, cancer patients are receiving traditional treatments such as chemotherapy, hormonal therapy, radiation therapy, etc. Along with benefits, they have numerous drawbacks. Cancer nano vaccines are thought to be the best replacement of traditional cancer therapies with less deleterious effects (Sung et al., 2021).

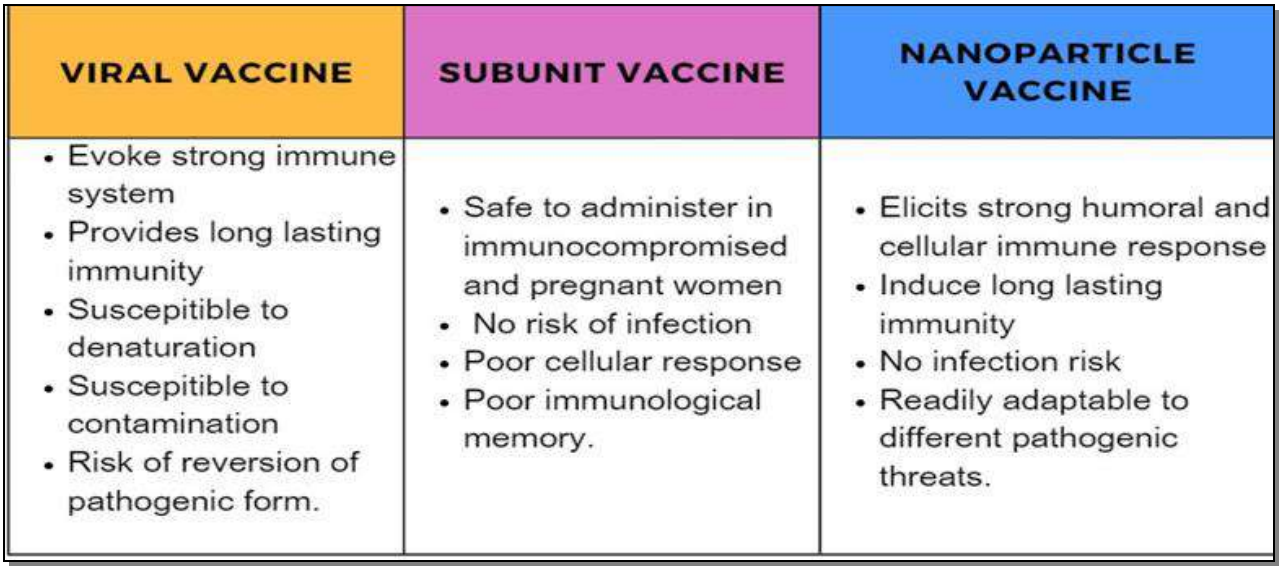

Fig. 1: Advantage of nano vaccines over other vaccines (Retrieved from Canva)

Nanocarriers

Nanomaterials that are used in the transportation of substances (such as drugs, and vaccinations) are called nanoparticles/nanocarriers. Multiple types of nanocarriers are being used in cancer immunization and escalate the positive results against cancer. The use of nanotechnology in the medical industry offers multiple strategies to enhance the efficacy of vaccines. It is considered the best cancer treatment (i.e. nano vaccines). Nano vehicles induce both cell-mediated and humoral immunity completely and are considered best when it comes to high antigen stability, immunogenicity, and antigen presentation. Moreover, it also helps to limit the drawbacks of treatment (Gurunathan et al., 2024). Nanoparticle formulations that are delivered intravenously organic nanoparticles, predominantly fall into two categories: (a) nanoparticles for gene therapy applications or (b) nanoparticles for delivery of small molecule drugs for cancer treatment e.g., head and neck, melanoma, breast, metastatic (Petros et al., 2010).

Based on their origin, Nanoparticles are typically divided into different groups.

1. **Synthetic Nanocarriers:** These include

- **Inorganic nanocarriers (**such as gold, silica, iron oxide): It is found from preclinical research that inorganic nanoparticles are an effective tool to study the various medical fields which include thermal ablation of tumors and, intraoperative sentinel imaging of lymph nodes. Applications for these nanocarriers in the treatment of anemia and imaging have already received approval (Anselmo et al., 2016).
- **Organic nanocarriers** such as polymeric, liposomes, micelles, etc. Currently, organic nanocarriers are being developed for broader fields such as vaccination, topical agents for systemic delivery through the skin and long-lasting depot delivery systems (Fig. 2).

Biogenic Nanocarriers

The nanomaterials which are produced by organisms. These include exosomes and bacterial outer membrane vesicles (OMVs). Exosomes are the unique carriers that are generated from most cells and found in biofluids (plasma, milk, saliva, urine). Bovine milk is the most economical source of exosomes (Kandimalla et al., 2021). OMVs is a new turn in the era of cancer vaccination that is naturally released by gram-negative bacteria. It contains periplasmic components and an outer membrane. It can easily be used as a delivery system of tumor-antigen for the design of a cancer vaccine to generate enough immune response (Zhang et al., 2019).

Bacterial OMVs contain immune-stimulating danger signals such as flagellin, and lipoproteins as lipopolysaccharides, which stimulate TLR4 (Toll-like receptor 4) and TLR5. They can be used as carriers and adjuvants for vaccine development (Kuzmich et al., 2017). The development of OMV-based tumor nano vaccines depends on numerous factors, including OMV heterogeneity, type of strain, safety, efficacy of tumor antigen loading, immunogenicity, and suitability for mass production (Gao et al., 2022).

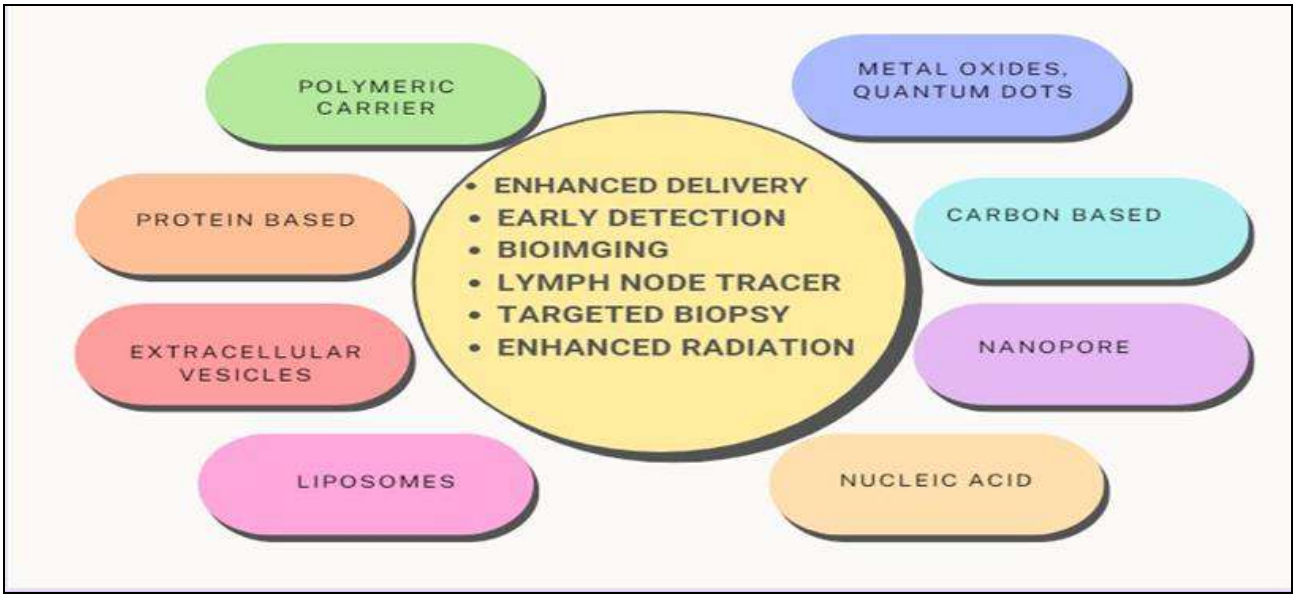

Fig. 2: Clinical application and benefits of nanocarriers (Retrieved from Canva)

Semi-biogenic Nanocarriers

These are partially composed of biogenic and synthetic components that comprise biocompatibility and low toxicity of biogenic nanocarriers. Moreover, the involvement of synthetic components allows the large-scale manufacturing of nanomaterial (Rana et al., 2023). These further includes

- **Cell membrane-coated Nanocarriers**

Cell membrane-coated nanocarriers are covered with membranes derived from tumor or immune cells to harness tumor-specific antigens and homotypic tumor-targeting properties for a strong anti-tumor response (Luk et al., 2015). Initially, red blood cell (RBC) membrane-coated NCs were fabricated by using a combination of RBC membrane-derived lipid vesicles and poly (lactic-co-glycolic acid) NCs (PLGA-NCs) via a co-extrusion approach.

Further advances have led to outstanding progress in cell membrane-coating technology, resulting in NCs coated with membranes derived from platelets, cardiac stem cells leukocytes, and mesenchymal stem cells. Among all these cell sources, cancer cells have gained prime importance due to their homologous binding to the source cells and their natural immune-evading properties (Bose et al., 2018).

- **Endogenous-protein based Nanocarriers**

These nanocarriers such as albumin-based nano vaccines, have the advantage of extending the in vivo half-life that plays an important role in efficient uptake of APCs and activation of APCs for priming of T-cells (Fitzmaurice et al., 2017). Similarly, some nanocarriers that mimic endogenous- protein-based nanocarriers (synthetic high-density lipoprotein nanodiscs) proving themselves as a promising nanocarriers for cancer therapy (Kuai et al., 2018).

Diverse Role of Nanocarriers in Boosting Anti-tumor Immune Response

Nanocarriers have played an important role in tumor vaccines by supplying the antigen or adjuvant to antigen-presenting cells for the activation of tumor-targeting cytotoxic T lymphocytes. Using nanoparticles to co-deliver antigens and adjuvants to immune cells like dendritic cells, macrophages, monocytes, and neutrophils is effective in anti-tumor immunotherapy (Li et al., 2016).

- The lipid-coated zinc phosphate hybrid nanocarriers were formulated to co-deliver multiple antigenic peptides (second tyrosinase-related protein and human glycoprotein 100) that facilitate immune cells with multiple epitopes and prevent tumor cells escape from the surveillance of the immune system. Nanocarriers targeted by specific targeting motifs C-type lectin receptors (Dec-205) may increase the access to the cytoplasmic major histocompatibility complex I loading machinery and boost immune response by stimulating anti-tumor CD8+ T cells (Hu et al., 2017).
- Subcutaneous immunization of nano vaccine results in the establishment of antigen depot. Continuous release of antigen from the depot stimulates mature dendritic cells, enhances infiltration of effector T cells, and boosts the anti-tumor immune response. (Yin et al., 2022). Nanocarriers loaded with immune agents result in precise targeting of tumor tissues and reduce toxic side effects. By surface modification of target molecules or Enhanced Permeability and Retention Effect, nanocarriers can effectively deliver drugs, antibodies, and immunomodulators however enrichment of drugs and antibodies, regulation of local immunity, and improvement of immunosuppressive microenvironment can also be achieved (Li et al., 2022).
- Use of organic and inorganic nanocarriers as short interfering RNA (siRNAs) delivery agents aims to alter the expression of particular genes in cancer cells in order to cause tumor regression. A flexible and powerful strategy for fighting tumors and changing the tumor microenvironment from a pro-oncogenic to an anti-tumoral state is the

combination of RNA interference (RNAi) and nanomaterials (Conde et al., 2016).

- Magnetic nanoparticles (MNPs) serve as a platform for developing combinatorial immunotherapies with enhanced efficacy, by concurrently addressing different tumor immune-evasion mechanisms (Williams, 2017). MPN used as heat mediators in magnetic hyperthermia leads to knocking down tumor priming of anti-tumor immunity. The design of new nanosystems is aimed at triggering tumor-specific responses for T cell priming by controlling the natural tropism of nanoparticles toward secondary lymphoid organs. The enhanced permeability and retention (EPR) effect is exploited by providing nanoparticles to tumors. Surface functionalization with tumor-targeting molecules facilitates the loading of immunomodulators that can boost immune responses (Persano et al., 2021).
- Reactive species are signaling messengers involved in immune suppression by aiding the accumulation of regulatory T cells (Tregs). Production of reactive oxygen species (ROS) has been used to induce immunogenic cancerous cell death, marked by the release of molecular patterns linked to damage and antigens specific to tumors (DAMPs) exposure for activation of anti-tumor immunity. Cancer immunotherapy can be boosted by accurate spatial-temporal control of reactive species in targeted bio-systems (Zhang et al., 2021).

Preparation of Nano Vaccine

The introduction of vaccination in the field of medicine has revolutionized and sped up the healing process against infectious diseases (Sheerin D et al., 2017). Nano vaccination consists of three components

1. Tumor antigens could be either tumor-associated antigens (TAA) or tumor-specific antigens (TSA) (Yarchoan M et al., 2017).
2. Nanocarrier, which can activate the immune response (Le Gall C et al., 2022).
3. Adjuvants that are immune boosters. Tumor antigen is used to produce immunogenicity adjuvant is added to enhance the immunogenicity so the vaccine can effectively provoke the immune system and produce antigens (Yuksel S et al., 2020).

Based on the separation of antigen from tumor, the vaccine is of four types

a) Peptide vaccine that contains an amino acid sequence that provokes immune response it must contain CD8+ T cells epitopes and CD4+ T cell epitopes to activate T helper cells and CTL anti-tumor immunity (Su et al., 2021) b) DNA vaccine that is plasmids c) mRNA vaccine d) Cell vaccine (Sha H et al., 2022) Nanocarriers are further liposome, polymer, inorganic and biomimetic type. It reduces drug-related side effects and increases the delivery of drugs (Hashemi V et al., 2022)

Types of Nano Vaccines for Cancer

Multiple types of nano vaccines are used in the immunotherapy of cancer.

- **STING Activating Nano Vaccines**

STING is an agonist of the stimulator of interferon genes protein; transmembrane protein 173; (TMEM173) and is used in nanoparticle vaccines. These vaccines encourage the inflammatory cytokines to strengthen the anti-tumor T-cell responses and to restructure the tumor microenvironment. (Hines et al., 2023). The PC7A NP is a synthetic antigen polymeric nano vaccine based on STING activation and is used in colon cancer, human papillomavirus-E6/E7 tumor models and melanoma. (Luo et al., 2017).

- **Multicomponent Nanovaccines (MCNVs)**

The STING agonist alone cannot obtain an intense cytokine response so the combination of agonists can be used to make a multicomponent nano vaccine such as STING and TLR7/8 is a MCNV used for tumor regression in melanoma. This vaccine contains the Toll-like receptor 7/8 (TLR7/8) agonist 522 and STING agonist CDG^{SF} to target tumor-infiltrating lymphocytes. This vaccine activates BMDCs (bone marrow-derived dendritic cells); promoting several anti-inflammatory variables to enhance antigen cross-presentation and produce a specific anti-tumor T-cell response. (Zhang et al., 2022).

- **Neoantigen Nanovaccines**

Neoantigens are tumor-specific antigens (TSA) found in tumor cells only as they arise from DNA mutations. The neopeptides are administered to the patients that as result activate the immune system's $CD8^+$ and $CD4^+$ T-cells to identify and destroy cancer cells having neoantigens. These vaccines are used for breast cancer, pancreatic cancer, malignant melanoma, and bladder urothelial cancer (Biswas et al., 2023).

- **The mRNA Nano Vaccines**

The dendritic cells (DCs) are targeted antigen-presenting cells in mRNA-based nano vaccines. As mRNA NVC containing lipid nanocarriers with self-adjuvant properties is injected into the body, the ribosome makes mRNA-encoded protein and modifies it post-translationally into functional protein in the cytoplasm (Deng et al., 2022). Then this protein targets sequences or transmembrane structural domains and enters subcellular compartments, such as the cell membrane, secretory pathway, mitochondria, peroxisomes and nucleus mRNA NVC is used in metastatic prostate cancer (Zhang et al., 2021).

- **The Artificial APCs Nanovaccines**

aAPCs are artificial antigen-presenting peptide cells that bind co-stimulatory molecules and TCR with their related receptors at the immunological synapse and activate T-cells by expressing APC-associated biomarkers in the tumor-infiltrated lymphocytes (TILs). These T-cells are 10 times more in number than that of free vaccines and help in raising the levels of IFN- γ and TNF- α (Fang et al., 2022).

- **Metal-based Nano Vaccines for Cancer Immune Therapy**

Metallic nanoparticles (MeNPs) are non-biodegradable. They have rigid structures and simple methods of production. MeNPs have the capacity of immunostimulation and can induce different reactions in all phases of vaccine development. According to studies, metallic nanoparticles can help in the production of T helper cells type 1 (Th1) and T helper cells type 17 (Th17). There are no findings about cellular immunity (Marques Neto et al., 2017).

Metal-based nanomaterials deliver antigens to antigen-presenting cells for producing immunity. Metal ions play essential functions in immune response which includes Ca^{2+} (activates T cell), Ca^{2+}, K^{+}, Na^{+} (collectively activate inflammasome), Zn^{2+} and Mn^{2+} (cGAS-STING signaling), Zn^{2+}, $Fe^{2+/3+}$, Mn^{2+} and Cu^{2+}(involve in pathogen-host interaction) (Li et al., 2022). Mq^{2+} restores the cytotoxicity of T cells and natural killer cells in chronic Epstein-Barr virus infection (Chaigne-Delalande et al., 2013).

Metal oxide nanoparticles have a tendency of internalizing into different cell types. They are found suitable to deliver antigens to Antigen-presenting cells (APCs). The research focused on using transition metal nanoparticles for biomedical applications e.g. Co, Ni. Cobalt is found to have a role as co-factor of vitamin B_{12}. Later in vitro, study showed that Phosphonomethyl iminodiacetic acid (PMIDA) conjugated cobalt oxide nanoparticles bonded to tumour lysate antigen and activated TNF-α to trigger an anticancer immune response. (Chattopadhyay et al., 2016).

Metal nanoparticles possess unique optical, magnetic, and electric properties. Cobalt oxide was used as nanoparticles for biomedical applications because of its special characteristics that depend on size and shape, as well as its uses in pigments, catalysis, sensors, electrochemistry, magnetism, etc. (Chattopadhyay et al., 2015).

Metal nanomaterials can inhibit virus infections by using three different pathways

1. Inactivating the virus directly
2. Inhibition of virus entry and its adsorption
3. Intracellular virus suppression (Yang et al., 2021).

There are findings that some metal elements are involved in major Cancer Immunity Cycle (CIC) steps which include the processing and presentation of antigens, Priming and activation of T cells, T cell infiltration into tumor cells, Cancer cells immune recognition, Tumor cells killing and release of antigen (Luo et al., 2024).

The specific valences of some metals like Fe, Mn, Cu, Co, Zn, Ca, Pt, etc. can induce Fenton, Fenton-like reactions, and other signal pathways that produce large amounts of reactive oxygen species (ROS) and induce endothelial reticulum (ER) stress; thus, it causes immunological cell death and activates the innate and adaptive immune systems to destroy cancer cells (Cao et al., 2021).

Coordination Polymers CPs consist of metal ions and organic ligand framework forming either 2 or 3 dimensional structures. Based upon metal ions CPs has been used as therapeutic anti-tumor vaccines (Pena et al., 2023). According to a study, Europium metal ion when combined with organic ligand Guanine monophosphate, forms a polymer that inhibits tumor growth and enhances survival in an OVA-expressing melanoma model (Duan et al., 2017).

Mechanism of Action of Nanocarriers for Treating Cancer

Research in the cancer field has proven that the formulation of vaccines including nanoparticles as delivery agents can enhance antigen presentation by antigen-presenting cells (APCs) to effector lymphocytes and increase antigen delivery to APCs (Jiang et al., 2020). Medications and techniques that are used for the treatment of cancer should be efficient enough to cross several layers and barriers to reach the target site (Gavas et al., 2021). Nanoparticles (NPs) should have the following qualities: (a) Stability in the vascular system. (b)To clear the reticuloendothelial system (RES)(c)Ability to escape mononuclear phagocyte system (MPS)(d) ability to penetrate in tumor fluid(e)Should be highly specific (only act on cancerous cells) (Omidi Yand Barar J, 2014). In general, there are two targeting mechanisms as shown in Fig 3:

Passive Targeting

Inflammation is observed in a cancer cell permeability of blood vessels increases in case of hypoxia and inflammation (Matsumura Y and Maeda H, 1987; Singh N et al., 2019). NPs easily move out of vessels, the lymphatic drainage of the affected vessel is also not efficient and the NPs stay at the target site and kill the cancer cells. Further, the drug is encapsulated in nano-sized particles to increase their selectivity and EPR (Enhanced permeability and retention**)** effect (Padera TP et al., 2004). Passive targeting depends on vascularity, leakiness, size, and circulation time of carrier molecules and does not possess ligands (Melody A et al., 2007).

Active Targeting

It is also called ligand-mediated targeting, the use of specific molecules (ligands) i.e. transferrin, folate, and epidermal growth factor receptor (EGFR) that bind to receptors or molecules that are overexpressed on the cancer cell (Mukwaya G et al., 1998; Peer D et al., 2007) binding of a ligand with the receptors followed by the receptor-mediated endocytosis as transferrin that in normal cells aids in transporting of iron but in cancer cells, it is overexpressed so NP's are made to attach to this receptor site in the core these particles release the drug that acts on the cancer cell and due to its specificity it only acts on cancerous cells and so protects the healthy cells (Saha RN et al., 2010).

Advantages of Nano Vaccination

Nanovaccines can induce an immune response that can overcomes multiple challenges in the cancer immunity cycle (Xie et al., 2022).

- It improves therapeutics standards and enhances early diagnosis through in vitro. Nanocarriers can stimulate both innate and adaptive immune responses through numerous mechanisms like enhanced antigen presentation, toll-like receptor signaling, and the activation of the complement system. This immune response is dose-dependent and can lead to an increase in the invasive response of immune cells and cytokines in the microenvironment of the tumor (Chehelgerdi et al., 2023).
- Nanocarriers reduce the toxic side effects and enhance drug delivery, particularly in case of poorly soluble or sensitive molecules in addition to increasing the efficacy of vaccines by both EPR (enhanced permeability and retention) effect allowing a passive targeting by NPs and ligand-based targeting reducing unwanted interactions and drug localization in peripheral tissues (Cassano et al., 2021).

Evidence suggests that the multivalent effect of self-assembled nanoparticles and multiple antigen-conjugated nanoparticles prompts a stronger cellular and humoral reciprocation of the immune system. In addition to that, nanotechnology can manipulate antigen density and orientation providing the opportunity to investigate its optimization and underlying mechanisms of the multivalency effect. (Feng et al., 2022).

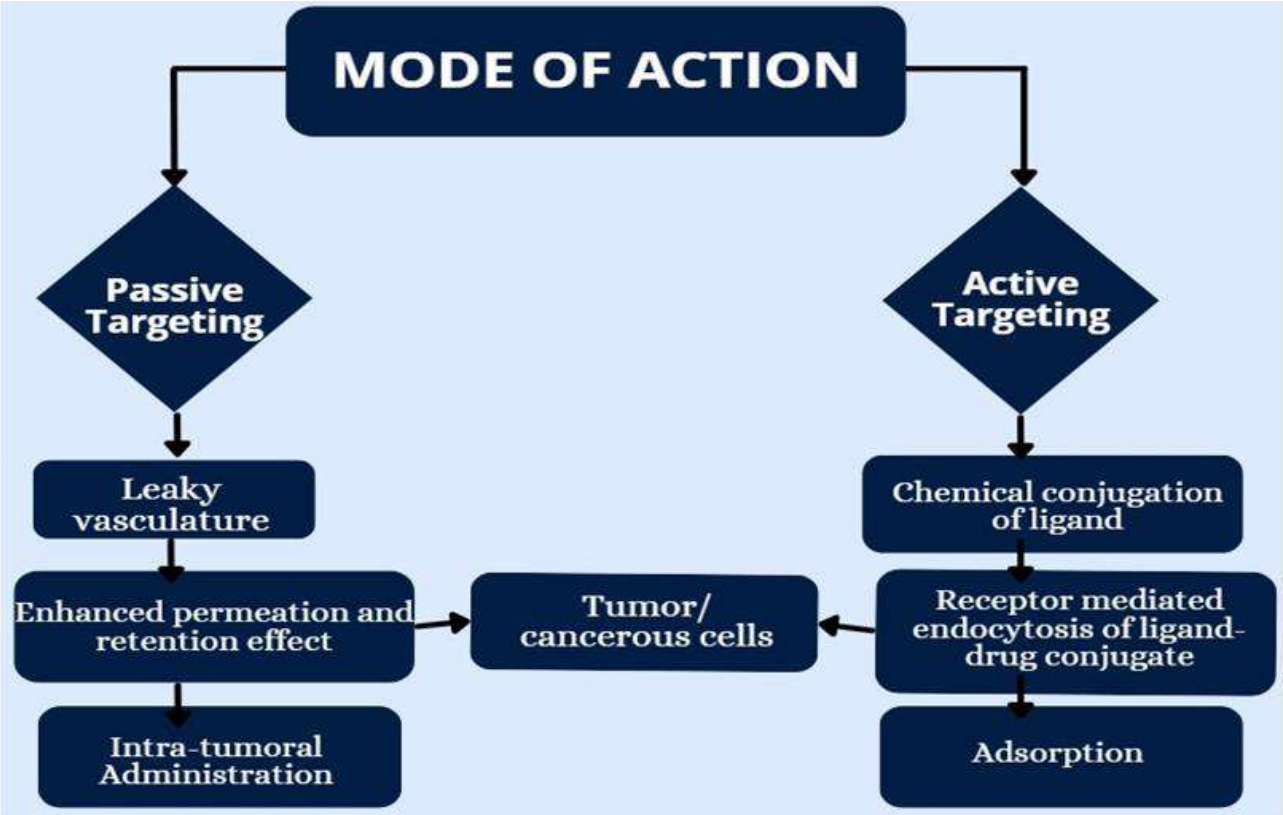

Fig. 3: Mode of action (Retrieved from Canva)

Challenges of Nano Vaccines

Nanoparticles have several potential benefits in future vaccination approaches. Despite that, they also have certain limitations and challenges. To overcome these challenges, the field requires innovations and progress in the research area (Sun et al., 2024).

- Nanomedicine, specifically protein-based faces a challenge in sterilization especially, contamination by endotoxins which leads to serious health issues (Rodríguez et al., 2022)
- Because of endotoxin contamination, almost 29.5% of nanoformulations fail during the early stages of preclinical research. (Li et al., 2016)
- The controlled manufacturing of nanomedicine is challenging because small variations in the production process can alter the properties like shape, composition, size, toxicity, biocompatibility, drug release, and in vivo outcome which necessitates the characterization of the products on batch-to-batch basis making nanomedicine production time consuming and costly (Dobrovolskaia et al., 2007; Aillon et al.,2009; Nel et al., 2009).

Future Prospects of Nanotechnology

- For the time being, nanotechnology is proving itself as a promising field and providing future evidence from mass of preclinical and clinical studies proving the efficacy of nanotechnology in terms of diagnosis, cancer imaging and treatment. however, this advancement in technology must be clinically translatable (Kemp et al., 2021).
- In the future, it is crucial to regulate the physical and chemical characteristics of nanoparticles to enhance their

targeting capability, focusing on their shape, size distribution, and surface chemistry (Tian et al., 2022). Even though cancer's dynamic character won't go away, innovation is still advancing and the convergence of several technologies shows potential for success.

Conclusion

In conclusion, nanotechnology represents a promising future in terms of cancer immunization and cancer therapy aiding the traditional cancer treatment options like chemotherapy and radiation therapy with its target specificity and reduced side effects. Various kinds of nano vaccines like STING activating nano vaccines, multicomponent nano vaccines, neoantigen nano vaccines, mRNA nano vaccines, artificial APCs nano vaccines, and metal-based nano vaccines, have specific properties that are being explored further to aid the immune system in recognition and destruction of cancer cells by enhanced antigen presentation, immune response, and tumor targeting. Despite these advantages, nanovaccination presents challenges such as endotoxin contamination and complex manufacturing processes for which further technological advancement and optimization of the nanovaccination is needed. In short, nano vaccination has immense potential in the future for which continued efforts are required to improve its design, targeting, and manufacturing process. This will improve nanotechnology and revolutionize cancer therapy while significantly enhancing patient outcomes.

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Chapter 23

Mapping the Dynamics of Vaccination in Parasitology

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ABSTRACT

The parasites play crucial role in causing fatal diseases in both humans and animals which need to be controlled or eliminated at human-animal interface The dynamics of vaccination in parasitology encompass a wide range of topics, from the understanding of parasites and their life cycles to challenges and advancements in vaccine strategies. The concept of one health holds that the health of humans, animals and the environment are all connected. Advances in the preventive approaches in veterinary parasitology are essential to reduce the foodborne illnesses and zoonoses along with an effective medical parasitology vaccine program to fulfill the urgent requirement for immunization against major human protozoal infections. To address recently identified and resurgent parasitic diseases globally, the dynamics of the antiparasitic vaccine represent a constantly evolving field requiring interdisciplinary collaboration, close observation, and creative thinking. Despite the tremendous progress, there are still obstacles to parasitic vaccination, including the complexity of their antigen variety and the challenges of producing target parasites *in-vitro*. This chapter provides an insight into the current update of vaccines development against parasitic diseases of medical and veterinary significance.

KEYWORDS
Vaccine, Parasites, Human health, Animal health, One health

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INTRODUCTION

The acronym "parasite" refers to a diverse group of organisms, such as helminths, arthropods, and protozoa that exist on or within another organism (the host). Comprehending the parasite life cycles, host relationships, and the undesirable consequences that arise are all linked to the controlling the parasites (Wood and Johnson, 2015). The parasites significantly affect both human and animal health because parasites have developed various tactics to take advantage of host resources, the host's immune system and the parasite's capacity for evasion frequently interact in intricate ways (Lopes, 2022; Chulanetra and Chaicumpa, 2021). The ecology and evolution of the parasite and host populations are both impacted by this dynamic interplay, which has wide-ranging effects (Shim and Galvani, 2009). Evolutionary change is probably going to be a key factor in a lot of basic biological processes since parasites are so common in the natural world and because they frequently affect the host's health. The development of both natural and adaptive defenses, for instance, has been linked to host-parasite coevolution (Mayer et al., 2016). The significance of eco-evolutionary feedback and its qualitative impact on the mechanism of the host-parasite evolutionary process has been emphasized in the recent years by studies that directly compare similar models with and without population variation (Ashby et al., 2019).

Among the strategies to control parasitic infections, chemotherapy is foremost option but due to irrational use of antiparasitic drugs, resistance has developed against these drugs (Dziduch et al., 2022; Rizwan et al., 2021). The novel drugs have not been developed or being discovered on the same pace as that of development of antiparasitic drug

resistance therefore it necessitates the development of vaccines to control the parasitic infections both in humans and livestock (Rao et al., 2023; Selzer and Epe, 2021). Vaccination is intended to take preventative action to create an enduring immunological response, reducing the degree of illness to the point where sickness is prevented upon subsequent exposure to the specific contagious pathogen. The immunological memory activation, which creates the capacity to react quickly, is essential for the efficacy of vigilant vaccination (Stern, 2020). The vaccination given to animals are more helpful, however, in more ways than one because many of them shield people from anthropozoonoses, or illnesses that both humans and animals can contract (Lombard et al., 2007).

Edward Jenner's 1796 demonstration of the vaccinia virus's ability to ward against had employed the smallpox virus itself (Plotkin, 2003). Most parasitic illnesses are lifelong or persistent, and they are not usually immediately fatal. This suggests that, in most situations, immunological reactions against parasitic illnesses are adequate to manage the disease and guarantee the individual's existence. The scientific approaches with liable efficacy, which have demonstrated potential in several microbiological programs are currently being extended to parasitic programs and can be used to directly screen pools comprising hundreds of thousands of genes for vaccine potential (Rainczuk et al., 2003). There are limited number of vaccines which are commercially available for parasitic disease. In addition, the production of vaccines for parasitic infections are more challenging than that of against viral or bacterial infections (Selzer and Epe, 2021). Within cells, *Plasmodium* spp. exist. Consequently, distinct immune system reactions may be needed to regulate infection patterns. One of medicine's ongoing objectives is to develop a very effective malaria vaccine that both prevents illness and ends the cycle of infection. One of the most promising options for vaccination against parasites is a whole vaccine based on the sporozoite forms of the parasite, which targets the clinically inactive pre-erythrocytic phases of illness (Goswami et al., 2019).

Challenges in Developing Antiparasitic Vaccines

The vaccination usage causes a significant change in the ecosystem that parasites inhabit. The aim of vaccine is to protect specific hosts and so lower the incidence of parasites. In the end, this might even result in the disease's eradication (Fenner et al., 1987). The immune system's reaction to several simultaneous infections is probably going to be enhanced or synergized. Research on trematode, nematode and *Plasmodium* infections have demonstrated reduced vaccination efficacy and capability to prevent new infections in both human and animal models (Urban Jr et al., 2007). In addition, the production of antiparasitic vaccines is more challenging and complex compared to vaccines against viral or bacterial infections (Selzer and Epe, 2021). To date, a very little success has been made in developing vaccines against parasitic diseases of veterinary and public health significance (de Barros and Koutsodontis Cerqueira-Cézar, 2023; Vercruysse et al., 2018).

The warm-blooded species including humans and domesticated animals are susceptible to infections by the obligatory intracellular protozoan parasite, *Toxoplasma* (*T.*) *gondii*. Consequently, the parasite is dispersed throughout the globe (Dubey (Dubey, 2008), 2008). By releasing proteins, especially rhoptry (ROP) and dense granule (GRA) effector proteins that alter host transcription channels of signaling, *T. gondii* manipulates the host immunity (Rastogi et al., 2020). Type I strains of *T. gondii* activate host immunity-related GTPases (IRG) to circumvent interferon-induced guanosine triphosphate hydrolysing enzymes (IFN γ-inducible GTPase) mediated host protection (Tomita et al., 2021; Zhao et al., 2009). The type II strain ROP54 was discovered as well to disrupt the canonical IFNγ-dependent mechanism of autophagy by limiting guanylate-binding protein 2 (GBP2) immunological modulation to evade the GBP2-mediated immune response (Kim et al., 2016). In humans, trematode infection (schistosomiasis) has been linked to a reduced response to tetanus toxoid vaccination, which is correlated with markedly different tetanus toxoid-induced Th1- and Th2-type immunological responses *invitro* (Sabin et al., 1996).

The reduced typhoid, tetanus, and *Haemophilus* influenzae type B response levels against vaccination have been seen in the context of coexisting malaria infection, demonstrating the detrimental impact of persistent protozoan infections on vaccination response (Usen et al., 2000). The immune system's "imprinting" during pregnancy may also be a major factor in the decreased efficacy of vaccinations in regions where parasite infestation is common. In coastal Kenya and other emerging nations often, one or more parasites affect fertile women (Hillier et al., 2008). Research on the mutual infection of HIV and parasites indicates that anthelminthic medication may slow the loss of CD4 cells and increase the viral load in HIV cases (Walson and John-Stewart, 2008). The *Plasmodium* parasites are the cause of malaria. There has been research on subunit vaccinations that target particular malaria antigens, like the circumsporozoite protein. One vaccination that has shown some efficacy in clinical studies is the RTS, S/AS01 combination. Targeting several *Plasmodium* parasite life stages, such as the sporozoite, liver, and blood stages, is the goal of developing multi-stage malaria vaccinations. Greater protection and more durable immunity are the goals of this strategy (Skwarczynski et al., 2020).

Infectious illness control has greatly been benefited from vaccination. Additionally, being the least expensive means of preventing infectious diseases, vaccination campaigns can, given the right circumstances, completely eradicate a disease, as smallpox did. The impact of vaccination on human parasite illnesses has been negligible, despite their enormous potential. This is due to a variety of factors, including the genetic and biological complexity of these eukaryotic pathogens some of which have intricate life cycles and highly developed immune evasion mechanisms (de Barros and Koutsodontis Cerqueira-Cézar, 2023). The live parasites are regarded as too harmful to be used as vaccines, while killed vaccines have generally proven useless. Since many parasitic pathogens cause live infections, the immune system's reaction is thought to

be inadequate. Hence, using a portion, or subunit, of the pathogen may be the most effective way to achieve the goal of a more robust reaction than what's feasible with a live infection (Ballou et al., 2004).

To select vaccine targets, gene pools or protein sequences can be screened using antisera. Western blot, pathogen lysis, infection inhibition, and ELISA are simple methods for determining the potency and selectivity of immune responses. The T-cell tests are used to determine which peptide epitopes T-cells are capable of identifying. The targets of T-cell responses are peptides that bind to the major histocompatibility complex (MHC) are predicted by algorithms that are becoming more accurate (Bukhari et al., 2022).

In tropical and sub-tropical climates, metazoan and protozoan parasites are the primary cause of disease in humans and animals, resulting in higher illness and mortality rates. The World Health Organization (WHO) believes that one in four people have parasitic worms. Despite significant advancements in the identification of potential protective antigens, the development of vaccines is hampered by the inability or difficulty to cultivate many of the main target parasites *in-vitro* the fact that most exhibit a significant diversity of antigens, and the mounting evidence that parasites directly modulate the host immune system to their benefit (Knox, 2010).

In order to lower the childhood mortality in nations like Bangladesh, vaccination against *Entamoeba histolytica,* the agent that causes amoebic colitis and liver abscesses, is essential. The mucosal antibodies against *E. histolytica* Gal/GalNAc lectin are linked to protection against amoebiasis. However, since the vaccine is intended for underprivileged individuals living in poorer nations, raising funds for its research becomes more difficult (Miller-Sims and Petri Jr, 2002).

The circumsporozoiteg gene of *P. falciparum*, which is essential for the production of a vaccine and can be cloned and produced, was first discovered twenty-one years ago (Dame et al., 1984). It was established in the 1960s that attenuated *Dictyocaulus* larval infection may induce a great degree of resistance to infections from typical infectious larvae. The vaccine "Dictol" which is still in use today, was developed as a result of this finding. Any vaccine's success may depend much on the adjuvant selected. Using *Haemonchus* ES as an example, lambs treated with alhydrogel as an adjuvant showed significantly higher antibody responses and protection compared to those treated with dimethyl dioctadecyl ammonium bromide (Smith and Zarlenga, 2006).

It has been suggested that vaccination be used in conjunction with an approach that combines public health and veterinary medicine to control the helminth infections. Several vaccine candidates are presently being developed and/or made accessible for use in treating helminthic illnesses (Matthews et al., 2023). The lead subunit vaccine targets that are currently available were mostly found using the traditional biochemical methods on parasite protein fractions (for example, 28 kDa glutathione S-transferase from *S. mansoni* and 14 kDa protein antigens that bind to fatty acids) (Hagan et al., 1995).

The metazoan parasites, such as helminths and mites, can strongly trigger inflammatory responses. These parasites have also been linked to the development of many allergies in humans and host sensitization (for instance, allergies to meat caused by ticks). When helminth infections occur, the host response is heavily biased toward Th2 immunity. By secreting bioactive substances, the parasites actively regulate the host's Th2 (and other) reactions which ultimately results in a reduction in the host's antiparasitic IgE and cellular response. Clear product profiles are less obvious when it comes to animal vaccines. Bovilis Huskvac, Barbervax, and GoTick are examples of native vaccines that have proven to be effective. Pure recombinant antigens, on the other hand, are favored for human vaccinations and may be found in vaccine formulations such as Providean Hidatil EG95, Cysvax, Providean Aquatec Sea Lice and GAVAC (Stutzer et al., 2018).

Recent Progress in Vaccines against Human Parasitic Diseases

The majority of deaths globally are caused by parasitic illness, and the rise of multi-drug-resistant forms of these diseases frequently makes therapeutic care of them difficult. The best line of action to combat them at this time is immunization as a preventative measure (Rueckert and Guzmán, 2012). For any human protozoal disease, there is currently no readily accessible vaccine, despite the urgent need and significant research (Palatnik-de-Sousa and Nico, 2020; McAllister, 2014). The effective and commercially available vaccines against some of the more significant human protozoal infections including giardiasis, vivax malaria, chagas disease, visceral leishmaniasis, cutaneous leishmaniasis, African trypanosomiasis, falciparum malaria, and cryptosporidiosis need to be developed (Zhang et al., 2022; Palatnik-de-Sousa and Nico, 2020; Armijos et al., 2003).

The parasitic disease toxoplasmosis affects humans, animals, and cats. Preventive measures would help to avoid infection. The goals of a one health vaccine strategy would be to prevent neonatal diseases and minimize *T. gondii* tissue cysts in women and cattle which are used for food production, and prevent oocyst shedding in cattle (Mévélec et al., 2020). Following the consumption of *T. gondii* oocysts, the sporozoites or bradyzoites are released into the lumen of the small intestine through an intermediate host, where they promptly undergo endocytosed-mediated proliferation within the parasitophorous vacuole (PV) of various kinds of cells after crossing the epithelial barrier of the bowel. The tachyzoites propagate throughout the organism after infecting circulating cells like dendritic cells, natural killer cells, monocytes, and macrophages (Persson et al., 2009). The intraerythrocytic protozoan parasites *Babesia* (*B.*) spp. spread to both humans and animals, causing bleeding disorders and flu-like symptoms, and vaccine is not available for humans at this time. Older people, immunocompromised people, and splenic individuals are the most prone to serious sickness. Among the species that mostly impact people are *B. microtian* and *B. divergens*. $CD4^+$ T cells and macrophages mediate immunity, although B-cells are not necessary for it (Al-Nazal et al., 2021).

The exceedingly prevalent mosquitoes referred to as *Anopheles gambiae* and *Anopheles funestus* are the carriers of *Plasmodium* (*P.*) *falciparum*, which is responsible for the deadliest form of malaria. Three phases make up *P. falciparum*'s life cycle in humans: the pre-erythrocytic stage, which initiates infection; the asexual erythrocytic stage, which causes illness; and the gametocyte stage, which infects mosquitoes that subsequently transmit the parasite. When a female *Anopheles* mosquito injects a small quantity of *P. falciparum* sporozoites into the skin, or both into the skin and blood, the pre-erythrocytic cycle begins. A tiny number of hepatocytes are infected by sporozoites once they reach the liver. The merozoites are asexual parasites that can multiply into tens of thousands from a single sporozoite. When infected hepatocytes rupture, releasing merozoites into the bloodstream and eliminate any remaining parasites in the liver, approximately one week after the original infection (Shortt et al., 1951).

To date, there has only been one effective vaccine for a parasite disease in human which is RTS,S malaria vaccine, which was authorized for use in children from age 5 months with its strong safety profile in regions with endemic malaria but further studies are required to assess the safety of this vaccine (Björkman et al., 2023; Laurens, 2020). Because of the complexity of their life cycle and the various immune escape mechanisms that different parasites show, human immunity to them is distinct, making the development of vaccines against them challenging (Draper et al., 2018).

Recent Progress in Vaccines against Animal Parasitic Diseases

The foodborne illnesses (FBDs) are a global health problem due to their higher mortality and sickness rates in the human population. Among the foodborne parasites that cause foodborne diarrhea (FBDs) include *Taenia solium*, *Echinococcus granulosus*, *T. gondii*, *Cryptosporidium* spp., and *Trichinella spiralis*. These parasites are ranked in the highest worldwide risk category. Animal vaccination against foodborne parasites not only prevent illnesses in animals but also enhance public health by reducing a major cause of FBDs (Sander et al., 2020).

The pig tapeworm (*Taenia solium*) is a parasitic flatworm that lives in the small intestine of humans. It contaminates the environment by releasing eggs in excrement. Pigs are intermediate hosts; the larvae develop in the muscles of the pigs after they are consumed. Eating undercooked pork that contains larvae can lead to human infection. When cysticerci mature into adult tapeworms in the human intestine, the life cycle is completed (Sánchez-Torres et al., 2019). A more recent study showed that the helminth was eradicated in pigs vaccinated against *T. solium* endemic in Nepal for 10 months at 3-month intervals using Cysvax®, the first and only commercially licensed cysticercosis vaccine based on the TSOL18 antigen. The vaccine was administered in conjunction with oxfendazole medications (Poudel et al., 2019). According to FAO/WHO estimates from 2015, *T. solium* infects about 50 million individuals globally, resulting in 2.8 million (DALYs) (De Coster et al., 2018).

The dog tapeworm (*Echinococcus granulosus*) is a parasitic flatworm. Adult tapeworms live out their life cycle in the small intestine of dogs, where they deposit eggs into their excrement. Sheep are examples of herbivores that become intermediate hosts when they consume polluted vegetation. The intermediate host's interior organs are where larvae, or hydatid cysts, develop. Inadvertent egg consumption can introduce this tapeworm into humans, resulting in the formation of cysts primarily in the liver and lungs (Kakundi, 2020). Numerous investigations have demonstrated that the EG95 vaccine immunizes bovine hosts against cystic echinococcosis with remarkable protective effectiveness (Heath et al., 2012).

A protozoan parasite (*Cryptosporidium* spp.) causes diarrheal sickness in both humans and animals. It goes through both asexual and sexual phases to finish its life cycle, mostly in its hosts' intestine (Hatam-Nahavandi et al., 2019). For passive immunization of young calves, the recombinant P23 protein has undergone vaccination trials. The study showed that when immunized dams gave improved colostrum and Freund's adjuvant-emulsified recombinant protein in 300 µg then the release of oocysts in calves feces was suppressed over 90% (Askari et al., 2016).

A zoonosis known as trichinellosis, which is considered to be a disease that is both emerging and re-emerging in some regions of the world, especially in Asia and Eastern Europe, is mostly brought on by the parasitic nematode that lives inside cells e.g. *Trichinella spiralis* which can spread to a large range of hosts that are both carnivores and omnivores. (Devleesschauwer et al., 2017). The *T.* spiralis antigens provide the host with systemic and enteral protection that is both safe and effective. Several potential vaccines, which can be administered alone or in conjunction with adjuvants, are relied on individual, combined, or multiple antigens from different *T. spiralis* stages. They can be used as DNA vaccines, recombinant proteins, or crude protein extracts (Zhang et al., 2018).

One Health Approach and Antiparasitic Vaccine Development

The one health concept, which recognizes the connection of ecological, animal, and human health, encourages interdisciplinary collaboration in the search for answers to challenging medical problems. The importance of the environment and the assessment of host-parasite relationships are two traditional veterinary parasitology characteristics that are associated with One Health's focus on the links between animal, human, and environmental health (Krecek et al., 2020).

Over the past few years, neglected tropical illnesses and zoonotic diseases have drawn more attention, and new objectives for control and eradication at the local, national, and international levels have been established. The *Taenia solium* taeniosis/cysticercosis (TSTC) is one such neglected zoonosis that the WHO has chosen to target for elimination in some endemic areas since it is deemed an eradicable illness (Johansen et al., 2017).

Table 1: List of effective vaccines against animal parasitic diseases

Parasitic Disease	Causative agent	Vaccine / Company Name	Efficacy	Availability	Reference
Tick infestation	*Rhipicephalus* (*Boophilus*) *microplus*	TickGard® (Hoechst Roussel Vet, New Jersey, United States)	Limited protection	Not Available	(De la Fuente et al., 2007)
Coccidiosis	*Eimeria* spp.	Attenuated: Paracox® 8 (MSD Animal Health, Madison, NJ, USA) Hipra Evalon® (E) (Amer, Girona, Spain) Huveguard® Start/ Mmat and Huveguard® Plus/NB (Huvepharma, Sophia, Bulgaria) Non-attenuated: Advent™/ Inovocox™/ Immucox®/ Coccivac®	Significant protection	Available	(Zaheer et al., 2022)
Cattle trichomoniosis	*Tritrichomonas foetus*	TrichGuard® (Boehringer Ingelheim, Germany)	Significant protection	Available	(Edmondson et al., 2017)
Canine babesiosis	*Babesia* spp.	Pirodog® (Boehringer Ingelheim, Germany)	Significant protection	Available	(Schetters, 2005)
Canine leishmaniasis	*Leishmania infantum*	Letifend® (LetiPharma, Barcelona, Spain)	Significant protection	Available	(Iborra et al., 2018)
Cattle lungworm infection	*Dictyocaulus viviparus*	Bovilis Huskvac® (MSD AH, Dublin, Ireland)	Significant protection	Available	(Jarrett et al., 1958)
Haemonchosis	*Haemonchus contortus*	Barbervax® (DAFWA parasitology laboratories in Albany, Australia)	Significant protection	Available	(LeJambre et al., 2008)

To prevent and control parasitic infections, one health concept's main goal is to create and implement collaborative measures that focus on treatments like immunization, vector control, better hygiene, and environmentally friendly land-use planning (Blake and Betson, 2017). The idea of "one medicine" encompasses the positive exchange of information and methods between veterinary and human medicine. However, knowledge mostly flows from human medicine to veterinary medicine due to the preponderance of financing for medical research in human medicine (Mcallister, 2014).

Conclusion

The raw material for vaccine creation has come from genome sequencing efforts. Techniques for triggering the appropriate kind and degree of immune responses are emerging. Researching vaccine adjuvants that trigger the appropriate natural immune responses as well as other methods to increase immunization effectiveness. More research is required to understand how the immune system reacts to parasites. The majority of parasite systems remain substantially unknown to us in terms of the variety of immune responses necessary for efficient infection control. Gene-based vaccines have shown great promise as a replacement for conventional vaccination strategies. Recombinant living vectors, such as rAd vectors, and plasmid DNA vaccines are two examples of gene-based vaccine technology. There will inevitably be new diseases and re-emerging diseases as the world's population rises and human-wildlife interaction increases. The WHO will continue to lead efforts to promptly detect, look into, and contain such outbreaks. The CDC in the United States and other recognized authorities in other nations will provide valuable support to this effort.

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Chapter 24

Bovine Babesiosis: The Role of Vaccination in Cattle Protection

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ABSTRACT

Babesiosis causes a significant loss in livestock productivity especially in tropical and subtropical areas. Various species of protozoan parasites cause the disease belonging to the genus *Babesia*, which are intraerythrocytic pathogen. Bovine Babesiosis has a major impact on the cattle industry through disease detection, prevention, treatment and vector control costs. *Babesia bovis* causes severe infection that characterized by anorexia, hemoglobinuria, fever, hemolytic anemia, and frequently death. Vaccines play a vital role in preventing those infections where treatment is limited or difficult, also for controlling diseases, especially in prevalent areas. Vaccine has a prolog history in protection effects against pathogens, by vaccination the disease burden can be reduced and the health status of animals can be maintained. Babesiosis prophylaxis has been achieved through the widespread use of live attenuated *Babesia* vaccine for calves since the mid-sixties. An achievement in veterinary vaccination successfully allowed the eradication of some devastating diseases. Challenges for developing and innovating in vaccine against new strains of various pathogens are continued. Recently vaccines are available for various parasitic infection in different hosts including bovine babesiosis, which they act on activation of host immune system, and reducing the effects of special pathogen through providing the protective response, some of them are lifelong. The lack of effective medications and the diversity of causative agents makes it difficult to control bovine babesiosis, however, various procedures were followed for developing of effective vaccine against *Babesia* species in cattle from different endemic regions globally.

KEYWORDS
Babesiosis, Vaccination, Immunization, *Babesia*, Cattle

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INTRODUCTION

Babesiosis is a parasitic disease with great significance, caused by protozoan parasites belonging to the genus *Babesia* and transmitted by ticks (Hunfeld et al., 2008). They are obligate intra-erythrocytic parasites (Westblade et al., 2017).

Victor Babes was the first who discover *Babesia* parasites in 1888 while investigating cattle herds detected with hemoglobinuria and initially named them *Haematococcus bovis* (Babes, 1888), while he didn't notice the presence of ticks in diseased cattle, but in 1893, Theobald Smith and Frederick Kilborne from United States demonstrating that *Boophilus annulatus* tick was responsible for transmitting of the disease and termed the tick fever (Smith and Kilborne, 1893).

There are around 100 species of *Babesia (B.)* known to exist in mammals (Schuster, 2002). Cattle are the main host and reservoirs for four species *B. bovis, B. bigemina, B. divergens*, and *B. major* (Iseki et al., 2010). In the tropics and subtropics, *Babesia bovis, B. bigemina, and B. divergens* are known to induce severe clinical bovine babesiosis (Bock et al., 2004). The disease is endemic in tropical and temperate regions of the world, where an estimated 500 million cattle are at danger of being infected (He et al., 2021). The infection initiates when parasitic sporozoites that are secreted from the salivary glands of ticks invade RBCs and then grow, multiply, and exit. This cycle continues when newly formed RBCs are invaded by the egressed merozoites. It is anticipated that stage-specific gene expression in the parasite will be the cause of these modifications (Elsworth and Duraisingh, 2020). When it subverts the hosts' immune systems, resulting in both transient and long-term infections (Alzan et al., 2022).

The most severe form of the disease is caused by *B. bovis* (Brown and Palmer, 1999). The acute babesiosis in bovine is characterized by fever (> 40°C), varying degrees of anemia, and hemolysis. Clinical indications of anemia can appear suddenly and include pale mucous membranes, inappetence, decreased milk production, weakness, lethargy, and elevated heart and respiratory rates (Shkap et al., 2007). The disease's acute phase may result in death or a lifelong infection. Adhesion of infected red blood cells in the capillaries of the brain, liver, and lungs, among other essential organs, is another sign of acute *B. bovis* infection (Sondgeroth et al., 2014).

Prompt diagnosis and anti-babesial agent treatment are essential for recovery, delays in therapy may cause the infected animals to pass away (Sivakumar et al., 2018). The mortality rate due to *B. bovis* infection is high, particularly in susceptible breeds or animals that have never been infected, however, species like *Bos indicus* cattle, which are native to *Babesia* endemic regions, exhibit mild to moderate clinical symptoms as a result of their innate resistance to the illness (Bock et al., 2004).

Babesiosis may result in either death or persistent infection in recovered animals from the acute phase. Animals with persistent infection serve as reservoirs for competent tick transmission (He et al., 2021). Detection of infected carrier cattle with *Babesia* parasites is crucial for risk evaluation since uninfected cattle can contract the parasites from ticks (Alvarez et al., 2019). A comprehensive calculation of the enormous expenses associated with babesiosis has taken into account mortality, abortions, lost milk and meat output, and control methods (Reyes-Sandoval et al., 2016).

In endemic areas, prevention of babesiosis in bovine is rely on the usage of acaricides for controlling of ticks, and live attenuated vaccines. Animals that have received vaccinations or have recovered from infections acquire adaptive immunity, but they still harbor the infection and could act as carriers of the disease (Hakimi and Verocai, 2023).

Administration of anti-babesial drugs ensuing the residues of chemicals in milk and meat which accompany harmful effects on public health. Furthermore, the use of acaricidal and anti-babesial medications at random doses leads to the development of resistance to the causing organisms and vectors. Therefore, strengthening immunization programs is crucial, especially in areas where a large number of animals are at risk (Maqbool et al., 2018).

The innate immune response in the spleen is responsible for clearing naive animals from acute babesiosis produced by *B. bovis*, while protection of continuously infected cattle from clinical illness (concomitant immunity) or vaccinated cattle depend on rapid activation of memory and effector CD4+ T cells for IFN-γ secretion and supporting in protective antibodies production (Brown et al., 2006a).

Recent sight about adaptive immune response against *B. bovis* involves a various response from both T and cells which characterized by the activation of CD4+ Th1-lymphocytes, and the production of neutralizing antibodies directed against parasite surface antigens at the erythrocyte membrane and extracellular merozoites. In addition, IFN-γ is necessary to stimulate macrophages to generate babesiacidal molecules and to enhance the opsonizing IgG2 antibody response (Brown et al., 2006a).

Applying live, attenuated organisms as immunogens has allowed for the development of solid immunity subsequent to infection (Maqbool et al., 2018). Consequently, for countries where the disease is enzootic, the development of dependable, safer, and equally protective vaccine against bovine babesiosis would be extremely helpful (Ortiz et al., 2019).

Bovine Babesiosis

After trypanosomiasis, babesiosis is the second most frequent disease transmitted to animals by ticks (Yabsley and Shock, 2013). *Babesia* species have an impact in tropical and subtropical regions where Ixodidae ticks are prevalent, however *B. bovis, B. bigemina* and *B. divergens* are more prevalent having a significant influence on the cattle sector (Bock et al., 2004). Bovine babesiosis commonly caused by *Babesia bovis*. It can be found in Asia, Africa, Australia, portions of Southern Europe, Central, South, and Southern North America. One of its main vectors is *Rhipicephalus microplus*, via transovarial transmission, infected female ticks transmit the infection to their offspring (Hakimi and Verocai, 2023).

Babesia species have a complicated life cycle that includes sexual reproduction in the gut of the definitive tick vectors, and asexual reproduction when the parasite is present inside the erythrocytes of its mammalian hosts. The parasite can also change into other distinct stages during its development in the tick's body, such as gametes, kinetes, and sporozoites. It can also invade various tissues, such as the tick progeny's ovary and egg tissues, which are essential steps for the parasite to spread transovarially (Alzan et al., 2022). The invasive sporozoites in the tick's salivary glands are transmitted during a blood meal, to a new vertebrate host (Jalovecka et al., 2018).

In vertebrate hosts, the parasite multiplies asexually through invading erythrocytes. Merozoites can re-infect other RBCs in the host after they are liberated from these RBCs. A portion of these parasites develop into gametocytes, both male and female. The sexual phase of the infection starts in the tick's body, when it consumes vertebrate host blood that contains these gametocytes through zygote development. The formed zygote in the tick's midgut goes through sporogony, a process that produces sporozoites (Jalovecka et al., 2018). Asexual replication of *babesia* merozoites within the bovine red blood cells is responsible for parasite pathogenesis (Hakimi et al., 2021), it induces significant intravascular hemolysis hence manifest clinical symptoms (Hunfeld et al., 2008).

Clinical bovine babesiosis is characterized by severe hemolysis of red blood cells, persistent fever, anemia, and frequently hemoglobinuria, which gives the disease's urine a reddish-brown hue and gives it the term "red water." Agalactia, or total milk loss in dairy cows, is an early warning sign (Githaka et al., 2022). Additional symptoms include temporary reduced fertility in bulls and abortion in pregnant cows. When the clinical indications are less severe, jaundice may occasionally be visible, and infected animals with *B. bigemina* frequently have hemoglobinemia (Shkap et al., 2007).

Infection by *B. bovis* are frequently acute or subacute, have a shorter course, and cause more severe symptoms quickly, either resulting in death in fatal instances or a longer recovery period in non-fatal cases (Githaka et al., 2022). Infrequently *B. bovis* can produce additional symptoms by altering erythrocytes, which accumulate in capillaries, particularly in the brain. This condition is known as cerebral babesiosis, and symptoms include hyperesthesia, nystagmus, circling, head pushing, aggressiveness, convulsions, and paralysis (De Vos et al., 2004).

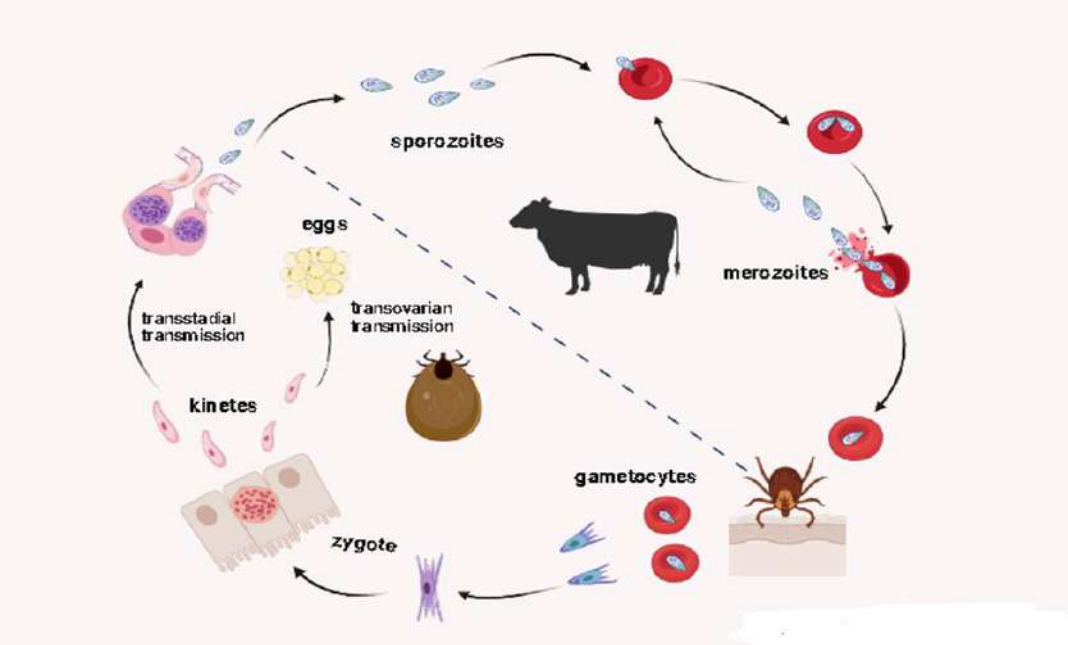

Fig. 1: Life cycle of *Babesia* spp. in bovine

Babesiosis causes high rates of morbidity and mortality in susceptible animals, particularly in exotic and cross-bred cattle. The mortality rates of 30% for *B. bigemina* infection, and 70–80% for *B. bovis* infection have been reported, and are strongly affected by a variety of factors (Githaka et al., 2022) including: previous exposure to *Babesia* species/strain, animal breed, age, sex, flock size, current applied treatments, vaccination status, feed density, seasonal management, tick infestations, domestic pet availability, and management of grazing area (Simking et al., 2014).

Owing to the persistent and hazardous of chemical residues in the environment (Wolstenholme et al., 2004). Development of resistance in ticks as a result of repeated usage of acaricide, as well as the associated costs has restricted acaricide use as a preventative measure (Palmer and McElwain, 1995). Conversely, vaccinations are safe, don't leave any chemical residues (hence don't require withholding periods in animals), are friendly to the environment, and are well-liked by consumers (Dalton and Mulcahy, 2001).

An Overview of Immune System

The immune system is a multifaceted, cohesive network of tissues, organs, and cells that plays specific roles in protecting the body against foreign chemicals and harmful microbes (Nye, 2004).

In mammalian vertebrates, there are two forms of immunity: innate and acquired. The innate immunity is the initial line of defense for a host against infections, which is mediated by phagocytes such as dendritic cells and macrophages. Acquired immunity involve the development of immunological memory and the removal of pathogens during the latter stages of infection (Akira et al., 2006).

Various anatomical barriers to infection together make up the innate immune system, such as physical barriers (the skin), chemical barriers (the acidity of stomach secretions), and biological barriers (the normal microflora of the gastrointestinal tract) (Nye, 2004). Furthermore, phagocytic cells and soluble factors are components of the innate immune system. Soluble factors comprise the acute-phase proteins, messenger proteins known as cytokines, and the complement system (Delves and Roitt, 2004).

The complement system is avital elements of innate immunity, which is made up of a biochemical network of over 30 proteins in plasma and on cellular surfaces. By encouraging phagocytosis or causing direct lysis (cell rupture), the complement system triggers reactions that destroy invasive infections, the complement proteins also control inflammatory responses (Dunkelberger and Song, 2010).

The immune response is regulated by chemical messengers called cytokines (Delves and Roitt, 2000). They are important for phagocytic cell induction into specific infection sites. The primary immune cells engaged in the phagocytosis process are neutrophils, monocytes, and macrophages, these cells engulf and break down invasive pathogens (Iwasaki and Medzhito, 2010).

The recognition of microorganisms done via a variety of germline-encoded pattern-recognition receptors (PRRs). These PRRs share characters: Initially, recognition of microbial components by PRRs identified as pathogen-associated molecular patterns (PAMPs) that are important for the survival of the microorganism. Second, constitutive expression of PRRs in the host and pathogens detection at any stage of their life cycle. Third, PRRs are nonclonal, expressed on all cells of a certain kind, germline-encoded, and immune memory-independent (Akira et al., 2006).

A second line of protection against infections is acquired or adaptive immunity, which takes several days or weeks to fully develop. Since adaptive immunity involves both immunologic "memory" and antigen-specific responses, adaptive immunity is far more sophisticated than innate immunity. The development of immune cells that target and destroy an

invasive pathogen is stimulated by exposure to a specific antigen on the pathogen (Nye, 2004). Immunologic "memory" refers to the fact that antigens are "remembered" and thus, immune responses are more powerful and rapid upon re-exposure to the same infection (Parkin and Cohen, 2001).

The main mediators for the adaptive immune response are B lymphocytes (B cells), and T lymphocytes (T cells). Antibodies are specialized proteins produced by B cells that attach to foreign proteins or pathogens and recognize them in order to destroy them or mark them for destruction by macrophages. The response mediated by antibodies is refers to humoral immunity. In contrast, T cells-lymphocytes that grow in the thymus are responsible for cell-mediated immunity. Various T cell subgroups play distinct functions in adaptive immunity. For instance, killer T cells, or cytotoxic T cells, target and eliminate infected cells directly, whereas helper T cells boost immune responses and support the activity of other lymphocytes (Parkin and Cohen, 2001). Regulatory T cells, also known as suppressor T cells, reduce the activation of the immune responses (Parham, 2005).

Apart from its essential function in innate immunity, the complement system also regulates adaptive immunological responses. It serves as an illustration of how the innate and adaptive immune systems interact (Kohl, 2006; Dunkelberger and Song, 2010). It is commonly known that the Th1/Th2 balance plays a crucial role in the fate of parasites. Th1 responses are linked to the removal of protozoan parasites, while Th2 responses are linked to the uncontrolled proliferation of parasites (Akira et al., 2006). The acquired or adaptive immunity include both passive and active immunity which obtained either naturally or artificially.

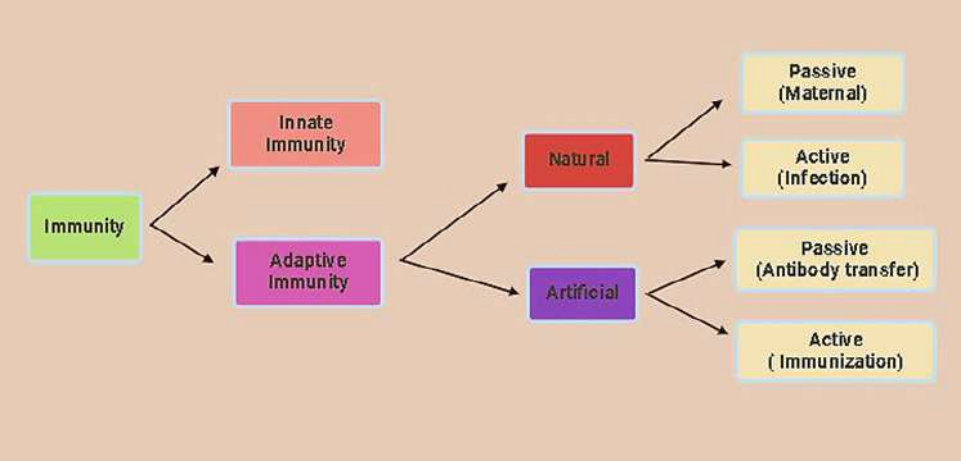

Fig. 2: Diagram of Immune response

Passive immunity is obtained through transferring of pre-made antibodies from one individual to another. Passive immunity can develop spontaneously when maternal antibodies are passed from the mother to the fetus or artificially, when high concentrations of particular antibodies against a virus or toxin are given to non-immune individuals. Immunization with passive components is used to lessen the symptoms of chronic or immunosuppressive conditions, or when there is a significant risk of infection and not enough time for the body to mount an effective defense (Hunt, 2015). Although passive immunity offers instant protection, because the body does not form memories, the patient remains vulnerable to future infections by the same pathogen (Janeway et al., 2001).

Active immunity arises when a pathogen activates B and T cells; memory B and T cells then grow; this leads to the development of the primary immune response. These memory cells will "remember" every unique pathogen that an individual encounters during their lifetime and be able to produce a potent secondary reaction in the event that the pathogen is discovered again (Glenny and Südmersen, 1921).

Due to the body's immune system's ability to adapt, this form of immunity is active and adaptive. Both the humoral and cell-mediated immunity components are frequently involved in active immunity. A natural infection leads to the development of active immunity. Immunological memory results from an individual being exposed to a live pathogen and developing a basic immune response. While vaccination, or chemical containing antigen, can produce artificially developed active immunity. A vaccination, instead of causing the disease's symptoms, elicits a primary response against the antigen (Hunt, 2015).

Immunity against Intra-erythrocytic *Babesia* Parasite

Bovine babesiosis is considered as an acute hazardous disease, when naive animals that are older than a year contract (He et al., 2021). Cattle's immunological response to *B. bovis* infection is crucial to the disease's course, the degree of parasitemia, the intensity of clinical symptoms, and the emergence of immunity (Santos et al., 2023).

In comparison to adults, younger animals exhibit greater resistance, which is spleen-dependent and linked to a robust innate immunity (Hakimi and Verocai, 2023). Additionally, calves have a certain level of immunity due to age-related characteristics that lasts for six to eight months, although adults are more susceptible to the risk of clinical disease (Santos et al., 2023). Concomitant immunity is the term used to describe the resistance that persistently infected cattle typically exhibit against reinfection with similar parasite strains (Brown et al., 2006a).

Both an innate and an adaptive immune response are necessary for defense against intra-erythrocytic protozoan parasites of the genus *Babesia* (Ortiz et al., 2019). The adaptive immune response comprises two aspects: the generation of neutralizing antibodies, and the appearance of parasite antigens to CD4+ T cells by specialized antigen-presenting cells (Montenegro et al., 2022).

The mechanism of protection has been reviewed and illustrated in different ways; a model represented that immunologically naive animals must have a sufficiently robust innate immune response to recover from an acute infection with virulent *B. bovis* parasites. This response triggers the activation of macrophages through IFN-γ, and parasite-derived products which kills the organisms through phagocytosis and produces toxic macrophage metabolites, such as Nitric oxide (NO) (Estes and Brown, 2002). When IFN-γ coexisted with *B. bovis* merozoites or infected erythrocytes, NO generation was triggered (Goff et al., 2002).

Antigen-specific CD4+ T cells play a crucial role in the adaptive immune response by producing IFN-γ in animals that are both well vaccinated and continuously infected yet have controlled parasitemia. Furthermore, IFN-γ stimulates the generation of the neutralizing IgG2 antibody and activates macrophages for effective organism clearance (Estes and Brown, 2002). In cattle, IgG2 is the most effective opsonizing antibody isotype for targeting extracellular parasites and parasite antigens that are visible on the surface of erythrocytes (Ortiz et al., 2019), that avert cattle from being challenged by homologous strains passively (Mahoney, 1986).

Other model has demonstrated that macrophages and NK cells are necessary for recovery from initial infection opposed to CD4+ T cells and antibodies (Aguilar-Delfin et al., 2003). To trigger this kind of innate reaction, the cytokines IL-12 and IFN-γ had to be produced (Brown et al., 2006a). An analysis of cytokine responses during infection represented that recovery from infection depends on early production of IFN-γ and IL- subsequently formation of IgG linked to an IL-4 and IL-10 response (Chen et al., 2000).

In response to *B. bovis*, activated macrophages produce inflammatory cytokines that are crucial for initiating both the innate and acquired immune responses consisting of IL-12, TNF-α, and IL-18. IL-12 stimulated the synthesis of IFN-γ by differentiated Th1 cells and boosted the production of IFN-γ by natural killer (NK) cells, which in turn produced higher amounts of IFN-γ (Brown et al., 1996). TNF-α and IFN-γ work together to stimulate macrophages' production of NO (Goff et al., 1998). Moreover, IL-18 and IL-12 work together to enhance the synthesis of IFN-γ (Shoda et al., 1999).

In cattle younger than six months old, there was an age-related resistance to Babesiosis; these animals are resistant to clinical illness after *B. bovis* exposure. The age-related resistance is rather paradoxical, as infants' innate immune systems are not as established as adults' (Petty and Hunt, 1998). Additional probable explanations for improved resistance of young animals may be related to the abundance of Gamma delta (γδ) T cells, which account for more than 70% of circulating T lymphocytes in ruminants (Hein and Mackay, 1991), or as a result of a diminished pro-inflammatory response for the disease's pathophysiology (Clark and Jacobsen, 1998). This age-related immunity also has a cellular component (Montealegre et al., 1985) in spite of a soluble babesiacidal component (Levy et al., 1982).

Mononuclear phagocytes (MP) are involved as a key effector cell for both innate and primary immune responses and Nitric oxide has been discovered as one of the babesiacidal molecules produced by activated mononuclear phagocytes (Goff et al.,1996). It has been suggested that because NO has a very short half-life, its microbicidal actions are localized to lymphoid organs like the spleen rather than being systemic (Jacobs et al., 1995). Spleen plays a major role in an infection management, since splenomegaly develops during acute babesiosis and splenectomized adult and young animals have significantly greater levels of parasitemia (Goff et al., 2001). The splenic red pulp has the potential to have microbicidal effects because it is sufficiently restricted to allow for NO activity. Furthermore, blood would be expected to show the residual babesiacidal effects of NO (Goff et al., 1998).

The complicated immunological and inflammatory environment in which mononuclear phagocytes operate often determines the MP's functional response, which is influenced by the first regulatory cytokine that the MPs encounter (Erwig et al., 1998). In response to *B. bovis* infection, young calves' protective innate immune response shows type-1 characteristics. This includes early induction of splenic IL-12 and IFN-γ mRNA production, which is followed by a brief period of inducible nitric oxide synthase (iNOS) expression. Conversely, in the spleens of adults who died from the infection, IL-12 and IFN-γ messages appeared later and there was no iNOS present. Furthermore, compared to calves, adults' spleens exhibited larger levels of IL-10 message induction and sustained expression longer. Finally, relative splenic transforming growth factor (TGF)-b mRNA expression levels, and kinetics differed between calves and adults, suggesting a dual regulatory role for this cytokine (Goff et al., 2001).

Numerous factors, such as developmental stage, nutritional stress, and concurrent infection, may affect a cattle's susceptibility to primary *Babesia* infections and possibly of protective immunity formation (Bock et al., 1997). The establishment of protective immunity is also influenced by the host's genotypes for example, purebred *Bos taurus* cattle are more susceptible to *B. bovis* infection than cross-bred or *Bos indicus* cattle (Bock, 1999).

Vaccine and Vaccination

The word "vaccine," comes from the Latin word "vacca," which means cow, was first used by Edward Jenner to refer to the process of vaccinating humans against the related human smallpox virus by inoculating them with the cow pox virus. This illustrates the close relationship between the sciences of infectious diseases in humans and animals (Meeusen et al., 2007).

Vaccination aims to imitate the development of naturally acquired immunity by inoculating immunogenic

components of the pathogen or closely related organisms. The vaccinations may be used to help control, or completely eradicate an infection at the population level, or they may be used to avoid clinical indications of illness following infection (Meeusen et al., 2007).

Vaccinology has emerged as a recognized scientific field that integrates immunology, microbiology, protein chemistry, and molecular biology with business-related concerns such as production costs, regulatory compliance, and profit margins. Developing a vaccine that will defense people and animals from illness is the ultimate goal of each new vaccination (Kahn, 2006).

Substantially the majority of vaccinations continue to use live, attenuated pathogen strains, even though commercial companies typically do not want this kind of immunization because it exposes them to mitigating risks. Additionally, the short shelf life and strain/region specificity of many vaccinations result in production losses. Meanwhile, the live organisms offer greater protection than many subunit vaccines (Lambert et al., 2005).

To increase the efficacy of killed or subunit vaccinations, a deeper comprehension of the immunological and molecular disease processes is probably needed. It is well known that the immune system uses a variety of effector pathways to fight different infections according to the microenvironments and life cycles of each pathogen. The majority of killed and subunit vaccines still primarily depend on the production of neutralizing antibodies (Lambert et al., 2005).

An important development in immunology is the increasing recognition of the pivotal function that innate immunity performs in the functioning of vaccination adjuvants that will influence a frequently overlooked aspect of vaccine development (Pulendran and Ahmed, 2006). Innate immune receptors that have recently been identified are being searched for novel adjuvant chemicals that are active (Pashine et al., 2005). The ligands linked with these receptors, known as pathogen-related molecular patterns, are then employed to either enhance or decrease vaccine responses (Huleatt et al., 2007). When compared to immunizations for humans, the use of adjuvants in veterinary medicine is significantly less limited. Currently, veterinary vaccines that are permitted for use include a wide variety of adjuvant kinds and formulations, while human vaccine use limited to only three adjuvants (Pashine et al., 2005).

Vaccine in Veterinary use

The main objectives of veterinary vaccinations are to enhance companion animal health and wellbeing, boost livestock productivity in an economical way, and stop the spread of diseases from domestic animals and wildlife to humans. Due to these varied goals, the creation of veterinary vaccines has been approached differently, these approaches range from simple yet efficient whole-pathogen preparations to molecularly specified subunit vaccines, or genetically altered organisms, vectored antigen formulations, and naked DNA injections. The creation of a product that will be sold or utilized in the field to accomplish desired results is the ultimate effective result of vaccine research and development (Meeusen et al., 2007).

Mahoney (1967) proved that splenectomized calves infected with *B. bovis* did not exhibit clinical illness after receiving serum from infected donors. The level of protection matched that of cattle recovering from a natural illness, and it was determined that humoral components played a significant role in the effector mechanism that destroyed *B. bovis* in the immune animal. Ultimately, the study results showed that antiserum was highly protective against several infections in donors and that the protective effects were strain-specific (Mahoney, 1979).

Hyperimmune serum appears to be mediated by antibodies in protecting splenectomized calves. It is possible that the merozoites were eliminated upon their emergence from erythrocytes, while the organisms present in the red blood cells in circulation remained unaffected by antibodies. Despite the fact that the initial rate of elimination was higher than the rate of multiplication, indicating that the infected erythrocytes were similarly susceptible to antibody attack, this trait seems to support the concept proposed by Mahoney in 1972 (Mahoney, 1979).

Curnow (1968, 1973) discovered that antibodies against the varied agglutinogens on the surface of infected erythrocytes directly provided protection. To ensure a successful passive transfer, the serum should have antibodies against every type of antigen that the parasite's homologue strain was able to create (Mahoney, 1979).

Live attenuated *Babesia* parasite vaccinations have been used extensively since the mid-1960s to prevent the disease in calves. These vaccinations are widely used in Australia, Argentina, Israel, and South Africa as a preventative measure against bovine babesiosis since they provide a high level of protection (Florin-Christensen et al., 2014).

Relapse of parasitaemia is a characteristic of the hyperimmune serum passive transfer trials. This can happen if the administration of the serum is postponed until the parasitaemia level reaches 103-104/mm^3, or given in modest dose (24 ml/kg) when the level of parasitaemia is between 1 and 102/mm^3. Relapses appeared to be caused by the quantitative relationship between parameters such as the initial antibody concentration and its rate of loss due to antigen-antibody responses, the number of parasites in the blood at the time of injection, and normal catabolism (Mahoney, 1979).

Global climate change-induced increases in animal movement and wildlife-human interactions will necessitate ongoing monitoring for disease outbreaks across the globe, as domestic, farm, and wild animals serve as major reservoirs for a variety of vector-borne human illnesses (Hayes and Gubler, 2006).

Veterinary vaccines have already a significant impact on public health as well as the health, welfare, and productivity of animals, vaccines have recently been used in animal reproduction and industrial procedures (Kahn, 2006). They are meant to increase overall productivity in livestock animals, they lessen the usage of veterinary medications and hormones and the residues of these substances in the human food chain (Meeusen et al., 2007). In order to stay ahead of the

constant threat of newly emerging diseases, there must be constant communication between scientists, animal and human disease control authorities (Kahn, 2006).

The process of generating veterinary vaccines comparing to that of developing human vaccines, has benefits and drawbacks. Considering that animal vaccines have smaller markets and lower sales prices than those for humans, compared to human vaccinations, the possible profits for animal vaccine manufacturers are far smaller. Consequently, despite the complexity and diversity of hosts and pathogens are higher for animal vaccines than for human vaccines, a lot less money is spent on research and development for them. However, the requirements for preclinical trials and regulations, which can account for the majority of the costs associated with developing a human vaccine, are typically less onerous in terms of creating veterinary vaccinations. Moreover, a faster time to market launch and a return on investment in research and development. Veterinary experts have a clear advantage over human vaccine developers in that they can quickly undertake research in the relevant target species (Meeusen et al., 2007).

Although only about 23% of the global market for animal health products is made up of veterinary vaccines, the industry has been growing steadily due to new technological advancements in vaccine production as well as the emergence of new diseases and the ongoing development of drug resistance by pathogens (Meeusen et al., 2007).

Vaccine trials against Bovine Babesiosis

Various procedures have applied for controlling of babesiosis in cattle, although innovation in vaccine preparation make them back word drawing in some procedures that previously applied for immunization against *Babesia* parasite in bovine. In order to combat *B. bovis*, several vaccine approaches were developed, including live attenuated and viral vector vaccines. These strategies used *B. bovis* proteins or whole live parasites, with the latter offering the best protection against babesiosis in cows (Santos et al., 2023).

The live vaccines provide a highly protective effect against babesiosis within a single dose production (de Waal and Combrick, 2006). The immunity is long lasting for at least four years if *B. bovis* vaccine is used, and may be last for less time in case of *B. bigemina* (De Vos and Bock, 2000). It has been documented to endure even after *Babesia* infections are eliminated. Study on drug cured cattle also suggested that the degree of acquired immunity is related to the degree of antigenic stimulation (duration of prior infection) rather than the presence of live parasite (Dalgliesh, 1993). Due to the vaccine's short shelf life, distribution and post-production testing for contamination and efficacy are not feasible in certain nations (De Vos and Jorgensen, 1992).

Despite the fact that there are several essential inadequacies such as the high production costs, the logistics of distribution, and the possibility of pathogen contamination during manufacture (de Waal et al., 2006). In addition, potential for co-infection with contaminating species, especially viruses; persistence of infection in the field; reversion to virulence, temperature liability; storage and transportation a short shelf life of four to seven days at 4˚C, are other drawbacks of receiving a live vaccine (Maqbool et al., 2018).

Developing a vaccine against *B. bovis* using in vitro *Babesia* parasites is known as the "killed vaccine" approach (Florin-Christensen et al., 2014). Which consist of adjuvant and antigens extracted from infected calves' blood or cultured material (Maqbool et al., 2018). The vaccines created using in vitro methods allow for more controlled and consistent environments and have a lower risk of spreading infections (Shkap et al., 2007). However, the duration and the degree of immunity against heterologous challenge lacks sufficient documentation (Maqbool et al., 2018), and the culture-derived organisms may loss its virulence and immunogenicity (De Vos, 1978). Another constraint points of this technique are its requirement for continuous supply of erythrocytes and serum from donor animals, as well as enough laboratory supplies and personnel skill (Florin-Christensen et al., 2014).

The development of novel vaccines that could target many stages of the *Babesia* parasite's life cycle will be greatly aided by recently developed genetic editing techniques (Santos et al., 2023). Following the release of the first whole genome of *B. bovis* in 2007, and sequencing of other *Babesia* spp. genomes important insights into the biology of the parasite have been gained (Brayton et al., 2007). The creation of transfection methods for gene modification and functional analysis has enabled these developments and accelerated the hunt for possible vaccines (Suarez et al., 2019).

Another strategy for the management of bovine babesiosis is vaccination with recombinant protein antigens. The expression of the *B. bovis* MSA-1 and MSA-2c antigens on the merozoite surface aids in the parasite's entry into the bovine erythrocyte (Brown et al., 2006b; Florin-Christensen et al., 2014). The 42 kDa membrane glycoprotein is encoded by a single-copy gene with an open reading frame of 961bp and no intron found in the msa-1 gene locus (Hines et al., 1995). A single copy of the msa-2c gene, which lacks an intron, has a coding sequence that is 795 bp long and produces a 30-kDa protein (Florin- Christensen et al., 2002).

The effectiveness of recombinant vaccines has been doubtful predominantly when fusion proteins have been used such as Thioredoxin. Fusion assembly may alter the recombinant protein's quaternary conformational shape, which may impact the antigenic determinants' interaction and, in turn, its ability to stimulate or protect the immune system. When many recombinant proteins are used simultaneously as immunogens, the results may be superior than when a single protein is used (Willadsen, 2008).

Conclusions

Babesiosis as one of protozoal infection has significant effect on livestock industry, through losses and cost of treatment. Prevention and management of babesiosis in bovine required an effective controlling strategies especially in

endemic areas. The natural resistance in some indigenous bovine breeds may reduce the mortality rate. And persistent immunity is a measured aspect for protection of an infected animals against the next attract of babesiosis caused by virulent *Babesia bovis*. Development of anti- babesial drug resistant as well as absence of effective vaccines are the obstacles impairing babesiosis control in cattle. Immunization have been used for protective purpose against losses caused by bovine babesiosis, although various factors might reduce its application. Vaccination as an advanced procedure can be used for protection against bovine babesiosis. Innovation in manipulation techniques for vaccine preparation is continue for targeting the different pathogen stage and assistance in controlling the devastation diseases.

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Chapter 25

Recent Advances in mRNA Vaccine Development and Their Control against Parasitic Diseases

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ABSTRACT

This chapter covers a thorough examination of the rapid growth of mRNA vaccine technology and its potential uses in battling infectious diseases, notably parasitic disorders. It emphasizes the outstanding safety, rapidity, and repeatability of mRNA vaccines, making them viable tools in vaccinology. It explores the distinct advantages of mRNA vaccines, such as their potential to stimulate complex immune responses while avoiding the restrictions associated with standard live vaccinations. It also examines the obstacles and prospects for using mRNA technology to target different stages of the parasite's life cycle, from host establishment to dissemination. Moreover, the chapter examines current clinical and preclinical research that show the efficacy and safety of mRNA vaccines against parasitic diseases such as malaria, leishmaniasis, and schistosomiasis. It investigates the mechanisms underpinning mRNA vaccination induced immunity, shedding light on the development of next-generation vaccine candidates. Finally, the chapter discusses essential aspects of scaling up and making mRNA vaccination platforms cost-effective for worldwide parasitic disease management. Overall, it highlights the potential of mRNA vaccines to change infection prevention and control, fueling hope for long-term benefits in world health.

KEYWORDS
mRNA vaccines, Infectious diseases, Vaccine development, Vaccine scalability, Immunity

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INTRODUCTION

The recent advancements in mRNA technology have revolutionized the field of human vaccine development against parasitic diseases in animals because which has a significant impact on a number of animals, posing substantial economic, veterinary, and conservation challenges (You et al., 2023).

Parasites in animals are usually fought with methods like antibiotics and removing carriers. Due to limitation against disease prevention traditional ways has many issues. With mRNA technology advance anti-mRNA vaccine boost the immunity, also can target specific parasitic diseases (Versteeg et al., 2019).

mRNA vaccines bring notable- benefits compared to current methods. They allow quick development, flexible selection of antigens, and overcoming challe-nges from antigenic diversity and immune- regulation. Potential use of advance vaccine in veterinary field improved animal health against parasitic infections, public health protection and ecological balance maintenance (Le et al., 2022).

This chapter discuss the development of advance vaccine through new technologies i.e. mRNA vaccines. Controlling parasitic disease and improve the health status of animal's is application of mRNA technology. The goal, after reviewing relevant literature, is to highlight on the vast opportunities and hazards associated with veterinary RNA vaccines (Laczkó et al., 2020).

Overview of mRNA Vaccines

mRNA vaccines has potential ability to control viral illnesses such as as CV2CoV, can cause immunosuppression, increasing immune responses beyond initial doses. Offsite vaccine which given outside the infectious site, having the ability to activate B-T cells greater ultimately results in faster immunity and more aggressive immunity (Gote et al., 2023). Traditional vaccines have several advantages, one of which is their ability to deliver antigen proteins that can effectively stimulate the immune system to fight infection (Wang et al., 2015).

1. Mutation overview: Anti-mRNA drugs are well suited to combat new or re-emerging parasites because they can rapidly mutate to a specific pathogen (G. Zhang et al., 2023).
2. Simplified antigen selection: This technology combines modified, optimal antigens into a single vaccine to protect against a variety of parasitic diseases (Brisse et al., 2020).
3. Increased safety: Compared to traditional vaccines, mRNA vaccines do not contain live virus. Additionally, since messenger RNA cannot be integrated into the host's genome, there is no need to worry about genetic mutations (Rosenblum et al., 2022).
4. Immunity: The use of anti-mRNA is important to prevent parasites (as shown in Fig. 1) because they make cells long-lived and fun (Teijaro and Farber, 2021).
5. Consideration for the Service Center: These mRNA vaccines have the flexibility to be used with a diverse range of animal species, thanks to their multiple administration routes such as intradermal, subcutaneous, and intramuscular injections. (Le et al., 2022).

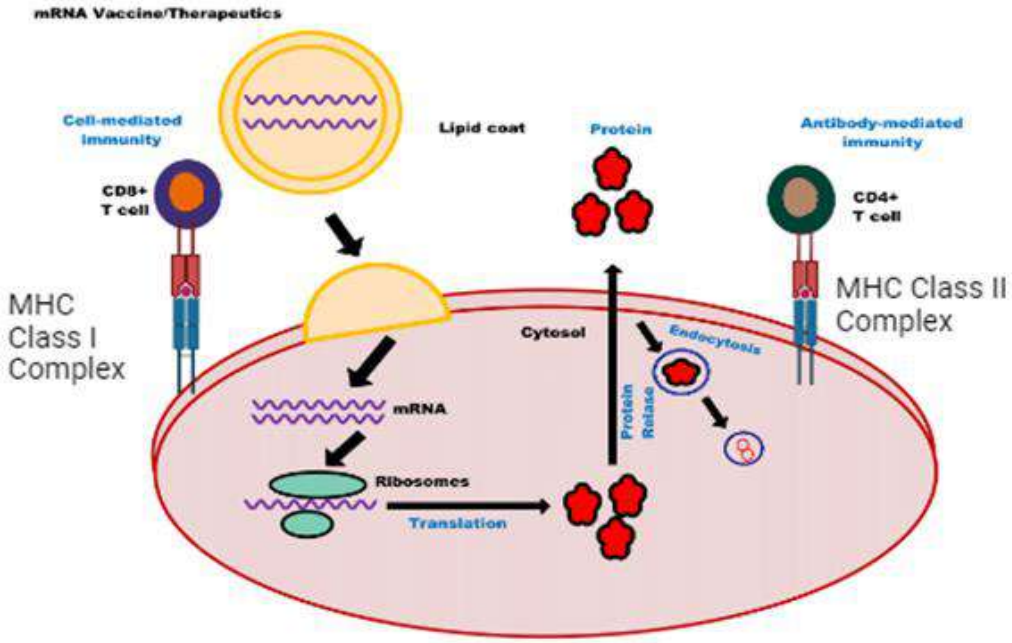

Fig. 1: Working of mRNA vaccine

Importance of the Treatment of Parasitic Infections

Parasitic diseases are an obstacle to animal health, welfare, and productivity, with important consequences for agricultural sustainability, food security, and economic development. Control of parasitic diseases in animals is important for the following reasons:

Animal Health and Welfare

Parasitic diseases has many consequences not only to animal health but also effect's country economic and human health (Campbell and VerCauteren, 2011).

Economic Impact

Infectious disease has impact on livestock production, treatment costs, mortality rates also significant economic impact on agricultural businesses (Lopes et al., 2016).

Food Safety

Parasitic diseases in farm animals can affect the human health with food supply through contaminated food and affordability by reducing the quality of meat, milk, and eggs (FAO, 2021).

Zoonotic Potential

Zootoinc infection has a bad consequences on the public health through intake of contaminated food or close touch with infected animal (Omeragic et al., 2022).

Antimicrobial Resistance

Antimicrobial resistance undermines the efficacy of remedy regimens and poses challenges for disease control and manage in each animal and people (Martin et al., 2015).

Sustainable Agriculture

Effective control of parasitic diseases is critical for sustainable cattle manufacturing systems, ensuring the health and resilience of animal populations, and minimizing environmental influences. (Lopes et al., 2016).

Innovation Required to Address the Gap in Parasitic Disease Control

Inequalities in the control of animal parasitic diseases is caused by many factors such as endemic parasites, vaccine use, limited alternative treatments and transportation limitations. Advance technologies possesses a quick response for detection and control strategies (Matos et al., 2015).

Anthelmintic Resistance

Overuse and abuse of anthelmintic drugs in cattle production has led to the modification of drugs dewormers. The emergence of anthelmintic resistance compromises the efficacy of remedy regimens and necessitates opportunity manipulate techniques, including vaccination, to lessen reliance on chemical interventions and preserve anthelmintic efficacy (Shalaby, 2013).

Limited Treatment Options

Developing novel healing procedures, which includes vaccines, that focus on precise parasitic pathogens and set off protecting immunity in animals is crucial for enhancing remedy consequences and lowering disorder burden (Sharma et al., 2015).

Accessibility and Affordability

Developing low cost and accessible vaccines, diagnostics, and treatment alternatives for parasitic diseases in animals is important for making sure equitable get entry to veterinary healthcare services and enhancing animal welfare globally (Diakou et al., 2023).

One Health Approach

Adopting a One Health technique that integrates interdisciplinary collaboration, surveillance, and control efforts is important for addressing the complicated challenges posed by parasitic diseases throughout species boundaries (Quaresma et al., 2023).

Objectives

The goals of exploring current advances in mRNA vaccine development for controlling parasitic diseases in animals are multifaceted and aim to address the challenges and possibilities present in veterinary medicine. The unique goals related to animals consist of:

Assessing the Feasibility of mRNA Vaccines in Animal Health

Evaluate the ability of mRNA vaccine generation in addressing the unmet desires and challenges in controlling parasitic illnesses immunogenicity, and safety in diverse animal species (Le et al., 2022).

Investigating the Efficacy of mRNA Vaccines Against Parasitic Pathogens

Examine the efficacy of mRNA vaccines in inducing defensive immunity towards various parasitic pathogens in various animal hosts, which include livestock, accomplice animals (You et al., 2023).

Exploring the Mechanisms of Immune Protection

Elucidate the immunological mechanisms underlying defensive immunity conferred by means of mRNA vaccines towards parasitic infections in animals, including induction of cell and humoral immune responses (Zhang et al., 2019)

Assessing the Safety and Tolerability of mRNA Vaccines

Evaluate the safety and tolerability profiles of mRNA vaccines in animals for systemic immune responses, and long-term safety assessments (Le et al., 2022).

Investigating Vaccine Delivery Systems and Adjuvant Formulations

Explore novel vaccine transport structures, adjuvant formulations, and vaccine platforms that optimize the immunogenicity, stability, and for controlling parasitic illnesses in animals (G. Zhang et al., 2023).

Common Parasitic Diseases and their Effects

The complexity of parasite existence cycles, their interactions with hosts and their capacity to keep away from immune responses make parasitic sicknesses a formidable obstacle.

Malaria

This is a disease unfold with the aid of mosquitoes that chunk humans with *Plasmodium* parasites. In human beings and different animals, it may cause quite a few symptoms together with excessive fever, chills, and anemia (Nkemngo et al., 2023).

Schistosomiasis

Organ harm and other extreme effects can result from this disease, that's caused by parasitic flatworms called schistosomes and affects both animals and human (Moriyasu et al., 2018).

Soil Transmitted Helminths

Infectious worms (*Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercoralis*) that parasitize animals by contaminating the soil. This can cause vitamin deficiencies and gastrointestinal problems in animals (Jourdan et al., 2018).

Protozoan Infections

Protozoan parasites are the cause of some devastating animal sicknesses, together with Chagas disease, leishmaniasis and snoozing sickness (McDougald et al., 2020).

Challenges in the Development of Vaccines against Parasitic Diseases

The development of vaccines towards parasitic diseases in animals presents unique demanding situations due to the complex biology of parasitic organisms, host-parasite interactions and the range of animal hosts. (Moriyasu et al., 2018).

Antigenic Range

The development of vaccines that focus on conserved epitopes at the same time as offering extensive safety towards different parasite lines is a undertaking because of the complex antigenic repertoire of parasitic organisms (Deitsch et al., 2009).

Host Immune Responses

Overcoming the host immunological challenges is important for the development of vaccines that set off protecting immunity in opposition to parasite infections in animals (Sacks and Sher, 2002).

Vaccine Delivery and Adjuvant Selection

The selection of appropriate vaccine transport structures and adjuvants is important for improving the immunogenicity and efficacy of vaccines towards parasitic illnesses in animals (Sharma et al., 2015).

Why mRNA Vaccines Hold Promise for Parasitic Disease Control?

As of remaining update in January 2022, mRNA vaccine technology has shown extremely good promise not only effective in fighting viral sicknesses but also in addressing parasitic diseases, together with the ones affecting animals. Here are a few reasons why mRNA vaccines preserve promise for controlling parasitic diseases in animals:

Customizability

mRNA vaccines can be tailored to goal unique antigens or proteins produced by means of parasites. This lets in for the improvement of vaccines that could successfully target various tiers of the parasite's lifestyles cycle, improving their efficacy in stopping infection (Versteeg et al., 2019).

Speed of Development

mRNA vaccine improvement is typically quicker in comparison to standard vaccine structures. This fast development manner allows for the short model of vaccines to rising or evolving parasitic lines, that is mainly important in controlling parasitic diseases which could show off antigenic variation (Chaudhary et al., 2021).

Safety

mRNA vaccines are considered safe because they do no longer comprise live pathogens. They work by using instructing cells to supply a protein that triggers an immune response, without posing a chance of inflicting sickness in vaccinated animals (Qin et al., 2022).

Potential for Targeting Intracellular Pathogens

mRNA vaccines should result in both cellular and humoral immune responses, which may be powerful in opposition to intracellular pathogens by using targeting inflamed cells and stopping the unfold of the parasite (You et al., 2023).

Scalability and Cost Effectiveness

The production of anti-mRNA medications can be carried out rapidly and efficiently, allowing for their widespread use in animals (Rosa et al., 2021).

mRNA Vaccine Development Fundamentals

Recent advances in the introduction of mRNA vaccines have led to an almost complete transformation of the vaccine industry. These developments have not been the easiest of changes in vaccination but have otherwise opened the door to new possibilities in pest management. There are several important groups involved in the occurrence of anti-mRNAs in animals, which may be as follows.

Antigen Target Detection

mRNA is the first step in the production of antibodies as it helps to identify the most appropriate target for a particular pathogen. Antigen targeting may also include proteins associated with surfaces, secretions, or important metabolic pathways (Versteeg et al., 2019).

mRNA Design and Optimization

Once an antigen target is identified, the corresponding mRNA is optimized to exhibit optimal activity in the desired species This fine coding function, modifying untranslated regions (UTR) are involved in complex processes such as nucleotide repair or secondary to provide structure, mRNA stability, translational efficiency, and immunity (Zhang et al., 2023).

Choose the Right Route of Administration

Several strategies exist to improve mRNA transport and cellular uptake, including lipid nanoparticles (LNPs), polymer-based nanoparticles, and electro filtration Once the appropriate transporters are selected for control, vaccination and disease protection the best of the best (Zeng et al., 2020).

Structure and Components of mRNA Vaccines

Recent advances in mRNA vaccines are two-fold in animal parasite control. To understand the mechanism of action and efficacy in healthy animals, it is important to understand the composition and structure of mRNA antibodies. Below you will find the most important animals with stored mRNA data and structure:

mRNA Sequencing

There are studies to modulate messenger RNA (mRNA) expression and develop antibodies because mRNA Sequencing is a key component of the anti-mRNA response (Kim et al., 2022).

Improved mRNA Translation

The part of the molecule that is not translated to molecules outside the coding level plays an important role in posttranscriptional regulation, initiation of translation, and mRNA stability Mutations in these processes can affect physiology of the immune system both through mRNA stability and translational stability (Tang et al., 2023).

Cap Shape Adaptation

To mimic the transcriptional structure of plant mRNAs, response mRNA is often modified to have a cap structure at the 5' end. The cap is replicated using a 5-triphosphate bridge in which the mRNA is bound to a 7-methylguanosine residue. (Fang et al., 2022).

Poly (A) Tail

Transcription of mRNAs is terminated by a poly (A) tail, which serves to stabilize the mRNA and prevent premature degradation by exonucleases. Messenger RNA (mRNA) stability and translation are affected by the length of the poly (A) tail. (Di Grandi et al., 2023).

Mechanism of Action of mRNA Vaccines

Recent advances in the development of anti-mRNA compounds have opened new avenues for parasite protection. To understand the impact of anti-mRNAs on the immune system, it is important to better understand their function.

Administration and Distribution

Anti-mRNA drugs are usually administered by intravenous and subcutaneous injections. During injection, mRNA molecules are taken up at the site of injection by mast cells, smooth muscle cells, and immune cells (including APCs and dendritic cells) (Meyer et al., 2022).

Antigen Translation and Presentation

The cellular machinery of host cells translates Messenger RNA (mRNA) molecules into protein antigens encoded by mRNA sequences Antigens can be surface or intracellular proteins expressed in bacteria in the 19th century (Cheng et al., 2016).

Antigen Presentation

Antigen-presenting cells, especially dendritic cells, play an important role in capturing, processing, and presenting parasite-derived antigens to T cells Dendritic cells process antigens and transport them to MHC magnificence II molecules so direct CD4 T helper cells. This interaction turns on and organizes CD4 T cells that help stimulate the immune system (Guermonprez et al., 2002).

The Benefits and Drawbacks of mRNA Vaccines

Recent advances in the development of mRNA vaccines are promising for the control of parasites in animals. However, like all generations, mRNA vaccines have each benefit and downsides while used in animal fitness. The description is as follows:

Advantages

Rapid Increase

Compared to conventional vaccine fashions, mRNA vaccines can induce surprising modifications. This ensures a well-timed response to rising infections or converting parasites, which is important for ailment manipulate in animals (Dolgin, 2021).

Customizable

mRNA vaccines provide flexibility in antigen choice and layout, permitting the vaccine to be tailor-made to a particular ailment or pressure (Tsimberidou et al., 2022).

Safety

mRNA vaccines are non-infectious and do now not contain living organisms, disposing of the hazard of antibodies. In addition, the mRNA vaccine is highly unique for the goal antigen, decreasing the quantity of off-target and adverse reactions inside the vaccinated animals (Rosenblum et al., 2022).

Scalability and Fee-effectiveness

The manufacturing of mRNA vaccines can be scaled up fast and efficiently, making them suitable for massive-scale vaccination of animals. This scalability will increase accessibility and affordability, mainly in resource-restricted environments (Rosa et al., 2021).

Drawbacks

Stability and Garage

mRNA antibodies are greater solid than normal antibodies and require special garage and managing conditions to hold their integrity. Bloodless administration and shelf life may be very limiting, particularly in faraway or rural regions (Uddin and Roni, 2021).

Delivery is Difficult

Delivering anti-mRNA pills efficiently to the target remains a mission. Although lipid nanoparticles (LNPs) have been developed to improve mRNA delivery, there is still a want to enhance delivery in precise animals and tissues to optimize the vaccine (Nitika et al., 2022).

Regulatory and Public Popularity

Regulatory approval and public calls for mRNA vaccines will also be difficult. Wider validation is required to demonstrate safety, demonstrate efficacy in multiple animal models, and meet regulatory standards (Le et al., 2022).

Recent Advances in mRNA Vaccine Technology

The latest release in January 2022 marks a breakthrough in the development of anti-mRNA drugs for parasite control. Recent advances in the development of anti-mRNA drugs include:

Efficient Drug Delivery

Scientists have spent a lot of time and effort finding better ways to deliver anti-mRNA drugs to animals without damaging the environment. One of these efforts is the development of lipid nanoparticles (LNPs), which have the potential to enhance drug resistance by improving cell uptake, tissue selectivity and mRNA stability (Gote et al., 2023).

Exciting Antigen Design

Recent advances in bioinformatics and computer modeling have opened new avenues for combining mRNA antibodies with parasite-specific antibodies. (Versteeg et al., 2019).

Adjuvants

Anti-mRNA antibodies can be added to the vaccine to boost the immune response of the animal. Recent studies have focused on the development of Toll-like receptor agonists or cytokine adjuvants to improve vaccine effectiveness and prolong the immune response (Di Pasquale et al., 2015).

Immune Enhancement

Scientists are developing ways to make an animal's mRNA more immunogenic. Lysozymes, also known as costimulatory ligands, enhance the immune response by binding to immune cell messenger RNAs. This triggers T-cell activation and memory (Xu et al., 2020).

mRNA Vaccine Platforms and their Development

Recent advances in mRNA vaccine development have caused the emergence of numerous structures tailor-made for controlling parasitic diseases in animals. These systems have developed over time with modifications in transport, antigen presentation, and immunomodulatory techniques. Some of the mRNA reactions and their changes in animal reactions are:

Anti-mRNA Pa

Anti-mRNA Pa consists of naked mRNA molecules that encode the goal antigen of hobby. Initially, those vaccines confronted issues with mRNA stability, immunogenicity, and transport. (Gote et al., 2023).

mRNA LNP Vaccine Combination

when mRNA and LNP are combined, they can co-transfer antigens and immune molecules, thus activating the immune system. Preferably in animals this method can utilize animals cytokines, or other anti-inflammatory agents in mRNA vaccines to induce severe immunity (Bevers et al., 2022).

Optimization of mRNA Vaccine Delivery Systems

Recent advances in mRNA vaccine development have focused on optimizing shipping systems for balance and efficacy (Gote et al., 2023).

The use of anti-mRNA drugs in the treatment of parasitic diseases is a promising area of research. The innate nature of infection and the immune system make it difficult to develop effective vaccines, but anti-mRNA drugs are promising to solve these problems. (Poveda et al., 2023).

mRNA Vaccines are Stable and Immunogenic

Recent advances in mRNA vaccines are increasing the safety and immunogenicity of these diseases, especially in animal studies. These vaccines have several advantages over traditional vaccines, including the ability to modulate expression, rapid development and low cost (Mahony et al., 2024).

Efficient mRNA sequencing technologies also play an important role in immunizing of the immune balance in veterinary medicine (Liang et al., 2021).

Applications of mRNA Vaccines in Parasitic Disease Control

The mRNA vaccine platform has been considered as an ability technique to the challenges of developing powerful vaccines towards parasites that are known for their complicated life cycles and capacity to keep away from the host immune machine. Several research have explored the usage of mRNA vaccines against parasitic diseases in animals, consisting of:

Malaria

In vitro transcribed (IVT) mRNA vaccines were proven to generate robust antigen-specific Tfh cell responses and long-lived high-affinity antibodies (Maruggi et al., 2021).

Leishmaniasis

Researchers have evolved mRNA vaccines concentrated on *Leishmania donovani*, a protozoan parasite that causes leishmaniasis (Duthie et al., 2022).

Toxoplasmosis

mRNA vaccines in opposition to *Toxoplasma gondii*, any other protozoan parasite, had been evolved (Chu and Quan, 2021).

Tick-borne Diseases

mRNA vaccines had been designed to goal the black-legged tick, *Ixodes scapularis*, which transmits diverse pathogens (Sajid et al., 2021).

Efficacy and Safety Profiles of mRNA Vaccines in Parasitic Disease Models

mRNA vaccines is a viable alternatives of traditional vaccines because of advanced technologies against many parasitic disease in animal model (You et al., 2023). Epitranscriptomic studies provide valuable information about mRNA expression patterns and the role of RNA therapy in protist gene regulation (Zhang et al., 2023).

Immunological Responses and Mechanisms of Protection

In fact, the mRNA vaccine technology vaccines have demonstrated impressive capacity to develop powerful immunity against different infectious disease targets in animal models like influenza virus, Zika virus and rabies virus (Rosa et al., 2021).

mRNA antibiotics is widely accepted and has the potential to be used as a parasite New antibiotics to target specific antigens (You et al., 2023).

Experimental data provide evidence the anti-mRNAs play better role in the immune system (Cagigi and Loré, 2021).

Immune Responses Induced by mRNA Antiparasitic Agents

Animal studies searching for mRNA antibodies against parasites have provided encouraging findings in the immune system that can be used to combat these diseases (You et al., 2023).

Scientific research shows that mRNA injections can protect against parasites. The effectiveness of mRNA antibodies in producing potent and specific antibodies against viruses is due to their ability to encode virus-specific antigens. (Versteeg et al., 2019).

Cellular and Humoral Immunity Elicited by mRNA Vaccines

mRNA vaccines have been found to induce both cellular and humoral immunologic responses in animals. Immune cells such as T cells and natural killer (NK) cells are responsible for cellular immune response, while B cells are the main producers of antibodies during humoral immunity (Cagigi and Loré, 2021).

mRNA vaccines in relation to parasites, it is obvious that these vaccines would not work without cellular immunity. By encoding antigens that are specific to parasites, mRNA vaccines can ignite an immune response which will generate T-cells able to identify and destroy virus-infected cells hence providing control over parasitic infection (You et al., 2023).

Challenges and Future Directions

The remarkable progress made recently in mRNA vaccine development for controlling parasitic diseases in animals offers opportunities as well as challenges for the future Rationale: mRNA vaccines promise a potential new approach to tackle a wide range of animal infectious diseases. (Versteeg et al., 2019).

There are significant research obstacles in the development and deployment of mRNA vaccines to parasitic diseases of animals. There are no mRNA vaccines for animal health industry use that are licensed by the US Department of Agriculture/Center for Veterinary Biologics, another encouraging challenge in the quest to address regulatory hurdles before the therapeutic products are deployed and used (You et al., 2023).

In the future, mRNA vaccines for parasitic disease in animals will need further work on efficacy, optimizing immunogenicity and durable protection, and attention will need to be paid to regulatory requirements.

Conclusion

The recent advent of mRNA vaccine development has demonstrated considerable promise for the prophylaxis of human and animal parasitic diseases. mRNA vaccines potentially replace far more traditional methods, such as attenuated parasitic vaccination and infection and treatment (IandT), where the susceptible parasites are killed within the host. mRNA vaccines have been developed for several emerging animal and zoonotic diseases, one of which is a parasitic infection like schistosomiasis or echinococcosis.

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Chapter 26

Malarial Vaccination's Compassionate Shield

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ABSTRACT

Malaria is a disease transmitted by mosquitos and caused by Plasmodium protozoan parasites. Although over the past 20 years, there has been a dramatic decline in malaria-related morbidity and mortality, the disease still poses a serious threat to public health in many countries. This emphasizes how vitally better prevention, treatment, and management techniques for malaria must be developed to move closer to the disease's eradication eventually. The creation and widespread use of a malaria vaccine that is both highly efficacious and capable of producing long-lasting immunity against malaria will ideally be part of this. In terms of malaria vaccination, there are over a dozen of these in clinical development. The most recent malaria vaccine, RTS, S/AS01 also referred to as Mosquirix, has been proven in phase III trials to have a modest efficacy against clinical malaria in infants aged five to seventeen months. It is advised for use in young children who are most vulnerable to infection by *P. falciparum*, the deadliest human malaria parasite. This recommendation comes from the World Health Organization. The need for more effective malaria vaccinations is widely acknowledged. The most advanced candidate for malaria immunization, the chemo-attenuated sporozoite vaccine showed high-level efficacy and feasibility.

KEYWORDS
Malaria, Vaccine, Plasmodium falciparum, WHO, RTS, Efficacy, Transmission

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INTRODUCTION

The immune system creates antibodies when it is exposed to a harmful disease, this is processed by a vaccine. There are several methods for the infection to enter a host (Toor et al., 2023). The vaccines have weakened or killed forms of bacteria and viruses. These microorganisms do not harm the living bodies, as they are present in their inactive form. According to WHO, an estimated 247 million people are affected by mosquito-borne diseases, and approximately 619,000 deaths are caused by malaria annually (Adefolalu et al., 2022). Given the fact that the most common parasite illness affecting both people and animals is malaria, it started affecting people in the 1940s to 1960s. In the 1970s people started using irradiated sporozoite vaccine (Richie and Saul, 2002). The effective vaccine development for malaria has been done for more than six decades. It is quite challenging to create a malaria vaccine that is highly successful, and this challenge has prompted the configuration and assessment of numerous new developments in the realm of vaccination (Hill, 2011).

Four distinct Plasmodium species—*P. falciparum, P. vivax, P. malariae*, and *P. ovale*—are typically responsible for human malaria. *P. falciparum* is usually responsible for severe malaria cases in sub-Saharan Africa, whereas *P. vivax* and *P. falciparum* are nearly always the cause of infections in southeast Asia (Skwarczynski et al., 2020).

Some vaccines against parasites are developed from mainly three categories – attenuated strains of bacteria or viruses, killed microbes, or protein units. A century later vaccine from killed microbes was introduced e.g. polio vaccine. More recent vaccines used against encapsulated bacteria are conjugated vaccines. These vaccines are proven to be highly efficacious in disease control. However, some protein subunit-based vaccines are rarely used because they are few and are particulate, they have a lot of repeating subunits of proteins that are like the immune cells e.g. the surface antigen of hepatitis B and human papillomavirus vaccine. Parasitologists have developed several vaccines that can safely grow and manufacture a parasite to induce immunity, but these vaccines are hard to develop and they do not have a sufficient number of whole parasites that can play a role in inducing immunity, this technique has been attempted for malaria

(Hoffman et al., 2010). Alternatively, for the generation of protective immunity, a huge amount of antigens are used, they are expressed mainly as proteins and less as vectors or vector system (Anders et al., 2010).

In case of malaria, the residents of endemic areas have the chance to develop natural immunity, this kind of immunity develops when one person is exposed to the endemic area for years. Comprehensive immune-epidemiological investigations have little insight into the most effective antigens for vaccines. Natural immunity targets many blood-stage antigens, with no single antigen being particularly crucial for protection (Marsh and Kinyanjui, 2006). The World Health Organization (WHO) recommended the RTS,S/AS01 vaccine (henceforth RTS,S) in October of 2021 in order to reduce the acute malaria in infants and toddlers living in areas with moderate to high prevalence. Targeting the immunodominant *P. falciparum* circumsporozoite protein (CSP) on sporozoites is the pre-erythrocytic vaccine (PEV) subunit RTS,S. Following thirty years of development, RTS,S was approved by the WHO, making it the first recommended vaccine against parasites for consumption by humans.(Duffy, 2022).

Development of Vaccine for Malaria

The malaria vaccine development began with vaccination trials on mice with irradiation sporozoites in the 1960s (Nussenzweig et al., 1967), followed by analysis of immunity mechanisms in the model (Doolan and Hoffman, 1997). The Clyde's challenge trials in humans found that while volunteers could achieve high levels of protection, it needed multiple bites from mosquitoes that were irradiated infected. The cloning and sequencing of the gene occurred in the early 1980s as a result of the isolation of the circum-sporozoite protein, a crucial part of the sporozoite coat, raising hopes for the sporozoites vaccine (Clyde et al., 1975). During this time, researchers made significant progress in discovering and expressing various blood- stage antigens, paving the way for a potential vaccine, Initial clinical trials showed mild immunogenicity and no substantial effectiveness against sporozoite challenge (Ballou et al., 1987). The Colombian vaccine SPf66, which is based on amino acids, first demonstrated effectiveness in both people and monkeys (Patarroyo et al., 1988). However, the follow-up field efficacy studies in Asia and Africa were unable to show any protection. Following that other vaccines were evaluated.

Parasite Vaccines

A biotech company, Sanaria in the United States configured and developed a pre-erythrocytic vaccine, this vaccine was developed from sporozoite (Hoffman et al., 2010). The PfSPZ Vaccine which is a *P. falciparum* sporozoite vaccine, a live-attenuated malaria vaccine, provides sterile protection against after the last dose of vaccination, parasites called *P. falciparum* (Pf) that are the same as the vaccine strain that can persist for a period of fourteen months (Lyke et al., 2017). The PfSPZ was extracted from the mosquito salivating glands that are aseptic, these mosquitoes were infected by cultured parasites from the laboratory. This culture was developed by a technology company Sanaria in 2010. Despite the limitations, irradiation sporozoites administered through mosquito bites have shown excellent levels of protective effectiveness, surpassing 90%, despite limited volunteer involvement in the experiments (Hoffman et al., 2002). Radiation-treated sporozoite can penetrate into liver cells and cause faulty schizonts. The defective schizonts exhibit markers that can set off a protective defense, but they are unable to burst and produce merozoites, which normally target RBCs and disseminate the disease. The generated $CD8^+$ T-cells may remove infected cells of liver in humans, alike how animal models provide complete protection. However, this has yet to be proven (Doolan and Hoffman, 1997). The Sanaria developed a production method that complies with regulations, which involves the sterile dissection of pathogens from glands of several thousand mosquitoes, since delivering a vaccination for the public health purposes would not be feasible given several thousand mosquito bites (Hill, 2011). The parasites were purified, radiation-treated, and preserved in liquid nitrogen to maintain their vitality and promote defense against increased temperature. Despite these limitations, the vaccine successfully completed phase I/II clinical trials in 2010. However, considerable efficacy is yet to reveal, and it's not apparent if the needle and the syringe could replace fluids from mosquito glands for producing sufficient immunity and effectiveness in people (Hill, 2011).

Protection through Antibodies

The majority of licensed vaccines work by triggering antibody responses to provide protection against bacterial and viral pathogens (Plotkin and Plotkin, 2008). Similarly, numerous vaccine formulations provide protection that lasts for a long time and doesn't require booster shots. Determining the protective threshold and an antibody-titer, the infection point at which antibody titers stabilize following the initial antibody spike brought on by immunization, is crucial to comprehending antibody-mediated immunity. The antibody titers typically decreased far more slowly after the inflection point (Amanna et al., 2007). The protective threshold for vaccinations that effectively prevent bacterial and viral illnesses is low and much below the vaccine's inflection point, meaning that protection lasts for a long time (Plotkin, 2010). Raising the inflection point would therefore be necessary to improve vaccinations that stop sporozoites from entering the liver or that stop the asexual cycle in the blood and lowering the antibody responses' rate of degradation. Improvements in vaccination schedules and delivery, as well as adjuvant strategies that support longer-lasting immunity, could meet these needs (Cockburn and Seder, 2018).

Pre-erythrocytic Vaccine (PEV)

The pre-erythrocytic vaccines were inspired by radiation-attenuated sporozoites (RAS), which centuries ago were shown to confer antibacterial immunity in both people and animals. The PEV targets liver-stage parasites and clinically

undetectable sporozoite pathogens. In humans, RAS immunity guards against infection with the same strain and *P. falciparum* sporozoites that were heterologous, but not blood-stage parasites (Duffy, 2022).

RTS, S/AS01 Vaccine

Although the principles behind eliciting immune reactions to the pre-erythrocytic phases of malaria remain unclear, it is known that vaccinations that elicit powerful humoral and immune cell reactions against the sporozoite phase of Plasmodium in the animals may protect them from subsequent infections. Even if the immunized host produces sporozoite-specific antibodies that block sporozoite invasion of the hepatocytes (Garcia et al., 2006). The parenchyma cells in liver infected by plasmodium presenting these antigens are eliminated when there are adequate numbers of T lymphocytes specific to the sporozoite antigen (Kurup et al., 2019). For the host hepatocytes and the sporozoites to interact, the Circumsporozoite Protein (CSP), which is an essential component of the sporozoite surface coat, must be present. Because of this, pre-erythrocytic stage antimalarial vaccinations may include the CSP as a target antigen (Marques-da-Silva et al., 2020). The Mosquirix vaccine consists of CD8 and CD4 T cell receptors (T) that are immunodominant and bind with the *P. falciparum* (NF54 strain) CSP central repeat region with the repeating unit (R) of amino acids with a B-cell receptor epitope, and hepatitis B virus surface antigens. Furthermore, vaccine formulation comprises three times the amount of "free" HBsAg antigen (S) to promote the immunogen's self-assembly into virus-like particles (Palatnik-de-Sousa and Nico, 2020).

R21 Vaccine

The RTS, S/AS01 (mosquirix) an enhanced vaccine, and R21 (Matrix-M malarial vaccine) is regarded as an antimalarial subunit vaccine of the future generation. The three times greater concentration of Hepatitis B surface antigen (HBsAg) seen in mosquirix is absent from the R21 design, which instead includes a larger percentage of *P. falciparum* CSP C-terminus coupled to Hepatitis B surface antigen N-terminus (Schijns and Lavelle, 2011). This mimics the greater CSP epitope excess on the surface of sporozoite by showing more CSP antigen on the vaccination surface of the particle (Langowski et al., 2020). The stronger humoral immune reactions against CSP were a result of this increased B cell activation (Caro-Aguilar et al., 2002). Even at extremely low doses, R21 was immunogenic in BALB/c mice. The *P. berghei* sporozoite challenge protection was evoked by R21 when given in conjunction with the assistance of Matrix-M and Abisco-100 (Collins et al., 2017).

Viral Vector Vaccine

Using viral vectors to transfer the antigens is another method for creating an antigen-subunit malaria vaccine. The cell-mediated immune responses would be elicited by prime-boost vaccines that are either heterologous or homologous employing pathogens expressing pre-erythrocytic antigens of plasmodium (Collins et al., 2017). The weakened vaccinia virus FP9 (Fowlpox Virus 9) MVA (Modified Vaccinia Ankara) was used as a carrier in one of the first attempts to apply this strategy, either separately or in combination (Swadling et al., 2014), and conveying the insert MVA ME-TRAP. Whereas, "ME" is a set of twenty epitopes primarily recognized by CD8 T lymphocytes as being present in the *P. falciparum* pre-erythrocytic stage (Tiono et al., 2018). The ME is linked to TRAP which is a thrombospondin-related adhesion protein, a pre-erythrocytic stage protein found in *P. falciparum* (T9/96 strain) to form ME-TRAP (Kimani et al., 2014), which targets several developmental stages of the plasmodium in mammals (Moorthy et al., 2003). Out of the five vaccinated participants, only two showed protection even though inducing with FP9-ME-TRAP and then MVA-ME-TRAP booster elicited CD4 and CD8 T cell reactions that are antigen-specific (Webster et al., 2006). As a result, the ChAd63/MVA ME-TRAP vaccination was switched to (Tiono et al., 2018). Furthermore, to the ME-TRAP and MVA antigen, the chimpanzee adenovirus 63 (ChAd63) is used in the vaccination of ChAd63/MVA ME-TRAP. The immunization of prime booster with ChAd63/MVA ME-TRAP produced exceeding effects of CD8 and CD4 T-cell responses (Ewer et al., 2013). During stage 1/2a clinical trial series, the immunized volunteers showed immunity against the heterologous sporozoite challenge in 21% of them and setback to blood-stage parasitemia in 36% of them (Tiono et al., 2018). Males from Kenya demonstrated a 67% lower chance of contracting malaria in the stage 2b clinical trial series using the immunization strategy; however, males from Senegal showed no such effect (Rampling et al., 2018). This indicated that in the genetically diverse malaria-endemic areas, the ChAd63/MVA ME-TRAP vaccination efficacy is not sure.

Transmission Blocking Malarial Vaccines (TBV's)

The goal of TBVs against malaria is to create resistance against the parasite stages that infect mosquitoes, preventing transmission of malaria in TBV-immunized patients. The majority of malaria infections are spread within a few hundred feet of a contagious human source, community-wide usage of transmission blocking vaccines would prevent infections in the vaccinated persons' surrounding environment (Carter, 2001). In 1950s chickens vaccinated against a combination of sexual and asexual phases of *Plasmodium gallinaceum* were unable to spread the parasite (Huff et al., 1958). Twenty years later, it seemed that the observed transmission-inhibiting effects were caused by antibodies against target antigens on sexual stages and that these antibodies acted after consumption by mosquitos (Gwadz, 1976). Antibodies can stop mosquito infectivity and can kill gametes and zygotes up to many hours after a blood meal (Eyles, 1952). The next few decades saw the development of monoclonal antibody technology and the

optimization of experimental mosquito membrane feeding systems. As a result, the standard membrane feeding assay (SMFA) was created, and a number of proteins associated with the sexual stage were identified as targets for antibodies that impede transmission (Nikolaeva et al., 2015).

Blood Stage Vaccine

The objective of the blood stage vaccination is asexual parasites that cause disease and death. In the 1960s, parasitemia and related symptoms were eliminated when sick children received passive transfers of adult African IgG. Rich merozoite parasite preparations protected monkeys against P. falciparum infection BSV focuses on merozoites (Siddiqui, 1977). Depending on the species, merozoites leave erythrocytes as one to two dozen children before quickly re-entering fresh erythrocytes, providing a little window of opportunity for neutralizing antibodies. Between 2000 and 2015, more than 30 BSV trials were finished (Dassah et al., 2021). Since then, advancements have been achieved in merozoite vaccinations that target non-redundant invasion pathways. PfRH5, or P. falciparum reticulocyte-binding protein homolog 5, is a highly conserved protein that binds the crucial receptor basigin and induces broadly neutralizing antibodies in animals (Douglas et al., 2011). In monkeys, parasitemia was regulated by the P. falciparum reticulocyte-binding protein homolog 5 vaccine (Douglas et al., 2015). These vaccines significantly reduced the multiplication of parasites.

Placental Malarial Vaccine

The PMVs, or placental malaria vaccinations, are a unique blood stage vaccine strategy for protecting expectant mothers. Although they may have developed immunity to malaria as children, pregnant women in malaria-endemic areas are more vulnerable to Plasmodium infection (Miller et al., 2002). The plasmodials that bind to the CSA and express VAR2CSA are the source of placental malaria (PM). As women become resistant to PM, they develop antibodies against CSA-binding parasites during subsequent pregnancies (Duffy, 2022). Targeting N-terminal VAR2CSA segments, two vaccines (PRIMVAC and PAMVAC) have successfully undergone testing on humans as potential protein-in-adjuvant options (in a stable emulsion containing TLR4-ligand GLA). They both generated antibodies that prevented the attachment of homologous parasites, but there was no evidence of any action against heterologous parasites. Similar outcomes have been observed in monkeys, where PM bouts increased heterologous functional activity but not VAR2CSA titers (Doritchamou et al., 2023).

Conclusion

The incidence of malaria has a profound effect on society, which drives up demand for vaccines to prevent the illness. This is particularly true for developing nations, where malaria poses the greatest threat. Among the main obstacles to the development of a malaria vaccine are low immunogenicity, side effects, and storage restrictions. Although RTS,S has received historical regulatory approval, its antibody-mediated protection should be strengthened. Improved merozoite vaccines have inhibited parasite development *in-vivo*, whole-sporozoite vaccinations are producing T-cell responses and providing protection, and a Pfs230 transmission-blocking candidate has outperformed the benchmark Pfs25 vaccine activity. After previous decades of disappointment, there is now optimism regarding malaria vaccines due to advancements being made in all techniques.

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Chapter 27

Overview of Vaccination against Babesiosis and Theileriosis

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ABSTRACT

Babesiosis and Theileriosis are major threats to livestock globally. No region of the world is safe from these diseases. Ticks play a crucial role in the transmission of these diseases. Among babesiosis, bovine, canine, and human babesiosis are the most important. Among theileriosis, tropical theileriosis, East Coast fever, and equine piroplasmosis are the most important. Currently, the application of antiprotozoal drugs and effective tick management are being employed for the prevention and treatment of these diseases. However, now the focus is on the development of effective vaccines against these diseases. In this regard, several antigens for vaccine development have been identified. The most important vaccines among these vaccines are the live attenuated vaccines. These live attenuated vaccines trigger the immune responses by CD4+ and CD8+ cells. However, most of these vaccines are not available yet for commercial purposes. There exists an antigenic variation among *Babesia* and *Theileria* that creates a challenge for the development of universal vaccines.

KEYWORDS
Babesiosis, Theileriosis, Diseases, Vaccines, Development, Live attenuated

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INTRODUCTION

Babesia and *Theieria* cause piroplasmosis which is a tick-borne protozoal disease (Kirman and Guven, 2023). They belong to the phylum Apicomplexa (Chisu et al., 2023). *Theileria* and *Babesia* are potential threats to the livestock, causing severe economic losses. These parasites are more prevalent in crossbred cattle as compared to domestic cattle (Hossain et al., 2023). They are transmitted by *Ixodidae* ticks (Kuibagarov et al., 2023). Babesiosis is commonly known as Texas fever or Red Water fever (Ali and Marif, 2023). *Babesia bovis* and *Babesia bigemina* cause Bovine babesiosis in cattle (Jaimes-Dueñez et al., 2024). *Babesia ovis* causes babesiosis in sheep and goat (Spotin et al., 2023). *Babesia canis* (Weingart et al., 2023) and *Babesia gibsoni* are the causative agents in canines (Karasová et al., 2022). *Babesia divergens* cause zoonotic disease (Hildebrandt et al., 2023). Humans are also affected by *Babesia microti* (Kumar et al., 2021). Equine piroplasmosis is caused by *Babesia caballi* (Bartolomé del Pino et al., 2023). Theileriosis is caused by *Theileria annulata* (Kernif et al., 2024) and *Theileria parva* (Ma et al., 2024) in cattle. *T. annulata* causes Tropical theileriosis (Verma et al., 2023), also known as Mediterranean theileriosis (Dolatkhah et al., 2023). The other species of *Theileria* that affects cattle and causes East Coast fever is known as *T. parva* (Surve et al., 2023). If we discuss the theileriosis in sheep and goats, then we come to know that *Theileria ovis* and *Theileria lestoquardi* (Norouzi et al., 2023) are the causative agents. Canines (Hegab et al., 2023) and equines are infected by *Theileria equi* (Jaimes-Dueñez et al., 2023). Camels are affected by a species of *Theileria* known as *Theileria camelensis* (Ali et al., 2024). The common clinical signs associated with babesiosis include pale mucous membranes, fever, anorexia, respiratory distress, ruminal atony, constipation or diarrhea, and dark red to brown urine (Chandran, 2021). On the other hand, enlargement of lymph nodes, fever, anorexia, inappetence, nasal discharge, lacrimation, pale mucous membranes, emaciation, and a high decrease in milk production are the clinical signs obvious to theileriosis (Jaiswal, 2023; Kumar et al., 2023; Onizawa and Jenkins, 2024). Moreover, in severe cases, diarrhea and dysentery are commonly present (Khan et al., 2021). A parasite in theileriosis targets both the lymphocytes and erythrocytes (Elati et al., 2024), while in babesiosis, only the erythrocytes are affected (Aguilar-Figueroa et al., 2023). Due to the emerging resistance against the drugs of choice against babesiosis (Weingart et al., 2024) and theileriosis (Steketee et al., 2023; Fadel et al., 2023), there is a focus on the alternative methods for these tick-borne diseases control. Vaccination against these tick-borne diseases can be a favorable method. This chapter describes it in detail.

Overview of Vaccination against Babesiosis

Overview of Vaccination against Bovine Babesiosis

To develop a potential live attenuated vaccine to prevent the bovine babesiosis caused by *B. bovis*, Att-S74-T3Bo is a potential antigen (Bastos et al., 2023). Similarly, an antigen of *B. bovis* named BASA-1, can be used for the same purpose (Flores et al., 2020). On the other hand, subunit vaccine development has remained a good option to prevent bovine babesiosis caused by *B. bigemina*. BbiTRAP-1 and HAP2/GCS antigens are being used for this purpose (Jerzak et al., 2023). Similarly, tick vectors can be vaccinated against *Babesia* to make them immune against *Babesia* and limit a pathogen's life cycle (Ribeiro et al., 2021). For example, *Rhipicephalus microplus* is vaccinated by a protein of *Babesia* known as BmVDAC. Such vaccines are called live vector vaccines (Ortega-Sánchez et al., 2020).

Overview of Vaccination against Canine Babesiosis

Only the pathogenesis of *B. canis* is limited by the vaccination (Halder and Gupta, 2021). The vaccine, which was created using the parasite antigens of *B. canis* and *Babesia rossi*, is currently accessible and can shorten the course of the disease and lessen the severity of its clinical symptoms. The vaccination appears to partially inhibit the development of the pathogenic process, even though it does not prevent infection. Preventive protection varies greatly (70–100%) and is ineffective against other species of *Babesia*. Research is being conducted on *Babesia gibsoni* vaccines, which are mostly being developed using DNA and recombinant antigens (Karasová et al., 2022). By the detailed study of the proteins expressed in the sexual life stage of *B. gibsoni*, we can recognize the potential vaccine candidates that will help us in the development of transmission-blocking vaccines (Li et al., 2023). These vaccines are not commercially available yet (Liu et al., 2022).

Overview of Vaccination against Human Babesiosis

Human vaccines can play an important role in the eradication of this disease (Al-Nazal et al., 2022). Whole-parasite vaccines have been developed to protect humans from *B. divergens* and *B. microti* (Al-Nazal et al., 2021). rBdP0 is a candidate for vaccine development against human babesiosis. It reduces the ability of *B. divergens* merozoites to invade the RBCs (El-Sayed et al., 2022). Even though many of the *Babesia* proteins are polymorphic, the glycosylphosphatidylinositol-anchored proteins that are found at the merozoite surface seem like promising vaccine-candidate antigens. The results obtained using recombinant versions of glycosylphosphatidylinositol-anchored *Babesia* proteins indicate that the immunodominance as well as expression of similar epitopes on geographically distinct *Babesia strains* are influenced by the 3D structure of the protein. The results also showed that protective immunity that is resistant to strain variation must be induced, and that enhanced relative hydrophobicity is essential for this. *B. divergens* appears to be protected by this (Weiser et al., 2019). For the prevention of *Babesia microti* infection, three surface antigens known as Maltese Cross Form Related Protein 1, Serine Reactive Antigen 1, Piroplasm β-Strand Domain 1, and *Babesia microti* Alpha Helical Cell Surface Protein 1 have promising results (Meredith et al., 2023). Both the inactivated and live vaccines are available for *B. microti* (Jerzak et al., 2023). Rhoptry proteins are not given the attention that they deserve, and evidence points to pRAP-1's role in parasite binding, attachment, and maybe immune response evasion. Humans infected with *B. microti* may be candidates for vaccinations and diagnostic testing because their antibodies recognize recombinant forms of the proteins (Montero et al., 2022). Following immunization and *B. microti* infection, analysis of the host immune response revealed that while both rN-BmRON2 and rC-BmRON2 improved the immune response, rN-BmRON2 provided greater protection. Rhoptry neck protein 2, particularly its N-terminal fragment (rN-BmRON2), provides immunological protection, is involved in the invasion of host red blood cells, and has considerable potential as a babesiosis vaccine candidate (Cai et al., 2021). In addition, r*Bm*SP44 can trigger an immune response that defends against *B. microti* infection. As a result, r*Bm*SP44 has potential as a vaccine candidate (Wang et al., 2020). Similarly, when animals are immunized with *Bm*8 polypeptide of *B. microti*, parasitemia is markedly decreased (Wang et al., 2023). Antigens that are potential candidates for vaccine development against various *Babesia* species are summarized in Table 1.

Overview of Vaccination against Theileriosis

Overview of Vaccination against Tropical Theileriosis

When tropical theileriosis is treated early in the infection cycle, buparvaquone is a successful medication; however, its relatively expensive cost restricts its use globally. Currently, many nations use low-pathogenic parasites that have been produced *in vitro* from infected cells as vaccines (Liu et al., 2022). Animals can be immunized with both live and killed vaccines against *T. annulata* (Ramzan et al., 2023). Live attenuated schizont vaccines (Zweygarth et al., 2020; Ma et al., 2020; Amira et al., 2023) and cell-culture-based schizont vaccines have been developed against *T. annulata* (Roy et al., 2021). These developed live attenuated vaccines give results only against the local strains (Kundave et al., 2021). A surface protein with *T. annulata* known as *Ta*SP is responsible for triggering an immune response and can act as a vaccine candidate (Saaid et al., 2021; Elati et al., 2022). For the prevention of this disease, sporozoite antigen SPAG1 of *T. annulata* can be an option for vaccine development (Agina et al., 2020). Rabbits, being unnatural hosts, can be used for the growth of *Theileria* for vaccine development (Ramzan et al., 2022).

Table 1: Antigens that are potential candidates for vaccine development against *Babesia* species

Babesia species	Antigens	References
Babesia bovis	Att-S74-T3Bo	(Bastos et al., 2023)
	GASA-1	(Flores et al., 2020)
Babesia bigemina	BbiTRAP-1, HAP2/GCS	(Jerzak et al., 2023)
Babesia canis	Serotransferrine, hemopexin	(Ritchoo et al., 2023)
	glycosyl-phosphatidylinositol	(Radzijevskaja et al., 2022; Delbecq, 2022)
Babesia gibsoni	Recombinant antigens	(Karasová et al., 2022)
Babesia divergens	rBdP0	(El-Sayed et al., 2022)
	glycosylphosphatidylinositol-anchored proteins	(Weiser et al., 2019)
Babesia microti	Maltese Cross Form Related Protein 1, Serine Reactive Antigen 1, Piroplasm β-Strand Domain 1 and *Babesia microti* Alpha Helical Cell Surface Protein 1	(Meredith et al., 2023)
	pRAP-1	(Montero et al., 2022)
	rN-BmRON2, rC-BmRON2	(Cai et al., 2021)
	r*Bm*SP44	(Wang et al., 2020)
	*Bm*8 polypeptide	(Wang et al., 2023)

Overview of Vaccination against East Coast fever

Various antigens of *T. parva* have been identified that can be used in the development of vaccines for the prevention of East Coast Fever. They are summarized in Table 2

Table 2: Antigens of *T. parva* for the development of vaccines against East Coast fever

Antigens	Kind of vaccine	References
Tp1, Tp2	Live vaccine	(Atuhaire et al., 2020)
Tp9	Live vaccine	(Bastos et al., 2019)
Tp10	Live vaccine	(Goh et al., 2021)
N36	Live vaccine	(Werling et al., 2022)
Whole parasite	Muguga Cocktail vaccine	(Allan and Peters, 2021; Chatanga et al., 2022; Surve et al., 2023)

Overview of Vaccination against *Theileria equi* in equines

For *T. equi,* the EMA-2 protein is a surface protein candidate for vaccine development. It modulates the immune responses against theileriosis (Santos et al., 2021). Similarly, the RAP-1 protein is an antigen of vaccine candidate (Onzere et al., 2022). None of these vaccines are currently available (Saliva et al., 2020).

Future Perspectives and Challenges

Babesiosis and Theileriosis are currently a significant threat to livestock worldwide. Although several vaccine candidates have been recognized, there is a need for a lot of research to develop them effectively and apply them in the field practically, as most of these vaccines are not commercially available yet. The major challenge lies in the fact that *Babesia* and *Theileria* show great variation in their strains region-wise and country wise. So, each country has to recognize the field strains present in their area so that the development of effective vaccines can be carried out specifically for that region.

Conclusion

Babesiosis and Theileriosis are tick-borne protozoal diseases. Ticks from the members of *Ixodidae* are responsible for the transmission of these diseases. Bovine babesiosis, tropical theileriosis, and east coast fever are major concerns for cattle health worldwide. Babesiosis clinical signs include high fever, anorexia, ruminal atony, respiratory distress, pale mucous membranes, constipation or diarrhea, and dark red to brown urine. Clinical signs associated with theileriosis include tick infestation, fever, pale mucous membranes, enlargement of lymph nodes, anorexia, inappetence, emaciation, respiratory distress, nasal discharge, lacrimation, and a high decrease in milk production. The life cycle of *Theileria* includes both lymphocytic and erythrocytic stages, while the life cycle of *Babesia* includes only the erythrocytic stage. Currently, available methods for the control of these diseases are the application of antiprotozoal drugs and effective tick management. However, during the past few years, there has been a focus on the development of effective vaccines against these diseases. A number of live attenuated vaccines are under investigation. Several antigens have been identified that trigger an immune response within the host. Among the immune cells, CD4+ and CD8+ cells are the most important for triggering an immune response against these diseases. So, any antigen that activates these cells can be used in the development of effective vaccines. However, the major problem is that there exists a strong variation among the strains of *Babesia* and *Theileria*. So, a specific antigen present in a specific region must be used for the development of a vaccine for that specific region. Also, the vaccines developed should be such inexpensive that they can be easily available for each farmer.

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Chapter 28

Vaccination Strategies against Malaria

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ABSTRACT

Malaria is a significant global burden that causes huge morbidity and mortality. Each year out of 50 million, 2 million deaths are due to malaria. Many techniques were developed for the eradication of malaria in the last century including insecticides, chloroquine, and many other drugs. However, due to the increased development of parasitic resistance in drugs, vaccines are considered the best way to prevent malaria. After the production of malaria vaccines, mortality and morbidity rates decreased. Therefore, the present chapter focuses on the strategies of malaria vaccine development. Due to the complexity of the plasmodium life cycle, different approaches are used for the production of vaccines like pre-erythrocytic, blood, and transmission-blocking stages. On the basis of the parasite's life-cycle, three types of vaccines are developed like, Pre-erythrocyte, blood-stage and transmission blocking vaccines. Although there are different challenges for malaria vaccine development, antigenic diversity, parasite mechanism of action, and immune invasion but despite of these challenges research is continuing towards the production of new, best, and efficacious vaccines against malaria that can decrease the malaria burden globally.

KEYWORDS
Malaria vaccine, Vaccine, Plasmodium, Strategies, Development, life cycle stages

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INTRODUCTION

Malaria becomes a burden from a public health perspective, about 229 million cases were observed globally in 2019. Almost 94% of people are affected by malaria in Africa (Fikadu and Ashenafi 2023), while in the US, almost 2000 malarial cases are reported annually (Daily et al., 2022). Malaria is a vector-borne, endemic, protozoal disease caused by different plasmodium species (Escalante et al., 2019). Overall150 plasmodium species have been discovered that affect mammals, reptiles, and some poultry species (birds), mostly these species are host-specific (Garnham, 1996; Butcher et al., 1970). *Plasmodium falciparum* (causes severe malaria), *P. malariae, P. knowlesi, P. vivax* (a second major cause of malaria), and *P.ovale* are well-known plasmodium species that cause malaria in humans (White 2008; Antony and Parija 2016). Signs and symptoms of malaria are fever, nausea, headache, diarrhea, vomiting, cough, abdominal pain, joint or muscle discomfort (Dondorp et al., 2009). The Life cycle of plasmodium parasite is complicated because this cycle is completed in the host (vertebrate) and vector (*Anopheles* mosquito) and has two types of reproduction (sexual and asexual). These reasons make drug and vaccination production difficult (Guttery et al., 2012).

Plasmodium protozoa are diagnosed by using a microscope in blood specimens however rapid diagnosis can be performed by examining the parasite-specific molecules in blood specimens (Walter and John 2022). For the control of malaria, different preventive measures are adopted such as insecticides, removing standing water and mosquito nets. Insect repellants, which contain DEET or picaridin and reduce outdoor exposure during dawn and dusk, for travelers, may also reduce the risk of malarial infection (Sluydts et al., 2016; Walter and John 2022). Chemoprophylaxis is recommended, daily or weekly, as preventive medication. It is recommended before or after a visit to the malarial endemic areas (WHO, 2022). 4-dose RTS, S malaria vaccine decreases infected children (malaria infected) death. According to WHO, in the areas that have moderate to high *P. falciparum*, children should be routinely vaccinated with RTS, S vaccine (Hogan et al., 2020; WHO, 2023).

Highly effective vaccines are required for the control and elimination of malaria because of the development of resistance in antimalarial medication and insecticides. For this purpose, the World Health Organization (WHO) and its companions developed a strategy that those vaccines will be used in endemic areas that will show more than 75% efficacy against malaria in 2030 (WHO, 2013, WHO, 2014). For this objective, different types of vaccine strategies are formed. Vaccine strategies may be classified according to the plasmodium-targeted life cycle stage. Pre-erythrocytic vaccines are those types of vaccination that target sporozoites (Beeson et al., 2019). Sporozoites are administered by the mosquitoes and are transported to the hepatocytes for initiation of infection. These vaccines avoid clinical signs and malarial transfer by preventing primary infection. Merozoites multiply asexually in hepatocytes and infect erythrocytes. Blood-stage vaccines are formed for the death of the merozoites so, that merozoites multiplication can be prevented. With the decrease of parasites in blood transmission of malaria is decreased. Transmission of malaria occurs when gametocytes are formed by the intraerythrocytic parasites, then they are taken up by the mosquitoes and utilized for sexual reproduction. Then these mosquitoes injected them into the humans. Transmission-blocking vaccines (TBVs) cause gametocyte mortality (Beeson et al., 2019). The aim of this chapter is to explain the malaria vaccine formation by using different approaches.

Vaccine Development Approaches

The development of partial immunization and successful therapy of clinical manifestation in children with amplified immunoglobins from semi-immune individuals against malaria are the signs of better malarial vaccine development (Cohen et al., 1961). This is verified by the development of sterile immunity by the induction of live or weakened sporozoites and infected red blood cells in experimental animals and controlled human malaria infection (Pombo et al., 2002; Hoffman et al., 2002; Roestenberg et al., 2009). Weakened sporozoites that still can penetrate liver cells, cannot maturation and transform into merozoites. When merozoites do not form then clinical signs of malaria are not developed (Nussenzweig and Nussenzweig 1989). There are three strategies for malaria vaccine development: pre-erythrocytic strategy (pre-erythrocytic vaccine), blood-stage (blood vaccine), and transmission-blocking strategies (transmission-blocking vaccine) (Beeson et al., 2019) as shown in Fig. 1.

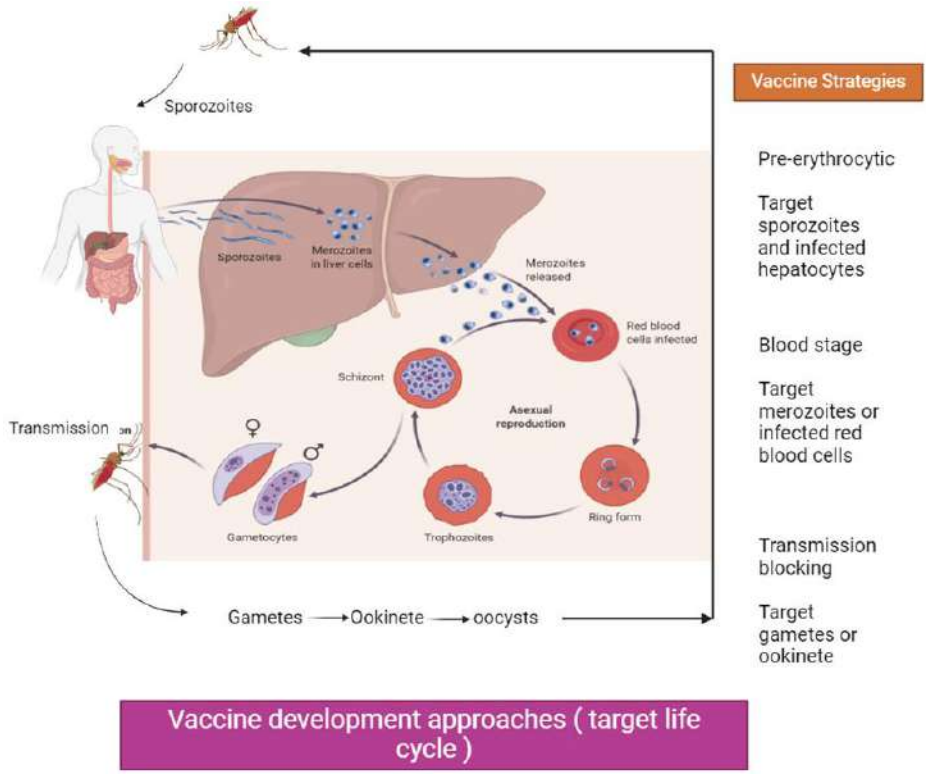

Fig. 1: Vaccine development approaches (that target life cycle) (retrieved from biorender)

Pre-erythrocytic Vaccines

This is the first target of vaccine development. In this stage, sporozoites's penetration occurs into blood and hepatocytes as shown in fig1. In hepatocytes, the parasite multiplies rapidly (schizogony). Penetration of red blood cells comes after this stage. The purpose of this type of vaccine is to prevent hepatocyte infection and parasite growth in liver cells. In this way, these vaccines inhibit the penetration of parasites into red blood cells (Nussenzweig and Nussenzweig 1989; Roestenberg et al., 2009). Antibody responses may be involved in this stage as a protective mechanism that inhibits the penetration of sporozoites into hepatocytes. Till now, highly advanced vaccines are the licensed RTS, S, and subunit vaccines. Other examples of whole parasite vaccines are PfSPZ (*Pf* sporozoite), PfSPZ-GAP (genetically attenuated parasite), and PfSPZ vaccination including chemoprophylaxis (PfSPZ-CVac) (Frimpong et al., 2018).

RTS, S is a recently produced malarial vaccine and its particles are virus-like, primary sporozoite surface Ag, circum sporozoite protein that consists of C-terminal and central repeat epitopes. Their purpose is to produce immunity that can prevent malaria. In vaccine trials, children (5-17 months of age) at the time of initial vaccination have 50.4% efficacy while in severe cases, 45.1% vaccine efficacy is reported, after 12 months of examination. Infants (6-12 weeks of age) show 30.1% efficacy. After 3-4 years of examination without booster vaccine efficacy in children is 28% while infants show 18% efficacy after administering booster dose efficacy in children is 36% and infants have 26% efficacy, at 18 months (Rts, 2015). With time efficacy of RTS, S is decreased that's why it is necessary to produce vaccines that have long-lasting effects. After 18 months, efficacy variability ranges between 40-77% in children and 0-49% in infants (Rts, 2014). Although RTS, S's efficacy is lower than the currently supposed level according to modeling studies (vaccination coverage, transmission intensity, therapy usage, etc.) it can prevent a substantial number of clinical cases, and serious outbreaks and enhance public health (Penny et al., 2016). In female children, a large number of mortality and meningitis cases are reported (Rts, 2015; Klein et al., 2016). European Medicines Agency considers RTS, S better vaccination for use, and in African countries, its trials are continuing for further assessment (Beeson et al., 2019).

Another vaccine strategy, for malaria vaccination is, by using a viral-vector heterologous initial booster that is applied in ChAd43 and modified vaccinia Ankara viral vectors carrying TRAP Ag and various cell epitopes. This strategy produces 21% sterile safety and 36% delay in CHMIefficacy (Ewer et al., 2013). But phase 2b examination shows that among Kenyan men, 67% infection rate is decreased, after a brief monitoring. While in Senegal adults it does not show any efficacy (Ogwang et al., 2015; Mensah et al., 2016). *P. vivax* is on its way to trials for efficacy (Bennett et al., 2016).

Whole sporozoite vaccine techniques proved their efficacy. PfSPZ vaccine is made up of irradiation-attenuated sporozoites which enter into liver cells but do not enter into the blood. Oppose to homologous strain in malaria-naïve individuals, PfSPZ gives 80% safety (Seder et al., 2013; Epstein et al., 2017; Lyke et al., 2017). However, it shows less efficacy in contrast to heterologous strains and has decreased efficacy and immunity in field examination (Sissoko et al., 2017; Olotu et al., 2018; Jongo et al., 2018). PfSPZ-CVac includes the administration of live sporozoites, combined with medication prophylaxis against malaria, to destroy the parasite at initial blood stages. Homologous strains show more efficacies in CHMI trials (Roestenberg et al., 2009; Mordmüller et al., 2017). Another vaccination (whole sporozoite vaccine) consisting of the genetically weakened parasite, is used that affects the liver but does not enter into blood (Kublin et al., 2017). However, the childhood Expanded Program for Immunization (EPI) requires various infrastructure and novel operational processes for the distribution of whole parasite vaccination. EPI aims to present the best vaccination for the control of contagious diseases in the affected population. EPI vaccines are administered subcut or intramuscular way, delivered, and stored at ~4°C cold chain. While nowadays, live weakened whole sporozoite vaccinations are stored and delivered in a liquid nitrogen vapor phase, without EPI favor (Beeson et al., 2019).

Blood-Stage Vaccines

The second stage of malaria vaccine development is the parasite blood stage as shown in Fig. 1. The idea for the discovery of this type of vaccine comes from the endemic areas because in these areas people are frequently infected with malaria, and acquired protective immunity. In this way, RBC invasion of parasites is limited (Cohen et al., 1961; Baruch et al., 1996). These vaccines target parasite outer membrane proteins like Rh (reticulocyte homolog proteins), merozoite membrane proteins, and AMA1 (apical membrane Ag 1) (Rodriguez et al., 2008; Baum et al., 2009; Kusi et al., 2012; Hill et al., 2016). Remaining blood-stage vaccines, such as PfEMP1 (*P. falciparum* red blood cell Membrane Protein-1) emphasize parasite antigens present in affected RBC membranes (Mkumbaye et al., 2017). Although these vaccines are extremely immunogenic and exhibit great potential their antigens are extremely variable and show responses specific to both antigen and parasite (Kusi et al., 2012; Hill et al., 2016). Conversely, Rh protein antigens exhibit great stability and typically have lower immunogenicity (Rodriguez et al., 2008; Baum et al., 2009; Partey et al., 2018).

AMA1 merozoite invasion causing protein gives better clinical results up till now. FMP2.1/AS02A in 2b clinical investigation against malaria in children, gives 64% efficiency compared to vaccine-like strains but does not have overall protection, probably due to antigenic variation (Thera et al., 2011). AMA-C1 (AMA1-based vaccine) does not show its efficacy against malaria in children (Sagara et al., 2009). MSP1 is mostly present on merozoite surface Ag, and Abs contrast toMSP1 are safe in experimental animals and linked to protection in few studies including human cohorts (Beeson et al., 2016). Despite generating significant antibody titer, FMP2.1/AS02A (MSP1–42) does not show greater efficacy (Ogutu et al., 2009). In the CHMI trial, If MSP1 and AMA1 utilizing viral vectors are joined together then they do not generate significant efficacy (Sheehy et al., 2012). The GMZ2 vaccination produces a high level of functional Abs in the primary trial but shows less efficacy in African children when they are tested in the larger trial (Jepsen et al., 2013; Sirima et al., 2016). Further, the multi-antigen vaccine gives significant results in Papua New Guinean children in the second phase. And show a 62% decrease in parasite levels, in those, who are not primarily treated with antimalarial medication (Genton et al., 2002). PvDBP-RII is primarily *a P. vivax* blood-stage candidate that generates antibodies and T-cell responses that prevent PvDBP-RII from connecting to its receptors (Payne et al., 2017; Beeson et al., 2019).

Transmission-blocking Vaccines (TBV)

This type of vaccine targets gametocytes (sexual parasite form) as shown in fig 1. These activate the host immune response against the parasite proteins e.g; pre-fertilization Ag (antigen) (Pfs230, Pfs45, Pfs48) and post-fertilization Ag

(Pfs25, Pfs28) by inhibiting the transfer of parasite between vector (mosquito) and humans. If there is availability of gametocytes in the peripheral blood, then malaria transfers successfully because mosquito takes blood as a meal. The effectiveness of penetrated gametocytes into mosquitoes depends upon some factors such as medication stress, gametocyte concentration, mosquito immune-mediated defenses, and clonal traits of infection (Dong et al., 2009; Bousema et al., 2010; Nsango et al., 2012; Churcher et al., 2013; Lefevre et al., 2013). One study demonstrated that gametocytes remain contagious to parasites if they are in low concentration (Churcher et al., 2013). Furthermore, it is also verified that if gametocytes are in low concentration, they can easily transfer in humans and this transfer does not depend upon the gametocyte concentration in peripheral blood (Hallett et al., 2006; Schneider et al., 2007). Transmission-blocking vaccines decrease parasite infection and transmission by taking the opportunity of the functional immune response against the sexual phase parasite proteins (Carter et al., 2000; Saul, 2008). Malarial transmission-blocking vaccines are of two types: sexual, SSM-VIMT (sexual, sporogenic, or mosquito stage VIMT candidates), and PE-VIMT (pre-erythrocytic VIMT candidates). SSM-VIMT stops human to mosquito transfer and PE-VIMT blocks mosquito-to-human transfer (Jones et al., 2015). Just Pfs25 and Pfs230 in TBV candidates have completed their research in humans (Wu et al., 2008; MacDonald et al., 2016; Talaat et al., 2016; Doumbo et al., 2018). Transmission-blocking vaccines have advantages over other types of vaccines. 1) Due to their reduced interaction with the human immune system, TBVs are not variably diversified. 2) TBV targets a decreased amount of parasites (commonly <10–100 oocysts per mosquito in nature) (Zollner et al., 2006; López et al., 2017; Duffy, 2021). Therefore, for the development of TBV, most research should be based on antibody response because the immunity of these vaccines is primarily antibody-mediated (de Jong et al., 2020; Tachibana et al., 2022). However, the failure of these potential vaccines creates an important barrier because they do not produce large amounts of antibody titers. Therefore, there is the possibility that if these vaccines are conjugated then they may show their good potential without any obstacle (Talaat et al., 2016; Sagara et al., 2018).

Potential TBV candidates containing Pfs230 and Pfs48/45 that are produced by gametocytes in humans as a host and Pfs25 are only produced by the mosquito are still now studied. Pfs25-based vaccination and Pvs25 (its ortholog) have demonstrated the administration of Abs that can inhibit mosquito infection (Wu et al., 2008). Potential transmission-blocking Abs are induced by Pfs25-EPA/alhydrogel, in healthy Malian individuals. However significant Ab titer can be attained after four dosages of Pfs25-EPA/alhydrogel (Sagara et al., 2018).

Promising Approaches to Malaria Vaccine Development

There are some other approaches of malaria vaccine development which mainly based on host or parasite immunity. These approaches are advanced that improved the production of malaria vaccines.

The Parasite-Focused Approach

This approach is mainly based on the detection of parasite's Ag that stimulates the immune system, through the study of proteome, parasite's genome or transcriptome. It may change the antigenic component's composition so, that they can attack different pathogen types. This approach also tests the security and immunogenicity of the candidate Ag so, that fresh and better vaccinations can be made. Different applications that are included in this approach are reverse vaccinology, immunoinformatics, and structural vaccinology (Frimpong et al., 2018).

Reverse Vaccinology

This technique is invented by Rappouli and his colleagues and it is first utilized in *Meningococcus* bacteria serogroup B, for the detection of current vaccine Ag. The pathogen's genome is assembled and evaluated to obtain the whole protein's repertoire and help in the identification of the same species of pathogens (Seib et al., 2012) as shown in fig 2. Genetic data is assessed by utilizing bioinformatics tools. Moreover, gene sequences that have similarities to humans are removed from the recognized vaccine candidates, by utilizing computational devices. While, the leftover genes are separated and placed into the appropriate vector, to gain proteins for experimental animals. Immunized mice are used for the analysis of vaccine Ag, for the verification of its effectiveness and immunogenicity. Moreover, molecular epidemiology researches are conducted utilizing different kinds of pathogens, so, that extremely unstable or conserved Ag is detected from the selected Ags (antigens) (Rappuoli, 2000). Reverse vaccinology is used for the detection of released or related to the signaling parasite proteins in malaria so, that potential vaccine candidates can be developed. Since 2002, *P. falciparum's* genome sequence has been used (Gardner et al., 2002). Comparative study has revealed homologs between species, of these sequences, with feasible similar functions. For example, functions of transmission-blocking vaccines (Pf48/45, PfHAP2 genes) have been discovered based on the function of their homologs in another *Plasmodium* spp. (van Dijk et al., 2001; Hall et al., 2005; Blagborough and Sinden 2009; Otto et al., 2014; Sundararaman et al., 2016).Despite various advantages of reverse vaccinology, there are some limitations. For example, it cannot detect non-peptide Ag but it can describe operons (that code for synthesis of such molecules) (Sette and Rappuoli 2010). In this technique, no successful malaria vaccine till discovered. For further progress, some other items are required like better predictive algorithms for the identification of T and B cell epitopes and precise quantitative evaluation before incorporation in vaccines (Frimpong et al., 2018).

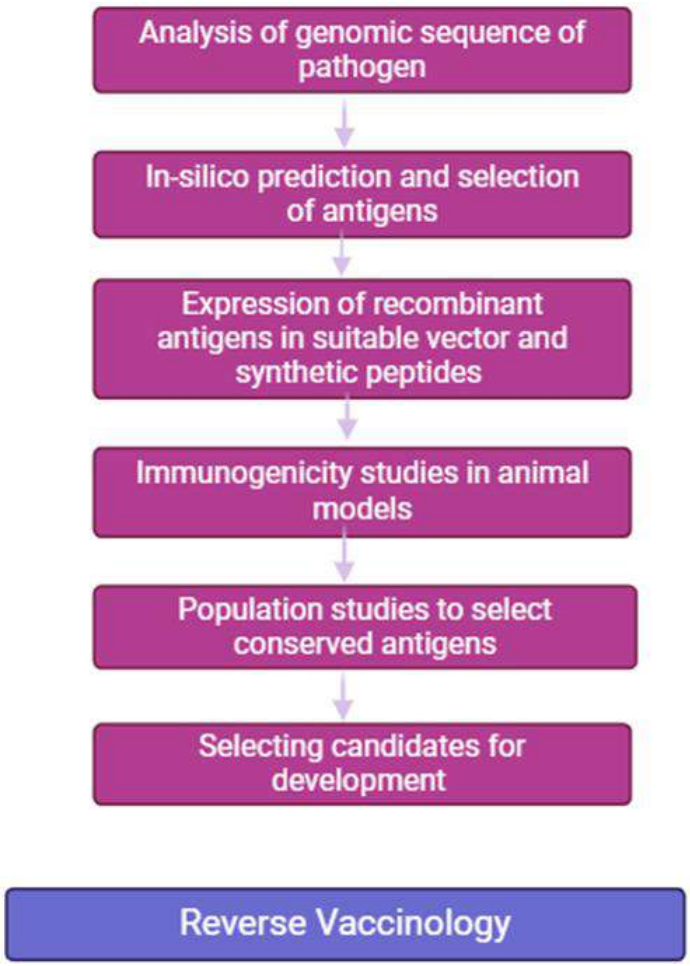

Fig. 2: Reverse Vaccinology (retrieved from biorender)

Structural Vaccinology

To detect the suitable epitope, there is a need for a deeper understanding of native structures of macromolecules like proteins, and how structural changes can affect their role in the body (Thomas and Luxon 2013; Delany et al., 2014). These epitopes are developed in such a form that they can easily be accepted by the immune cells. By creating changes in these structures, vaccine efficacy and safety are increased and genetic variation among various strains can be decreased (Nuccitelli et al., 2011). In the past, α-helical coiled-coil structural domains with limited preservation is identified from asexual *P. falciparum* by detecting the Plasmodium genome (Villard et al., 2007). Upon further investigation, a disorganized peptide (P27A) is selected that evolves in native confirmation. This peptide can detect that antibody that can limit the parasite reproduction (Olugbile et al., 2009). P27A has been considered to be safe and immunized (Steiner-Monard et al., 2019). Structural vaccinology assists *P. falciparum* to combat antigenic variation. For example, structural vaccinology facilitates the detection and confirmation of invasion ligand Cysteine-Rich Protective Ag (CyRPA) (a three-dimensional structure) and the analysis of less polymorphic DBL4ε domain of VAR2CSA to discover the novel characteristics in the pattern which impacts the functional characteristics of the Ag (Gangnard et al., 2015; Favuzza et al., 2017). Further, CyRPA is a protective epitope that gives a combined effect with Reticulocyte binding-like Homologous protein 5, in such a way that Abs can reduce the reproduction of parasites against *Pf* RH5 and CyRPA, in host erythrocytes (*Pf* RH5) (Favuzza et al., 2017). Luckily, malaria vaccines that contain these epitopes can generate effective immune responses that are effective. The efficacy of the malaria vaccine can be enhanced if hybrid protein vaccines are made by the interaction of these protective Ag. But for the formation of such types of vaccines, the detection of appropriate B and T cell epitopes is necessary (Frimpong et al., 2018).

Immunoinformatics-Based Approach to Vaccine Design

For the prediction of immunogenicity of Ags, there is a need for the formation of machine learning algorithms. For the development of this algorithm, computational techniques and experimental immunology are combined. These techniques may be theory or sequence-based and can be operated at an amino acid pattern or protein framework level. A model is formed based on immunogenicity, by utilizing physical concepts. While in the pattern-based technique, the problem is detected like immunogenicity related structural or sequence pattern. Different algorithmic tools are used in pattern-based techniques like statistical structural-activity assessment, artificially generated neural networks, and support vector machines (Wan et al., 2006; Liu et al., 2006). Whereas, in theory-based techniques, various statistics-based models and Markovian or Bayesian models are used (Degoot et al., 2018).

For the production of the malaria peptide vaccine, the Immunoinformatic technique has already been used on *P. falciparum*. In it, the prediction of cytotoxic T cell epitopes combined with HLA A/B molecules is already done (Doolan, 2011). For example, due to various changes and capacity to combine with various host receptors, the PfEMP1 gene (a var gene family member) is linked to parasite escape from the host defensive mechanism (Mkumbaye et al., 2017). For a few years, for the detection of antigenic epitopes from DBL-3γ and CIDR-1 (conserved domains of PfEMP1), experimental and in-silico techniques have been used. These epitopes have some characteristics in exposed people like excellent attachment ability to HLA molecules and capability to stimulate T cell proliferation, IFN-γ, and IL-4 production (Khan et al., 2017).

Peptides with an amino acid length ranging from 8–10 are considered best for administration to CD8 T cells by HLA class I molecules, while peptides with a length of 12–25 amino acids are thought optimal for administration to CD4 T cells by HLA class II molecules. Bioinformatics techniques for peptide binding to HLA I molecules have shown very high predicted accuracy levels; nevertheless, methods for peptide binding to HLA II molecules still need refinement. Predicting which peptides will bind to B cell receptors to produce effective antibody responses is an even bigger difficulty (Degoot et al., 2018; Jensen et al., 2018). However, not all HLA binders are favorable T cell epitopes, which provides a significant obstacle for methods that detect HLA binders without considering the whole picture of HLA-peptide-TCR interactions. However, these computational techniques, which are highly economical and serve as essential down-selection instruments when the number of peptides is too great for evaluation through experimentation, may contribute to the creation of malaria vaccines that work (Frimpong et al., 2018).

Immune-Focused Approach

As *P. falciparum* has a complex immune system, therefore, it can survive with the host. That's why, new novel approaches are needed for the development of vaccines as shown in fig 3. The immune-focused approach is a novel approach that has been made to tackle these infections. The parasite-focused approach focuses on the desired organism while the immune-focused approach accelerates the production of efficient vaccines by utilizing the host immune system as shown in Fig. 3. It emphasizes the investigation of the host immune system so, that it can easily recognize the protective immune-mediated signatures. It is believed that, in susceptible hosts, these protective signatures can be induced de novo to shield them against infection or illness. The immune-focused approach is better for a highly variable organism like malarial parasites than the pathogen-focused approach. Especially, it may be possible to create and recognize immune cells with enhanced cellular response and widely neutralizing antibodies that cannot easily obtained by traditional methods (Haynes et al., 2012).

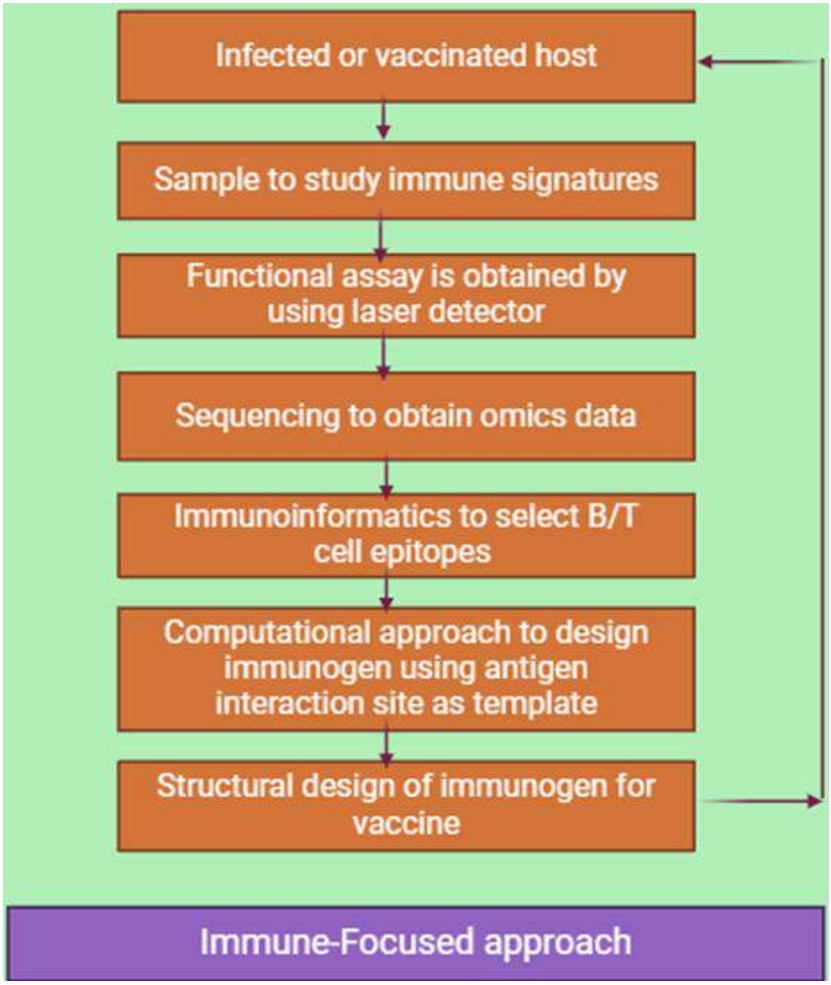

Fig. 3: Immune-Focused approach (retrieved from biorender)

Conclusion

Development of malaria vaccines that target the parasite's life cycle is continued. Pre-erythrocytic, blood-stage, and transmission-blocking vaccines have different advantages and challenges but some trials have proved successful. Moreover, the development of newly, high output techniques may increase the recognition of new vaccine candidates such as the structural layout of immunogens, lymphocyte repertoire pattern, and breadth of defense of old and new vaccine candidates. Further, vaccinologists developed vaccines that operate the immune system through different protective ways. Such as computational techniques and mathematical modeling to the data thus achieved, which will form new ways towards the production of highly efficacious and target-achieving malarial vaccines for 2030. Therefore, governments, health organizations, and researchers must continue to make collaborative efforts so, that the malaria eradication goal can be achieved.

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Chapter 29

Vaccination and Plant Extract to Control Ticks

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ABSTRACT

Parasites, being the global threat in animals are a widespread cause of the spread of various diseases. Among these, ticks are of significant importance in livestock and domestic animals. They have the tendency to spread the diseases by attaching to the skin of the animals and lead to the spread of the diseases. This chapter focuses on the structural adaptations of the tick species and covers the impact of ticks on livestock health. They are also responsible for huge economic losses. Various strategies are being used to control the infestation by the ticks and subsequently the spread of the diseases. This chapter highlights the limitations of various techniques and methods for preventing tick infestations and also sheds light on the emerging resistance of ticks against acaricidal agents. So, alternate strategies like vaccination, nanoparticles, and essential oils are focused in this chapter to be beneficial in reducing the spread of disease via ticks. Their mode of action specifies their effect on reducing the occurrence of tick infestations.

KEYWORDS
Ticks, Chemical drugs, Resistance, Alternatives, Plant extracts, Control

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INTRODUCTION

Globally, parasites serve as devastating agents relying on their hosts for continuous survival and reproduction (Perry et al., 2011). Endoparasites such as protozoans, nematodes, cestodes, and helminths exist inside the body of their host while ectoparasites like ticks, flies, mites, mosquitoes, midges and lice live outside the host's body and both can cause different kinds of disorders in animals (mammals, birds, reptiles, and even amphibians) (Rodriguez et al., 2024). Ticks are more common hematophagous (feed on blood) among these ectoparasites. Majority of ticks survive solely on specific host animals but certain ticks infest on a broad range of hosts (Munir et al., 2023). They act as vectors of multiple protozoal, rickettsial, bacterial and viral diseases (Madder et al., 2014). These diseases can spread in a number of ways and are serious threat to the growth or development of animals, humans, birds, and wild fauna (Estrada-Peña et al., 2014)

Ticks are opportunistic parasites of domesticated and wild animals distributed worldwide most often in tropical and subtropical regions including Pakistan (Ghosh et al., 2007). Ticks are generally in search for food in the wooded regions, among dense grass, around the piles of wood, beneath ground covered by plants or leaf debris and stone walls where small mammals are usually inhabited (Černý et al., 2020). They live in warm and humid environment comfortably and use oduors, movement, and breath and body heat of their host to access them. The preferable sites for tick infestation are ear, udder, tail, testes (Ramzan et al., 2018).

Ticks' Morphology

Ticks belong to phylum Arthropoda, order Acarina, and class Acari. The capitulum (head), idiosoma (flattened, oval shape body) and the legs are the major parts of tick's body. A base (basis capitula), paired chelicerae, hypostome which is coated with denticles or recurved teeth, and leg-like palps are located in capitulum. Certain mouthpart features such as the length of the palps, number of denticles and form of the basic capituli are used to distinguish tick genera and species (Stafford et al, 2024). There are anterior and posterior divisions of tick's body. Podosoma is the anterior region has four pairs of legs and a genital pore while opisthosoma, the posterior region comprises of an anal portion and spiracular plates. There are six segments in legs connected with body through coxae (Kuo et al., 1970). A sensory organ called Heller's organ is present in the first segment of leg and used to detect such external factors like smell, temperature.

There are two categories of ticks; Ixodidae (hard-bodied ticks) and Argasidae (soft-bodied ticks). A hardened,

sclerotized plate on the dorsal body surface called scutum (sometimes named conscutum) make the body hard, cover the entire dorsal body surface in males which limit their feeding ability and one third of the dorsal body surface in unfed females, nymphs (8-legged) and larvae (6-legged) (Estrada-Peña et al., 2004; Madder et al., 2014). Due to lack of hard covering in females, engorgement may increase up to three times their unfed size during feeding (Sonenshine et al., 2013). Larvae and nymphs perform molting or cuticle shedding in order to move to the next stage after digesting blood meal (Stewart et al., 2020). Only in adult stage sexual dimorphism is visible. Males are smaller in size than females due to the presence of sclerotized plate on dorsal surface, which prevents growth in males (Johnson, 2023). Compared to hard ticks, soft-bodied ticks have leathery cuticle instead of hard scutum (Anderson et al., 2008). They usually consume frequently, but in a lesser volume. They are incapable to expand during feeding to a comparable degree as the hard bodied ticks.

The most important internal organs are trachea, salivary glands, malpighian tubules, digestive system and reproductive organs (Lee et al., 2021). Hemolymph, a fluid equivalent to blood present in sinuses of ticks, contains hemocytes which are cells of immunity and nutrients provide to different body parts (Castillo et al., 2020). The midgut is the largest body part which is responsible for storage and digestion of blood meal (Maqbool et al., 2022). Salivary glands secrete saliva with various component which are anti-immunomodulatory, vasodilatory, complement factors and platelet aggregation factors (Neelakanta et al., 2022). Reproductive organs in adult occupy major portion rather than midgut (Shepherd et al., 2023).

Livestock Importance and Ticks

Livestock sectors are essential components of a country's economy. The economic worth of farming is raised 60.1% by livestock sectors. Furthermore, these sectors currently contribute 11.5% of Pakistan's GDP. The rural communities with great number consisting of over 8 million individuals and their families in the country have supported the growth of livestock field. However, this field employs 35–40% of rural populations (Maqbool et al., 2021). Livestock animals provide the nation with meat, eggs, milk, hides, and wool. They play a vital role in international trading. Import and export of animals may increase the chance of tick's infestation (Kumar, 2019; Rahman et al., 2022). Climate change, water availability, suitable environment or surroundings and Pakistan's physiographic areas are the main factors involved in the spreading of ticks (Ghafar et al., 2020). As the ticks infest animals, it results in significant losses because they disseminate pathogens, damage livestock products and anemia due to blood loss.

Chemical Drugs used against Tick

Several methods are being applied to control tick's population. Chemical acaricides are the main weapon generally used to reduce this population currently. To control ticks on livestock or in the surroundings, acaricides are used in such a way that the ticks are killed without damaging animals and applicators (Rajput et al., 2006). Acaricides such as arsenical preparations, chlorinated hydrocarbons (DDT and lindane), organophosphorus compounds (coumaphos), carbamates (carbaryl), formamidines (amitraz), pyrethroids (permethrin, flumethrin), formamidines (eamitraz), macrocyclic lactones (ivermectin), phenylpyrazoles (fipronil), and isoxazolines (afoxalaner, fluralaner, sarolaner) are used by pest controllers to manage ticks. Nowadays, among the safest and best-performing pesticides the synthetic pyrethroids are widely used for tick control. Fipronil is a less toxic broad-spectrum phenyl pyrazole insecticide, frequently used against ticks and other ectoparasites (William et al., 2019; Arshed et al., 2021). The efficiency of an acaricide depends on the quality and quantity of active substances deposited or delivered in animals besides the activity of a product. (George, 2000).

A variety of methods such as dipping, ear tagging, spraying and pour on have been used to administer chemicals to animals. But using acaricides directly to the animals is a popular method of tick control (Khan et al., 2022). However, the acaricide use has not proven to be extremely efficient in decreasing tick infestations and has serious negative impacts including acaricidal resistance, contamination of surroundings, milk and meat products (De la Fuente and Kocan, 2006).

Acaricidal Resistance

Drug resistance has been observed in the ticks due to decrease in the efficiency of acaricides over the past few decades. Resistance to acaricides is a natural consequence of evolution, which indicates the physiological and behavioral mechanisms that enable the survival of ticks with resistance (Waldman et al., 2023). Resistance develops due to genetic alternations in the population of ticks which leads to the changes in target site, increase in metabolisms with reduction of acaricidal penetration through outermost protective layer of tick's body (Guerrero et al., 2012). Investigations on the acaricide resistance mechanisms in ticks have revealed detoxification pathways based on increased enzymatic activity (p450, GST, esterase and cytochrome) which have a part in acaricides modifications biochemically towards reduced toxicity and greater removal of dangerous substances (Some 2008; Waldman et al., 2023)

In order to avoid the current issues, a greater awareness of mechanisms behind acaricide resistance is crucial for improving the efficiency of chemicals to control tick population. Furthermore, researchers are working on development of better tools to identify acaricidal resistance as well as other controlling strategies.

Vaccination

Ticks spread various disease such as cowdriosis, Lyme borreliosis, anaplasmosis, theileriosis, and babesiosis in animals and humans. Thus, these tick borne diseases are major threat for livestock as well as human health around the

globe. *Rhipicephalus microplus* is the most serious threat to livestock particularly the dairy and beef sectors in different areas of the world. It also spread tick fever (babesiosis and anaplasmosis) in bovine and piroplasmosis in equine (Jongejan et al., 2004; Lew-Tabor et al., 2016). The immunity against ticks and TBDs in livestock can be produced through vaccinations.

DNA-Based Anti-Tick Vaccines

Researchers have been working on creating a DNA vaccination to manage tick infestation for the past few years (Fan et al., 2022). These bacterial plasmid-based vaccination include benefits like easy to use, stability and secure delivery (Myhr, 2017). The plasmid DNA molecules that were injected constantly produce protective antigens, which permits repeated boosting to sustain a high antibody titer. DNA vaccine can stimulate CD4+ and CD8+ T cells and do not cause immunological responses to the vector DNA (Li and Petrovsky, 2016). The possibility of creating a DNA vaccine to prevent tick bites is further enhanced by the recent evaluation of a variety of different antigens, including lipocalins and Salp14, either alone or in combination with other antigens (Matias et al., 2021).

Vitelline and GP80 base Vaccine

Both the 80 kDa glycoprotein (GP80) from *R. microplus* larvae and the purified vitellin protein from tick eggs were found to be useful for vaccination. A vaccination including both antigens may be able to stimulate an immune response and offer sheep hosts some protection against *R.microplus* (Abbas et al., 2023).

Bm86 and Bm95 based Vaccine against Ticks

The Bm86/Bm95 based vaccinations were the first and only anti-tick vaccines to be licensed and registered for use in the commercial market at the beginning of the 1990s. Both TickGARD and Gavac are recombinant proteins attached to the midgut membrane of *R. microplus*, called Bm86. The administration of Bm86 based vaccines to cattle has been shown to positively correlate antigen specific antibodies with decreased tick infestations and fertility, indicating the effectiveness of the vaccine. However, it has been noted that the animals' age and reproductive status have an impact on the original antibody response to vaccination. Anti-Bm86 antibodies bind with Bm86 protein molecules to disrupt the proteins' biological function, which reduces the number, weight and reproductive potential of engorged female ticks and provides protection (Kasaija et al., 2022). But still, vaccinations have not completely eliminated the tick problem in the nations where they have been implemented, they are definitely good subjects for studies focused on tick management

Plant Extract to Control Tick

The extracts of various plants are used nowadays as alternative cheap and ecofriendly control strategy with low side effect to manage tick population. Extraction is a process of separating the parts of plant that are medicinally active by using established techniques and specific solvents. Different parts of plants such as seeds, bark, leaves and roots have been utilized to cure ectoparasites in animals and humans. The most abundant source of pharmaceutical intermediates, modern and traditional medicine's active ingredients, food supplements, folk remedies and chemical entities for synthesized medications is medicinal plants. Many methods such as maceration, percolation, infusion, decoction and others are used to extract medicinal herbs. Hydro distillation and some recent techniques including microdistillation and headspace trapping are used to extract aromatic plants (Alemu and Kemal, 2015). The medicinal plants are repellent, growth inhibiting, anti-molting and insecticidal. Tick feeding, fecundity, molting and egg viability all be decreased by certain plant extracts. Extraction duration, concentration of extract, solvent, age of extracted plant, organisms of concern such as ticks and exposure duration are some of the factors that influence the acaricidal activity of plant extracts. Plant extract can be used as a powder or diluted with a solvent (Habeeb, 2010).

Stylosanthes plants, which produce a particular fluid active against ticks, are among the various plants that have been reported to have anti-tick properties. It has been demonstrated that *Tephrorosia vogelii* leaf extracts are extremely poisonous to host ticks numbering one, two and three. After being sprayed with the extracts, the cattle had a 10 day residual protection against tick reinfestation. In addition, it has been reported that leaf extracts from *Calpurnia aurea*, which the Borana people of Southern Ethiopia and Northern Kenya employ to treat louse infestations in humans and calves, have anti-tick qualities. Phytochemicals are the cause of the activity seen in plant extracts (Kosgei, 2014).

Phytochemical Constituents

Phytochemical components found in plant extracts have the ability to suppress the tick population. According to phytochemical analysis, the presence of terpenoids, flavonoids, steroids, phenols, tannins and saponins in varying amounts has been found in plant extract (Arshed et al., 2021).

Mode of Action

The naturally occurring substances in plant extract have a variety of active components with various mechanisms of action (Aboelhadid et al., 2022). The phytoextracts have acaricidal effects by inhibiting the molting, fertility, hatching and viability of eggs in addition to death of adult ticks and reduced feeding (Kemal et al., 2020).

Antioxidant Activity

Any number of chemicals that can donate one of their own electrons to neutralize free radicals are collectively referred

to as antioxidants. Antioxidants function in several ways such as inhibiting the production of free radicals, scavenging free radicals, stopping the oxidative chain reaction from spreading, participating in the redox antioxidant network or controlling gene expression (Ahmad et al., 2013). Many helpful medicinal properties have been demonstrated by medicinal plants and their purified ingredients. A number of Indian and Chinese plants are among the herbs and spices that have been shown to possess antioxidant activity. Catechins, coumarin lignans, isocatechins, anthocyanins, flavones and isoflavones are primarily responsible for the antioxidant action. Drug formulations based on antioxidants are used to treat and prevent complicated diseases (Nooman et al., 2008)

Table 1: Plant-derived compounds activities against ticks

Plant species	Family	Main compounds	Tick species	Concentration or dose	Repellence (%)	References
Ammi majus L.	Apiaceae	hexadecanoic acid (38%)	Ixodes ricinus	0.015 mg cm^{-2}	68.3	(El-Seedi et al., 2017)
Ammi visnaga L.	Apiaceae	carvone (57%), apiol (18%)	Ixodes ricinus	0.015 mg c m^{-2}	62.4	(El-Seedi et al., 2017)
Artemisia herba-alba Asso	Asteraceae	piperitone (26%)	Ixodes ricinus	0.015 mg c m^{-2}	84.2	(El-Seedi et al., 2017)
Calendula officinalis L.	Asteraceae	α-cadinol (21%), carvone (18%)	Ixodes ricinus	0.015 mg c m^{-2}	82.0	(El-Seedi et al., 2017)
Citrus bergamia (Risso)	Rutaceae	limonene, linalool, linalyl acetate	*Dermacentor reticulatus*	1.0%	71.1	(Štefanidesová et al., 2017)
Cleome hirta (Klotzsch) Oliv.	Capparaceae	cedrol, phytol, pulegone, *n*-octacosane, terpinen-4-o	Rhipicephalus appendiculatus	0.1%	89.9 (5 min)	(Ndungu et al., 1999)
Conyza dioscoridis L.	Asteraceae	α-cadinol (10%), hexadecanoic acid (10%)	Ixodes ricinus	0.015 mg c m^{-2}	94.0	(El-Seedi et al., 2017)
Corymbia citriodora (Hook.) K. D. Hill and L. A. S. Johnson	Myrtaceae	citronellal, citronellol, geraniol, linalool, pinenes, limonene	*Dermacentor reticulatus*	1.0%	76.6	(Štefanidesová et al., 2017)
Cymbopogon winterianus Jowitt.	Poaceae	citronellal, geraniol, citronellol, geranyl acetate, limonene	*Dermacentor reticulatus*	1.0%	86.9	(Štefanidesová et al., 2017)
Foeniculum vulgare Mill.	Apiaceae	*p*-allylanisole (88%)	Ixodes ricinus	0.015 mg c m^{-2}	70.6	(El-Seedi et al., 2017)
Lantana camara L.	Verbenaceae	β-caryophyllene (32%), α-humulene (12%)	Ixodes ricinus	0.015 mg c m^{-2}	63.3	(El-Seedi et al., 2017)
Lawsonia inermis L.	Lythraceae	furfural (21%) 5-methyl-2-furfural (15%)	Ixodes ricinus	0.015 mg c m^{-2}	58.3	(El-Seedi et al., 2017)
Lippia javanica (Burm. F.) Spreng	Verbenaceae	bicyclo (3.1.1) heptanes-2-one (21%) and 2-butanone (13%)	Hyalomma marginatum	10.7 and 5.3%	100.0 and 69.2, resp. (3 h)	(Magano et al., 2011)
Matricaria recutita L.	Asteraceae	bisabolol oxide A (67%)	Ixodes ricinus	0.015 mg c m^{-2}	40.0	(El-Seedi et al., 2017)
Mentha spicata L.	Lamiaceae	carvone, limonene, myrcene	*Dermacentor reticulatus*	1.0%	61.4	(Štefanidesová et al., 2017)
		carvone (55%), pulegone (14%)	Ixodes ricinus	0.015 and 0.007 mg c m^{-2}	93.2 and 59.4, resp.	(El-Seedi et al., 2012)
Nerium oleander L.	Apocynaceae	hexadecanoic acid (17%), carvone (13%)	Ixodes ricinus	0.015 mg c m^{-2}	60.0	(El-Seedi et al., 2017)
Ocimum basilicum L.	Lamiaceae	methyl chavicol, methyl eugenol, cineole, linalool, eugenol	*Dermacentor reticulatus*	1.0%	84.6	(Štefanidesová et al., 2017)
		linalool (28%), 4-allylanisole (12%)	Ixodes ricinus	0.015 mg c m^{-2}	64.5	(El-Seedi et al., 2012)
Origanum majorana L.	Lamiaceae	terpineol, thujanol, linalool	*Dermacentor reticulatus*	1.0%	78.1	(Štefanidesová et al., 2017)
		4-terpineol (55.6%)	Ixodes ricinus	0.015 mg c m^{-2}	84.3	(El-Seedi et al., 2012)

Origanum onites L.	Lamiaceae	carvacrol (75.70%), linalool (9.0%)	Amblyomma americanum	0.413 and 0.103 mg c m^{-2}	100.0 and 66.7, resp.	(Carroll et al., 2017)
Ricinus communis L.	Euphorbiaceae	coumaran (24.7%)	Ixodes ricinus	0.015 mg c m^{-2}	61.2	(El-Seedi et al., 2017)
Rosmarinus officinalis L.	Lamiaceae	1,8-cineole (51.8%), borneol (17.5%)	Ixodes ricinus	0.015 and 0.007 mg c m^{-2}	100.0 and 68.3, resp.	(El-Seedi et al., 2012)
Syzygium aromaticum (L) Merr. et L. M. Perry	Myrtaceae	eugenol, caryophyllenes, eugenyl acetate	*Dermacentor reticulatus*	1.0%	83.8	(Štefanidesová et al., 2017)
Thymus serpyllum L.	Lamiaceae	geraniol, linalool, thymol	*Dermacentor reticulatus*	1.0%	82.0	(Štefanidesová et al., 2017)
Thymus vulgaris L.	Lamiaceae	thymol, linalool, caryophyllene, *p*-cymene	*Dermacentor reticulatus*	1.0%	87.1	(Štefanidesová et al., 2017)

Octopamine Receptors

Invertebrates use octopamine, a biogenic amine, as a neurotransmitter and neurohormons. Its receptors are widely dispersed throughout the central and peripheral neural systems, and it is implicated in numerous physiological and behavioral processes (Reynoso et al., 2020). Like the adrenergic receptors in vertebrates, the octopamine receptor belongs to the G-protein coupled receptor (GPCR) family (Audsley et al., 2015). Three types of arthropod octopamine receptors are identified; octopamine and tyramine receptors, alpha and beta adrenergic like receptors (Farooqui, 2012). Tyramine and octopamine influence a number of processes, including arthropod behavior and metabolism. It is likely that the pure constituents of oils taken from plants like eugenol, a-terpineol and cinnamic alcohol have deadly effects on arthropods such as ticks due to their binding to their octopamine and tyramine receptors which caused neurotoxicity and cytotoxicity (Salman et al., 2020).

Cytotoxic and Neurotoxic Effect

Cytotoxic refers to a material or procedure that has ability to harm or kill cells. The use of toxic plant material to repel and combat ticks is growing, which highlights the necessity for research aimed at clarifying how these compounds affect the populations and developmental stages of these arthropods. It has been observed how cytotoxic plant compounds affect the stages of developmental and egg hatch in *Hyalomma marginatum* ticks. The research indicates that cytotoxic substances have the ability to decrease tick populations and induce alterations in teratology (Buczek et al., 2019). Hence, there is need to find more cytotoxic effect of plants extract against ticks.

A neurotoxic impact is the result of chemical constituents of plant extracts acting on ticks' nerve systems. Many of the chemicals derived from plants used to control ticks have unclear mode of action. Certain essential oils derived from plants have neurotoxic effects, such as blocking acetylcholinesterase (AChE), competing with octopamine neurotransmitter receptors, or causing gamma-aminobutyric acid (GABA) to close chloride channels (Camilo et al., 2017).

Other Controls

Use of Entomopathogenic Bacteria, Fungi, Nematodes

Enterobacteriaceae (*Cedecea Lapagei*) were observed in Brazilian origin that infested naturally on almost 40% engorged females (*Boophilus Microplus*). These bacteria infected on vaginal area of ticks. When *Bacillus thurengiensis* sprayed on engorged females (*Argas persicus*) it showed 100% death. *Hyalomma dromedari* females and eggs as well as *Argas persicus* females and eggs appeared to be less vulnerable. Nematodes as entomopathogenic from Heteorhabditidae and steinernematidae families were discovered to be virulent to 2 argasid and 13 species of hard tick (Samish, 2000). Experiments against ticks have revealed that various types of entomopathogenic fungi are beneficial in controlling tick population. *Beauveria bassiana* and *Metarhizium anisopliae* among them caused highest ticks' mortality in both laboratory and field. They are able to penetrate through cuticle and usually appear to be virulent to the all stages of ticks (Ramzan et al., 2021).

Essential Oils

Essential oils are volatile compounds in nature. Various aromatic plants synthesize these as secondary metabolites. These have strong smell and produced by different part of plants including roots, seed, flowers, buds. (Ali et al., 2015; Khan et al., 2023). The widespread applications of essential oils in phytotherapy, cosmetics, nutrition, perfumes, aromatherapy have encouraged various scientists to investigate the properties of plants that yield essential oils, from chemical and pharmacological assessments to the remedial aspects. Many techniques such as hydro distillation, steam distillation, solvent extraction and liquid CO2 extraction are used to extract essential oils. The constituents of the extracted oil may change depending on extraction techniques (Abdel-Hameed et al., 2018).

Antioxidant, anti-inflammatory, spasmolytic, analgesic, anesthetic, anticancer, sedative and antimicrobial activities are the properties of essential oils (Mobeen et al., 2018). Due to these beneficial properties, essential oils are used to control or kill the ticks in livestock.

Nanoparticles

In bio nanotechnology, nanoparticles are widely use for imaging, diagnostics, sensing, tissue engineering, artificial implants, drugs and gene delivery (Aurel et al., 2007; Benelli et al., 2017; Khan et al., 2023). Green, chemical, and arthropod based synthesis are the methods to produce nanoparticles (Zaheer et al., 2022). The extracellular production of metal nanoparticles driven by plant-borne substances, is a less expensive approach than physical and chemical methods. Extreme pressure, temperature, much energy and harmful chemical ingredients are not required to accomplish it (Kumar et al., 2015). Numerous nanoparticles such as silver, nickel, copper, zinc and titanium have attracted a lot of attentions (Kausar et al., 2023) for management of pathogenic agents i.e. ticks. Various recent researches focused on the adverse effects of metal nanoparticles synthesized chemically or plant synthesized on tick vectors that are significantly associated with public health (Youssef et al., 2020; Zaheer et al., 2022).

Discussion

Keeping in view the emerging resistance, failure of acaricidal agents provides insights on the use of alternate strategies like vaccination, use of NPs and EOs (Some, 2008). EOs and NPs are emerging as most commonly used techniques to prevent the occurrence of the ticks. They offer various advantages as compared to the acaricidal use. Furthermore, they show less toxic effects and offer few or no environmental hazards like toxicities (Kumar et al., 2015; Mobeen et al., 2018). More research is required to overcome any kinds of minor demerits offered by these techniques also.

Conclusion

A multipronged approach to the control of ticks was discussed integrating novel and traditional methods each having their own benefits and demerits. While the acaricidal agents have been the most conventional and commonly used methods but some mentioned limitations make their use questionable. Due to these limitations and the rising resistance there has been a demand for use of some other control measures, for which vaccination, essential oils and use of nanoparticle have emerged very beneficial. By manipulating these and using them as antiparasitic agents, promising results can be achieved in the near future**.**

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Chapter 34

Immunization with Live Vaccines for Infectious Laryngotracheitis and Infectious Bronchitis in Avian Species

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ABSTRACT

Many avian species are prone to the infection with Infectious laryngotracheitis (ILT). Live attenuated vaccines of tissue culture origin (TCO) and chicken embryo origin (CEO) are used for efficient control of ILT. The TCO live vaccines are produced by continuous passaging of virulent strains of virus in the cell cultures. Live vaccines of CEO are produced by passaging the virulent virus in embryonated eggs. Recombinant types of live vaccines are also in use, which use fowlpox virus (rFPV) and herpes virus of turkey (rHVT) as vector. Commercially available recombinant viral vector vaccine of ILT is in combination with avian encephalomyelitis, by using fowlpox virus as a vector (FP-LT+AE). Infectious Bronchitis (IB) emerged as one of the prominent diseases of respiratory and reproductive origin in avian species. Vaccination with live virus vaccine in the day-old chicks is done. Beaudette spike protein can be substituted with spike protein of virulent M41 strain (Massachusetts serotype). This approach is known as reverse genetics approach. This challenge can be overcome using more advanced strategy of vaccination i.e., immunization with multi-epitope vaccines. Multiple epitopes are derived from one or more than one pathogenic microbe and constitute an epitope-based subunit vaccine.

KEYWORDS
Infectious laryngotracheitis (ILT), Tissue culture origin (TCO), Chicken embryo origin (CEO), Infectious Bronchitis (IB), Immunization, Multi-epitope vaccines

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INTRODUCTION

Infectious laryngotracheitis (ILT) is one of the most common diseases of viral origin in avian species that affects the chicken. Turkeys and pheasants are also susceptible to infection with ILT. This disease is associated with discharges from ocular and nasal passages, more precisely expectoration of blood in mucus, depression, dyspnea, and gasping. Severity of the lesions result in bronchitis, bronchopneumonia, pharyngitis with ulcers and syringitis. Sometimes, the confinement of lesions to nasal cavity, conjunctiva, and trachea with the involvement of mild exudates of mucoid or catarrhal nature. This is termed as the silent ILT, in which characteristic respiratory lesions of ILT are not present. Mortality due to ILT is associated with the age, vaccination history and individual immune status of the birds, and on the virulence of the strain of the virus. Histopathology is the method of choice for the ultimate diagnosis of ILT suspected case. However, diagnosis can also be done using tools like immunohistochemistry (IHC), which is used to detect the presence of ILT antigens in the cells (Carnaccini et al., 2022).

Infectious laryngotracheitis virus has the ability to create a carrier state in recovered birds. After seven days of acute illness, ILTV, like most herpes viruses, can potentially go into latency in the central nervous system's trigeminal ganglion. These viruses can be identified by tracheal organ culture and PCR analysis of live, recovered birds' ILTV DNA in the trigeminal ganglion. It has also been demonstrated that a few elements, including stress, onset of laying, and relocation can cause latent carrier birds to shed viruses (Kaur, 2021).

In 1931, a viral disease of respiratory and reproductive significance in young chicken flocks emerged in the United States which was termed as Infectious Bronchitis (IB). High mortality rates are observed in cases of infections with nephron-pathogenic strains of IB and resultant opportunistic bacterial infections. Diagnosis of IB can be made by recovering the infectious bronchitis virus (IBV) from lungs, windpipe, and kidneys of the sick young chickens. Antigens of IBV can be directly detected in pancreas and liver. Some other methods for the indication of IBV are Immunohistochemistry and Hemagglutination Inhibition. Most of the countries vaccinate the day-old chicks for the

protection against IB (Samad et al., 2021). Other serological methods employed for the efficient diagnosis of IBV infection are enzyme-linked immunosorbent assay (ELISA) and agar gel precipitation test (AGPT). Molecular techniques involve the use of restriction fragment length polymorphism (RFLP) and quantitative real-time polymerase chain reaction (qRT-PCR). Effective control of IB can be achieved by use of vaccination. However, vaccination cannot provide full protection, but it may lead to effective decline in the clinical signs of the disease. Vaccination strategies are made depending on the epidemiological data and genetic variations of infectious bronchitis virus (Legnardi et al., 2020).

Causative Agent

Infectious Laryngotracheitis

The etiologic agent of infectious laryngotracheitis (ILT) belongs to the family *Herpesviridae*, genus *Iltovirus,* and species *Gallid herpesvirus 1*. Infectious laryngotracheitis virus (ILTV) consists of a double stranded linear DNA genome of 150 kb. ILTV consists of an outer envelope, and it is sensitive to lypolytic solvents, chloroform, and heat. The virus maintains its viability at 13-23 °C for many days to months in tracheal exudates of chickens and carcasses of birds (Ou and Giambrone, 2012).

The surviving birds may carry this virus in their sensory neurons and the virus may reactivate to cause infection in young naive chickens. The ILTV has been studied with gG-deletion mutation, and when specific-pathogen-free (SPF) chicken flock was inoculated with gG-deletion mutants, it was then prevented from the clinical disease, following subsequent exposure with virulent strain of ILTV (Chen et al., 2011).

ILTV replicates in the tracheal mucosa and conjunctiva, leading to respiratory distress, inflammation and serous to mucoid discharge. In the case of lytic cycle of virus replication, it can maintain latency in trigeminal ganglion. Under stress conditions such as onset of lay, vaccination or shifting of birds, virus can reactivate itself. The cytopathic effect (CPE) due to ILTV is evident in case of the infection with vaccinal strains of the virus. Cell rounding, detachment and syncytium formation are the evident cytopathic effects. The ILTV stimulates an effective humoral and cell mediated immune response in the host chicken by potent immunogenic nature of glycoproteins of viral envelope (Gowthaman et al., 2020).

Infectious Bronchitis

Infectious bronchitis virus (IBV) belongs to the family *Coronaviridae,* genus *Gammacoronavirus and* species *Avian coronavirus*. The IBV has RNA genome, which is a single stranded, consists of 27 kb and has an envelope. The virus replication is affected by the two untranslated regions. Two open reading frames (ORFs) encode non-structural proteins (nsp). The autoproteolytic activity plays a key role in cleavage of the polyproteins into 15 non-structural proteins (nsps). The most widely studied protein of IBV is Spike (S) protein. The virus morphology is determined by Membrane (M) and Envelope (E) proteins (Legnardi et al., 2020).

Hemagglutination, and antigenic neutralization is determined by S1 subunit of S protein. Variations exhibited by IBV in pathotypes, and serotypes cannot be explained solely by the analysis of just S gene. Some accessory and non-structural proteins of coronaviruses including infectious bronchitis viruses, play a vital role in replication and immune evasion of viruses (Lin and Chen, 2017).

The Massachusetts 41 (M41) strain of IBV is involved in the etiology of highly contagious infection of respiratory tract in young chickens. The embryonated eggs and primary chicken embryonic kidney cells are the sites of replication for most clinically important strains of IBV. The IBV strain Beaudette can infect the cell cultures of baby hamster kidney (BHK-21) and other cell lines such as mammalian cell lines Vero (Promkuntod et al., 2013).

The gene sequence of S1 protein of the virus was determined for two different batches of the vaccines. Sequencing of S1 gene was also done for IBV isolated from vaccinated flock and the flock exposed to virus challenge (McKinley et al., 2008).

Immunization and Related Challenges

Infectious Laryngotracheitis

Strategies and Techniques

Live attenuated vaccines of tissue culture origin (TCO) are being used, which are produced by continuous passaging of virulent strains of virus in the cell cultures. The embryonated chicken eggs are also used to develop vaccines for ILT by passaging the virulent virus in embryonated eggs, known as the vaccines of chicken embryo origin (CEO). The novel strains of ILTV lead to widespread disease occurrence and these novel strains emerge because of spontaneous genetic recombination between vaccine strains of the virus. An effective control of ILT is done via a strategy that differentiates the wild-type strain from vaccine strain in any infected flock and the strategy is termed as differentiation between vaccinated and infected animals (DIVA). Whole genome sequencing of wild-type strains and vaccine strains of ILTV were compared by the advance techniques like next generation sequencing (Coppo et al., 2013).

Development of widespread clinical disease in commercial poultry flocks of Australia was because of the genetic recombination in the vaccine strains of ILTV. Thus, vaccine strains of ILTV revert to much stronger virulent strain causing significant clinical signs (Lee et al., 2012).

The comparison of two ILT vaccines of U.S. with Australian Serva vaccine was made to identify slight differences in the nucleotide sequence. The Illumina Genome Analyzer was used to sequence the genomes of vaccine strains of ILTV. The US vaccine strains are identical to Australian Serva strain with a slight difference in the sequences of nucleotides. Moreover, both vaccines are of chicken embryo origin (CEO) (Chandra et al., 2012).

Evaluation of Strategies for Vaccines

The CEO vaccines are proven to be effective and efficient in providing immunity to the clinical ILT infection. However, this type of vaccine leads to the development of virulent strain of the ILTV following consistent passages of the virus in the embryonated chicken eggs. Efficiency of vaccine is determined by the fact that it should limit the replication of virus in the trachea and maintain the weight gain when exposed to viral challenge. The live-attenuated vaccines and viral vector vaccines of ILT were compared for the level of immune response in broilers when challenged at 35 and 57 days (about 2 months) of age. The live-attenuated vaccines were incorporated via eye drop method with a full dose, while viral vector vaccines were incorporated *in-ovo* with a half dose. Evaluation of the protective immunity induced by vaccination is done by scoring of clinical signs at specific days after challenge. The Real-time polymerase chain reaction (RT-PCR) was used to quantify the ILTV present in the trachea of exposed birds. Analysis of blood samples was made before and after exposure to the virus for evaluation of the presence of antibodies in serum (Vagnozzi et al., 2012).

Recombinant Viral Vector Vaccines for ILT Prevention

The recombinant types of live vaccines are also available to be administered in poultry for protection against ILT. These vaccines are recommended to be administered *in-ovo* at hatchery or via subcutaneous route post-hatch. Secondary lymphoid organs of the chicken allow almost similar degree of replication of these viral vector vaccines, irrespective of the route of administration. One of the recombinant viral vector vaccines, licensed in Canada, uses fowlpox virus (rFPV) and two other vaccines use Herpes virus of turkey (rHVT) as vector (Barboza-Solis et al., 2021).

Trachea and the trigeminal ganglion are the sites of latency of ILTV. Many efforts had been made to prepare the genetically engineered vaccines for ILTV which could eliminate the chances of latency of ILTV. The commercially available recombinant viral vector vaccine of ILT is in combination with avian encephalomyelitis, by using fowlpox virus as a vector (FP-LT+AE). This vaccine was administered in leghorn pullet flocks and evaluation was done by challenging the flock with tracheal exposure of the ILTV, in collaboration with the National Veterinary Services Laboratory (Ames, IA). The consequences of this trial indicated that FP-LT+AE recombinant vaccine induced adequate immunity against ILT (Davison et al., 2006).

Specific pathogen free (SPF) and commercial broiler chickens were used to evaluate the recombinant turkey herpes virus vectored, infectious laryngotracheitis vaccine (rHVT-LT), following various protocols (amniotic route at embryonation day [ED] 18; intra-embryonic route at ED 19; and subcutaneous [SC] route at day 1 of bird). In one of three experiments, the chronological evaluation of rHVT-LT vaccine and its comparison with the replication of HVT in SPF chickens was done; in the second experiment, the replication of rHVT-LT vaccine was tested by *in-ovo* administration in SPF; and in the third experiment, the replication of rHVT-LT vaccine was tested by *in-ovo* administration in commercial broiler chickens having maternal antibody titers against ILT and HVT. The rHVT-LT vaccine replicated slower than HVT vaccine. However, both vaccines replicated to similar levels and detected at lower frequency in lungs with consistent detection in feather pulp and spleen. (Gimeno et al., 2011).

The recombinant ILTV was produced by deletion of thymidine kinase (TK) gene and insertion of a marker in the form of green fluorescent protein (GFP) gene in both virulent and low virulent strains of ILTV. The GFP gene was expressed in the chorioallantoic membrane of embryonated chicken eggs, leghorn male hepatoma (LMH) cell line and chicken kidney cells. The viral cytopathic effect (CPE) was produced by recombinant virus in chicken kidney cells and LMH cells. While the chorioallantoic membrane of embryonated chicken eggs was observed for the formation of pocks. It was observed that the rate of replication of recombinant virus was like the wild-type virus in chicken kidney cells. The recombinant ILTV showed a reduced virulence effect as compared to wild-type ILTV and induced immunity against virulent strain of ILTV in the specific pathogen free (SPF) chickens. Thus, these recombinants can be used as genetically engineered live vaccines for ILTV (Han et al., 2002).

Efficacy and Administration Methods of Vaccines

All the licensed vaccines for ILT in the United States, whether they are of tissue culture origin (TCO), chicken embryo origin (CEO) or recombinant, are live vaccines. Although recombinant vaccines are also widely used, but immunity induced by these recombinant vaccines is limited. The TCO and CEO live vaccines are administered to birds via oral route (in drinking water) or eye drop method, to make sure the contact of vaccine with the mucous membrane (epithelium) of trachea. A cell mediated immune response is initiated by the vaccine and this response prevents the latency of infection and replication of virus. Wing web puncture mode of administering the vaccine is used to deliver Fowl pox vector vaccines. On the other hand, the Herpesvirus of turkey (HVT) vector vaccines of ILT are administered via *in-ovo* route or post-hatch subcutaneous route. These recombinant vaccines of ILT are not potent enough to induce a strong cell mediated immune response (Koski et al., 2015).

Infectious Bronchitis

Controlling the spread of Infectious Bronchitis Virus (IBV) is a global challenge. The most efficient way to control IB in avian species is vaccination. Both live and killed type of vaccines are available worldwide for protection against IB. However, one of the challenging factors to the vaccination is the emergence of different variants (serotypes) of IBV. These variants exhibit no cross protection. Some studies indicated that new pathogenic serotypes of IB emerge because of the

administration of live attenuated vaccine in the birds. The commercially, live vaccines for IB are available to be administered at day 1 or during the first week of the age of bird via drinking water or coarse spray method. Virulent strains of IBV, for example, Dutch H52 and H120 strains, and Massachusetts-based M41 serotype are widely used in preparation of commercial vaccine for IB (Bande et al., 2015).

Optimizing Vaccine Strategies

During the preparation of vaccines, post vaccinal reactions should be kept in mind. Because live attenuated vaccines, after administration in young birds, can seriously damage the epithelium of trachea. Tracheal damage can trigger the development of secondary infections of bacterial origin. There is not much research on administering IB vaccine *in-ovo*. Because most serotypes of IBV are lethal to embryos and can cause death of the embryo. However, one of the groups has shown that *in-ovo* vaccination for IBV can successfully be done, even though, the strain is pathogenic. One of the advantages of *in-ovo* vaccination is that one can be sure that birds have received adequate and precise dosage of vaccine. It is observed that ciliated epithelium of trachea is not affected (damaged) by Beaudette strain of IBV (Massachusetts serotype). The Beaudette spike protein can be substituted with spike protein of virulent M41 strain (Massachusetts serotype). This approach is known as reverse genetics approach. This induced significant immune response in vaccinated flock against the virulent M41 strain (Tarpey et al., 2006).

Even though vaccination is consistently incorporated in commercial poultry for IBV, new variants of IBV are constantly emerging. These newly emerging variants can be used as a valuable tool for the vaccine development (Umar et al., 2016).

Combined Vaccination Approaches

The efficient control of IBV in poultry often involves the use of combination of killed and live vaccines depending on the challenging serotype prevailing in the field. The main reason for this approach is that currently available vaccines ensure little or no cross protection at all, and a combination of killed and live vaccines induce a wide range of protection against different strains. Moreover, the recombinant type of vaccines for IBV, having chimeric S gene, play a vital role in improving protection against the viral challenge, as confirmed by various means like antibody titers and cellular infiltration etc. (Ellis et al., 2018).

Regulatory Compliance in Vaccine Licensing

The manufacturers must follow the rules and regulations of a specific territory or country for licensing of vaccines. The regulations, instructed by the European Pharmacopoeia (PhEur) and the Food and Drug Administration (9CFR) in the USA, must be considered, and implemented on a priority basis. The PhEur guidelines consider either ciliostasis test to determine the damage to the ciliated tracheal epithelium or recovery of the virus from the swabs of trachea. On the other hand, 9CFR guidelines consider only viral recovery from tracheal swabs post-challenge (De Wit and Cook, 2014).

Emerging strategy: Epitope-Based Vaccines

The prevention of infectious bronchitis has become a challenge due to the emergence of new variants, genetic recombination, and pressure of the host selection. To cope with these challenges, new advancements have also emerged to control the spread of infectious bronchitis and many of the other viral infections of avian species. One of the advancements is the discovery of epitope-based vaccines. Designing a multi-epitope vaccine involves some crucial factors to be considered, such as screening and prediction of neutralizing B cell epitopes (functional) and T cell epitopes which are species restricted. The cleavage of IBV glycoprotein (S) is made into two subunits, 92-kDa S1 and 84-kDa S2 subunits. These subunits play a significant role in causing infection. The receptor binding domain (RBD) is present on S1 subunit, which plays a key role in the adsorption and entry of the virus into its host cell. The S1 subunit also possesses the epitopes of neutralizing antibodies. The experimental studies indicated that recombinant DNA vaccine, which consists of multi-epitope cassette of S1 protein of IBV, can stimulate a strong immune response against IBV exposure in chickens (Tan et al., 2019).

Multiple epitopes are derived from one or more than one pathogenic microbe and constitute an epitope-based subunit vaccine. These epitopes are composed of cytotoxic T cell epitopes, helper T cell epitopes and B cell epitopes. The B cell receptors (BCRs) on B-cells help in the recognition of thymus dependent antigens. These multiple epitope vaccines play an integral part in compensation of the shortcomings of classical vaccines including reversion of the vaccine virus to virulent form, autoimmune response, and allergies in the subjects (Lei et al., 2019).

The cytotoxic T lymphocytes (CTLs) are activated against virus infected cells by a combination of restricted major histocompatibility complex (MHC) class 1 molecules and the T cell epitopes. The receptor-binding sites of pathogens or antigens are recognized by antibodies (Abs). The T cells play a role in the co-stimulation of B cell epitopes which further activate the naïve B cells. The B cell epitopes mostly consist of discontinuous conformational epitopes but also comprise contiguous linear epitopes. The conformational epitopes of B cells usually produce neutralizing antibodies. This phenomenon provides cross protection against various serotypes of homologous viruses (Tan et al., 2016).

Future Perspectives

In spite of the development of different types of vaccines for the prevention of infectious viral diseases, there is constant emergence of variants of different viruses which are posing some serious threats to the susceptible host species.

Similarly, infectious laryngotracheitis virus (ILTV) and infectious bronchitis virus (IBV) are constantly emerging themselves and causing lethal infections in avian species. Keeping in view all current challenges, there is a need to develop and constantly apply strategies which will lead to the efficient prevention and control of ILT and IBV.

The nanoparticle based vaccines can be designed in such a way that this type of vaccine enhances the presentation of viral antigens to the cells of immune system. As a result of this type of enhanced presentation, there is efficient initiation and extension of immune response in the form of production of neutralizing antibodies and cytotoxic cells. In this way, nanoparticles can act as adjuvants. Nanoparticles can lead to the development of vaccines for multiple pathogens because nanoparticles can employ more than one antigens in a single formation. This type of enhancement is a key feature in the prevention and control of poultry infections of viral origin.

Another approach to innovative development of vaccines is Gene-edited vaccine. This technique holds promising future for the development of targeted antigen, thus leading to vaccine efficacy. Gene-editing technique like CRISPR-Cas is now being used in diagnosis of various infectious diseases and it promises a significant future in the development of vaccines for the prevention of various infectious diseases of avian species including ILT and IB.

Conclusions

Continuous challenge of infectious viral diseases is one of the emerging global problems. Avian species contract a lot of pathogens from their surroundings and can transmit these pathogens to adjacent birds, leading to a more lethal spread. Infectious laryngotracheitis (ILT) and infectious bronchitis (IB) are examples of the lethal viral infections of poultry. The sporadic outbreaks of ILT have been reported worldwide in poultry birds either because of direct contact of the healthy birds with diseased birds or via contact with the contaminated environment including fomites, feeding trays, drinkers etc. The ILTV undergoes continuous genetic variations thus leading to the emergence of new potentially more virulent strains, posing a serious threat to poultry health. The IBV has considerable genetic diversity and multiple serotypes and genotypes of IBV have been identified namely Massachusetts (Mass), 793B, and Arkansas (Ark) Variants. The strategic control of such infectious diseases must be done to meet the needs of modern day such as commercial products of avian origin.

The role of biosecurity cannot be nullified in preventing any type of incidence of infectious disease in poultry flocks. The biosecurity determines the overall health status and ultimate immunity of the flock; thus, biosecurity can be a key factor in determining the economy of any poultry flock. Vaccination is one of the promising phenomena to prevent the onset of such infectious diseases to limit their spread. The live virus vaccines are more commonly used as a preventive tool, which induce strong immune responses in hosts to cope with the challenges of wild type strains of virus. However, these live virus vaccines can revert to virulent forms and can cause serious disease in the hosts. Several advancements are being made in the live vaccines to limit their shortcomings and increase their utility and biosafety. Advance types of recombinant vaccines are being used for the control and prevention of ILT. The epitope based vaccines is one of the recent progresses in preparation of effective vaccines for the prevention of IBV.

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Chapter 31

Role of Vaccines and Immune Boosters in Preventing Coronaviridae: A Comprehensive Review

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ABSTRACT

Amidst the relentless battle against *Coronaviridae*, vaccines stand as our beacon of hope, illuminating a path towards an eventual triumph over the pandemic. Through tireless collaboration and scientific ingenuity, researchers have forged ahead, developing a diverse array of vaccines to combat COVID-19 and its variants. Yet, alongside these remarkable achievements, formidable challenges persist. Vaccine hesitancy, fueled by misinformation and cultural complexities, threatens to impede progress, underscoring the need for comprehensive strategies to foster trust and understanding. Moreover, the imperative of global cooperation looms large, demanding equitable vaccine distribution to ensure that no community is left behind in our shared pursuit of health and resilience. As we navigate these uncharted waters, let us draw inspiration from the resilience and compassion that define our collective humanity, forging ahead with determination and unity to overcome this unprecedented crisis.

KEYWORDS
COVID-19, Global cooperation, Humanity, Pandemic, Vaccine distribution.

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INTRODUCTION

The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has persisted since December 2019 (Zhang et al., 2020; Zhu et al., 2020), despite widespread vaccination efforts (Hein et al., 2022; Jackson et al., 2020). Coronaviruses belong to four genera—alpha, beta, gamma, and delta—with alpha and beta coronaviruses infecting mammals and gamma and delta primarily infecting birds, though some can also infect mammals (Trevisol et al., 2023). While some coronaviruses cause major pandemics or epidemics, others infect without obvious clinical signs. As of July 22, 2021, COVID-19 had resulted in over 192 million cases and 4.1 million deaths (Yap et al., 2021).

The late 20th century saw a groundbreaking shift in molecular biology, shedding light on microbiology and immunology, which deepened our understanding of how pathogens interact with the body and how the body responds to vaccines. Advances in molecular genetics and genome sequencing have empowered the creation of vaccines targeting RNA viruses, which harbour diverse epitopes. Examples include the live and inactivated influenza vaccines and live rotavirus vaccines (Rodrigues et al., 2020). This progress has led to the development of safe and effective vaccines against diseases that once posed significant threats to public health. Vaccination, alongside improvements in sanitation and access to clean water, stands as a cornerstone of global health, saving an estimated 6 million lives annually from preventable diseases (Rodrigues et al., 2020).

Positive-strand RNA viruses with an envelope that infect a broad range of mammals are known as coronaviridae. In the order Nidovirales, which is made up of enveloped, positive-strand RNA viruses, the Coronaviridae family is a member of the suborder Cornidovirineae, along with the Tornidovirineae family (Zmasek et al., 2022). The family is subdivided into six genera by the sub-families Coronavirinae and Torovirinae: Bafinivirus, Torovirus, Gammacoronavirus, Deltacoronavirus, and Alphacoronavirus (Phan et al., 2018). The nucleocapsid of the toroviruses is characterized by a helical, doughnut-shaped structure. Many animals are infected by members of the subfamily Coronavirinae; however, these infections are mostly moderate respiratory or intestinal (Payne and S., 2017).

The family Coronaviridae, suborder Cornidovirineae, order Nidovirales, realm Riboviria, and five genera and two subfamilies are recognized under the current categorization of coronaviruses, which consists of 39 species divided into 27 subgenera. A working group of the ICTV, the Coronaviridae Study Group (CSG), develops the taxonomy and family categorization (King and A. M, 2012). High pathogenicity is attributed to the coronaviruses that cause diseases like the severe acute respiratory syndrome (SARS) and the associated Middle East respiratory syndrome (MERS). The virus SARS-Cov-2, which is also a member of this family, is the source of the COVID-19, which the World Health Organization (WHO) designated to be a pandemic in March 2020 (Mavrodiev et al., 2020).

Vaccines have become a crucial defence against the pandemic, especially as other treatments have not been able to directly eradicate the virus. Traditionally, vaccine development has been a lengthy process spanning several years. However, Moderna Biotechnology, Inc. achieved a remarkable feat by producing the mRNA-1273 vaccine in just 42 days after the genetic sequence for SARS-CoV-2's spike protein was made public in January 2020 (Park et al., 2021). Vaccination stands as the most effective way to reduce the morbidity and mortality associated with infectious diseases. According to the WHO, vaccines save the lives of approximately 2.5 million children worldwide each year. These biological preparations enhance immunity against specific illnesses. Due to significant scientific advancements, over 70 vaccines now protect against more than 30 pathogens, with more expected in the future. This interconnected narrative provides a comprehensive view of the COVID-19 pandemic and the scientific advancements in vaccine development, emphasizing the crucial role of vaccines in public health and the ongoing efforts to combat infectious diseases (Donnelly, R. F., 2017).

Understanding Coronaviridae

Structure and Classification of Coronaviridae

The genomes of coronaviruses are single-stranded, positive-sense RNAs that range in size from 27,000 to 32,000 nucleotides (nt). The genome is generally arranged into five primary open reading frames (ORFs), each of which codes for a different set of structural proteins, including spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins, as well as many non-structural proteins for viral replication (ORF1a/1b) (Muzeniek et al., 2022).

Coronavirus 2 (SARS-CoV-2) is a positive RNA coronavirus that is single-stranded, enclosed, and a member of the betacoronavirus genus. The genome of this new virus is 29.9 kB and consists of 14 open frames that encode 29 proteins, 16 of which are non-structural proteins (nsps), 4 of which are structural proteins, including the envelope, membrane, spike, and capsid glycoproteins, and 9 of which are accessory proteins (Khreefa et al., 2023). The three main structural proteins of coronaviruses are the internal phosphorylated nucleocapsid protein (N), a unique transmembrane glycoprotein (M), and the extremely large (200K) glycoprotein S (for spike), which produces the bulky (15 to 20 nm) peplomers present in the viral envelope. Furthermore, there is a small transmembrane protein E (Burrell et al., 2016).

Four structural proteins are shared by all coronaviruses: an integral membrane glycoprotein (M; c. 250 amino acids); a small envelope protein (E; c. 100 amino acids, present in very small amounts in virions); a phosphorylated nucleocapsid protein (N; c. 500 amino acids). The hemagglutinin-esterase protein (HE; about 425 amino acids) is an extra structural glycoprotein present in several betacoronaviruses. This may have an impact on tropism in vivo and is not necessary for replication in vitro (Britton and P, 2019).

Common Strains and their Impact on Human Health

In the 1960s, scientists identified coronaviruses that could jump from animals to humans. Since then, several harmful coronaviruses affecting humans have been discovered. It all began in 2002 with the identification of SARS-CoV. Over time, seven human coronaviruses have been identified, with SARS-CoV-2 being the most recent. Among these, SARS-CoV, MERS-CoV, and SARS-CoV-2 are known to cause severe illness, while the others—229E, OC43, NL63, and HKU1—typically lead to milder infections (Malik and Y.A, 2020). These viruses belong to the delta, gamma, beta, and alpha genera of coronaviruses. In the alpha and beta genera alone, there are now seven coronaviruses known to infect humans. Common human coronaviruses like HKU1, OC43, 229E, and NL63 usually cause mild upper respiratory tract infections in both children and adults (Rajapakse et al., 2021).

SARS-CoV and SARS-CoV-2

The illness received the designation COVID-19 after the World Health Organization (WHO) proclaimed SARS-CoV-2 a worldwide pandemic on March 11, 2020. Industry, government, charity, non-governmental groups, and academics are working together to produce vaccines and other curative and preventive measures in response to this pandemic (Dagotto et al., 2020). Out of the three coronaviruses that have entered the human population in the last 20 years, the severe acute respiratory syndrome coronavirus (SARS-CoV) is the one that causes the most morbidity and mortality. SARS-CoV-2 is a potentially fatal respiratory disease that affects 1%–10% of the elderly population (Feng et al., 2022).

MERS-CoV

A new human disease linked to severe respiratory symptoms and kidney failure is the Middle East respiratory syndrome coronavirus, or MERS-CoV. Up to 536 laboratory-confirmed diseases and 145 fatalities have occurred since its debut in 2012 in at least 17 countries across Asia, Africa, Europe, and North America. The majority of these instances had their origins in the Arabian Peninsula or its surrounding nations, especially Saudi Arabia (Somarajan et al., 2014).

HCoV-OC43

In the 1960s, HCoV-OC43, a member of the beta-coronavirus genus, was initially identified from a patient suffering from a respiratory ailment. Furthermore, this virus is known to be the most common coronavirus in humans worldwide, with winter and spring being the peak incidence seasons. Notably, HCoV-OC43 is thought to be one of the most significant viruses causing upper respiratory infections and has been shown to be the most common subtype of HCoVs (Chen et al., 2022). It is commonly acknowledged that the intermediate hosts responsible for facilitating the transmission of HCoV-OC43 to humans were cattle and buffaloes (Forni et al., 2021). The human respiratory tract can be infected by HCoV-229E, OC43, NL63, and HKU1. These infections often result in mild to moderate sickness, but in children and people with comorbidities, severe symptoms including pneumonia have also been observed (Chen et al., 2022).

HCoV-HKU1

S protein with rod-shaped protrusions on the HCoV-HKU1 envelope selectively attaches to the human receptor, causing the infection, which was the cause of the first known cases of HCoV-HKU1 infection in the 1950s (Liu et al., 2022). Since HCoV-229E is a prevalent non-SARS HCoV, detectable antibodies are present in roughly 90% of persons. As previously mentioned, the majority of infections manifest as cough, sore throat, and nasal discharge. However, HCoV-229E infection has also sporadically been linked to lower respiratory tract infections in young children, the elderly, or people with impaired immune systems (Funk et al., 2012). It is hypothesized that, like SARS-CoV-2, the four endemic HCoVs have animal origins. However, they have been around for decades in humans, unlike SARS-CoV-2, and infection often causes non-life-threatening symptoms similar to the common cold (Sechan et al., 2022). In comparison to the SARS and MERS viruses, which are members of the same viral family (Coronaviridae), the SARS-CoV-2 is less pathogenic (Rabaan et al., 2020).

Common Strains in Animals

The oldest known coronavirus pandemic in animals stems from the avian coronavirus infectious bronchitis virus (IBV), dating back to 1931, which continues to afflict chicken populations despite widespread vaccination efforts using mainly live attenuated strains delivered via aerosol (Ardicli et al., 2022; Tucciarone et al., 2018). Both SARS-CoV-2 and IBV share similarities in their spherical pleomorphic electron microscopic appearance, size range, genome organization, and structural and non-structural proteins. IBV exhibits considerable genetic diversity, with seven genotypes and numerous recombinants arising from variations in the S1 gene sequence, responsible for major immunological traits (Rohaim et al., 2020; Valastro et al., 2016). Consequently, even slight amino acid changes (<5%) in the S1 protein of field strains can impact vaccine efficacy and cross-protection (Korath et al., 2022; Tizard and I. R., 2020).

As a result, vaccination-induced neutralizing antibodies targeting the S1 protein of each IBV genotype may not offer sufficient protection against infection by new genotypes and recombinants. Effective control of clinical IBV cases hinges on employing vaccine strains that closely match the circulating field strains. The coronavirus envelope features spike (S), membrane (M), and envelope (E) proteins on its surface, while the nucleocapsid (N) protein encases the viral RNA. Notably, a highly conserved segment between SARS-CoV-2 and IBV spike sequences lies within amino acids 807 to 830 (Ardicli et al., 2022).

Mechanisms of Transmission and Infection

The S glycoprotein mediates the viral particle's entrance processes, which include fusion and adhesion to the host cell membrane. The homotrimer S protein is made and placed into the virion's membrane in numerous copies, giving it a crown-like appearance (Jackson et al., 2022). There is mounting evidence that SARS-CoV-2 can spread through the air based on the transmission characteristics of cases that are currently being investigated. According to the WHO, the COVID-19 virus (SARS-CoV-2) is spread via large droplets, airborne particles, and surface contact. When a vulnerable person is near an infected individual, they can directly inhale the virus that the sick person is exhaling. This is known as droplet transmission. The inhalation of tiny aerosol particles exhaled by an infected individual ten or even hundreds of meters distant is known as "airborne transmission" (Zhao et al., 2022). Alveolar macrophages, vascular endothelial cells, and airway and alveolar epithelial cells are among the primary targets of viral entry for SARS-CoVs after they enter the host through the respiratory system (Harrison et al., 2020).

The human placenta functions as an immunological and physical barrier, keeping microbes from harming the developing semi-allogeneic fetus inside the mother's uterus. The two primary layers of the placental barrier are made up of syncytiotrophoblasts and stem-like, mononucleated cytotrophoblasts. Placental defense mechanisms can be compromised by immunocompromising microorganisms and high viral loads, which can facilitate fetal transmission (Gostomczyk et al., 2023). Rodents are the source of HCoV-OC43 and HCoV-HKU1, whereas bats are thought to be the source of HCoV-NL63, HCoV-229E, SARS-CoV, MERS-CoV, and SARS-CoV-2 (Guruprasad and L., 2021).

Vaccines: The Cornerstone of Prevention

Historical perspective: Development of vaccines against Coronaviridae

Since humans lack innate immunity to SARS-CoV-2, the development of vaccines and therapeutic treatments is necessary to control the present pandemic and inhibit COVID-19 from reemerging. To expedite the testing of vaccinations and treatment medicines, the WHO convened an international team in February 2020 to create animal models for COVID-19 (Muñoz-Fontela et al., 2020). The most significant development in significantly reducing the severity of the pandemic has been the development of vaccinations to stop the SARS-CoV-2 outbreak, particularly for the high-risk population of elderly people (Sánchez et al., 2023).

Types of Vaccines Available for Coronaviridae

The world has benefited greatly from the life-saving discovery of vaccines, which have assisted in the eradication and management of several infectious illnesses. The COVID-19 vaccine has the potential to dramatically reduce hospital stays and severe infections (Getahun et al., 2024). Researchers and pharmaceutical firms had to move quickly to produce vaccines utilizing either new or pre-existing technology due to the novel coronavirus's fast dissemination and high illness burden. Currently, three vaccines are widely used in the United States. Johnson and Johnson, Moderna, and Pfizer-BioNTech created these vaccines. mRNA vaccines that target the SARS-CoV-2 surface protein were produced by Pfizer-BioNTech and Moderna (Patel et al., 2022).

Different Designs of SARS-CoV-2 Vaccines

The concept behind inactivated vaccines is to offer the pathogen in a condition where its ability to cause disease has been lost. Cell lines that serve as a substrate for the generation of copious amounts of antigen are used for the viral culture (Strizova et al., 2021).

Inactivated Vaccines

Inactivated or live-attenuated organisms have frequently been used in traditional vaccines. A greater and longer-lasting immune response may be more likely with such a vaccination because of its resemblance to the natural illness. This is its main benefit. Vaccinations with mRNA: Made by Pfizer and Moderna, two of the vaccines with the earliest Phase III findings, are innovative messenger RNA (mRNA) vaccines. Initial findings from both suggested efficacies over 90%. By directly infusing mRNA encoding the SARS-CoV-2 spike protein into the host, they function (Soiza et al., 2021). An mRNA vaccine called BNT162b2 (Comirnaty) expresses a prefusion-stabilized spike protein (S protein) with substitutions K986P and V987P (S-2P protein). Adult recipients of the BNT162b2 vaccine develop both innate and adaptive immune responses. Th1 (Th1) polarized CD4 and CD8 T cells, which are antiviral and multifunctional, are generated (Xu et al., 2022).

Non-replicating Viral Vector Vaccine

Utilizing recombinant DNA from the SV40 virus, viral vectors were initially used in 1972. Based on adenoviruses, the majority of virally vectored candidates for non-replicating SARS-CoV-2 vaccines are found.

DNA Vaccines

The imbalance in the production of various immune cells is one of the factors contributing to the progression of the infection in COVID-19 illness; hence, while designing vaccines, care is taken to ensure that the intended immunological response is produced without causing this kind of imbalance. There are currently no licensed medications or vaccinations against hCoVs on the market. Prior studies mostly focused on SARS-CoV and MERS-CoV. These studies included the creation of inactivated virus vaccines, live-attenuated virus vaccines, viral vector vaccines, subunit vaccines, DNA vaccines, nanoparticle, and virus-like particle (VLP)-based vaccinations. Subunit vaccinations have fewer drawbacks than other vaccine forms, such as viral infection, worries about partial inactivation, virulence recovery, and potentially damaging immune responses. Instead, they are target-specific and may produce high-titer nAbs (Shi et al., 2021), as indicated in Table 1.

Efficacy and Safety Profiles of Approved Vaccines

Vaccine development typically involves many years of rigorous research, including extensive safety and effectiveness studies where vaccine recipients are compared to those who receive a placebo (Plotkin, S. A., 2021). Several COVID-19 vaccines have now been authorized or licensed for use in humans, with more nearing the completion of their clinical trials. However, simply having approved vaccines is not enough to control COVID-19 globally. They must also be mass-produced, affordable, and distributed worldwide to ensure everyone has access, and they need to be widely utilized in local communities (Wouters et al., 2021). Policymakers are now considering the implications of administering periodic or seasonal booster doses of COVID-19 vaccines to protect vulnerable individuals and reduce the healthcare and economic burdens. This comes as protection from SARS-CoV-2 infection may decrease over time following the standard two-dose vaccine schedule (Munro et al., 2021). Overall, COVID-19 vaccinations have proven to be highly effective and safe in the general population. However, the level of safety and effectiveness may vary between different groups depending on their risk of severe COVID-19 and SARS-CoV-2 infection, as well as the potential for adverse reactions after vaccination (Luxi et al., 2021).

Table 1: Role of Different Vaccines in Controlling Coronavirus

Vaccine Type	Description	Specific Role in Controlling Coronavirus	References
mRNA Vaccines	Include Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273). Utilize messenger RNA to instruct cells to produce viral antigens.	Generate strong immune responses, high efficacy in preventing COVID-19, and rapid adaptability to new variants.	(Thomas et al., 2021 and Polack et al., 2020)
Viral Vector Vaccines	Include Oxford-AstraZeneca (ChAdOx1) and Johnson and Johnson (Ad26.COV2.S). Use a modified virus to deliver genetic material.	Induce robust immune responses, single-dose options available, and good efficacy against severe disease and hospitalization.	(Voysey et al., 2021 and Sadoff et al., 2021)
Inactivated Vaccines	Include Sinopharm (BBIBP-CorV) and Sinovac (CoronaVac). Consist of virus particles that have been killed.	Elicit immune responses without causing disease, widely used in various countries, and have a good safety profile.	(Al-Jighefee et al., 2021 and Tanriover et al., 2021)
Protein Subunit Vaccines	Include Novavax (NVX-CoV2373). Contain harmless pieces of the virus (often protein) to stimulate an immune response.	High efficacy in preventing symptomatic COVID-19, well-tolerated, and strong protection against new variants	(Keech et al., 2020)
DNA Vaccines	Include ZyCoV-D. Use plasmid DNA to produce the SARS-CoV-2 spike protein.	Promising results in trials, easy storage and transport, and potential for rapid mass production.	(Azari-hamidian et al., 2023)
Live Attenuated Vaccines	Currently in development. Use a weakened form of the virus that causes the disease.	Potential for strong and long-lasting immunity, single-dose regimen, and stimulate both cellular and humoral responses.	(van Riel and de Wit, 2020)

Challenges in Vaccine Distribution and Acceptance

The diversity, complexity, and dynamism of human behavior define it. In order to carry out public health initiatives, such as vaccination campaigns, successfully, it is essential to comprehend human health-related behaviors and to possess adequate understanding of cultural and environmental factors. Considering the interconnected origins of religious, economic, and social forces, changing health-related behavior is extremely difficult and complicated (Ala'a et al., 2021). In the past, vaccines have shown to be a very successful tool for controlling epidemics. Nonetheless, in recent years, both Europe and the US have seen a rise in the anti-vaccine movement. The campaign against vaccinations, which promotes vaccine hesitancy, has become a major public health issue and is currently the biggest danger to world health. For instance, two prominent politicians in Pakistan increased vaccination hesitancy by expressing to the local population their opposition to the COVID-19 vaccine (Yin et al., 2021).

To achieve widespread immunity, it's crucial to develop and distribute safe and effective vaccines, ensuring they reach every corner of the globe. The demand for COVID-19 vaccine doses has never been higher (Yarlagadda et al., 2022). The emergence of new SARS-CoV-2 variants has underscored the need for innovative and adaptable diagnostic techniques to detect infections. However, these variations and the diverse symptoms displayed by infected individuals have made the development of rapid and sensitive diagnostic tools more challenging (Fernandes et al., 2022). The COVAX program, a collaborative effort between WHO, Gavi, and the Coalition for Epidemic Preparedness Innovations, aims to ensure fair distribution of COVID-19 vaccines worldwide, with a focus on supplying doses to West African nations. However, there are concerns that the poorest countries may lag behind this year due to the overwhelming demand for vaccines surpassing the available supply (Burki, T. K., 2021).

Immune Boosters and its Role in Combating Coronaviridae

COVID-19 has spread worldwide, instilling fear and panic since its emergence. The severity of the infection varies based on individual immune competence, which is influenced by daily activities (Michienzi et al., 2020). Nutritional deficiencies can compromise the immune system, increasing susceptibility to infections. Recent research suggests that nutritional supplementation, especially at doses higher than the recommended daily intake, could potentially reduce viral load and hospitalization among COVID-19 patients. Vitamins play a crucial role due to their antioxidant and immune-modulating properties. Some vitamins regulate gene expression in immune cells and support their maturation and differentiation. Vitamins C and E, for instance, act as potent antioxidants against free radicals. The body may experience a depletion of these essential nutrients during infections due to increased demand for immune activation energy, coupled with factors like stress, viral diseases, diabetes, and obesity, which directly affect nutrient status (Gombart et al., 2020; Mujahid et al., 2024).

Role of Vitamin A

Vitamin A plays a crucial role in the body's immune function, existing in three active forms: retinoic acid, retinol, and retinal. Because of its ability to bolster the immune system and fight infections, it's often called an "anti-infective" vitamin (Huang et al., 2018). Adequate intake of vitamin A is vital for a robust immune response, as deficiencies can weaken the

body's ability to fight off microbes (Kańtoch et al., 2002). Regular consumption of vitamin A has been linked to milder cases and lower infectivity rates of diseases like diarrhea, pneumonia, measles, malaria, and AIDS (Villamor et al., 2002).

Studies have shown that both natural and synthetic forms of vitamin A, known as retinoids, can protect against various viruses, including hepatitis B, influenza, norovirus, measles, and cytomegalovirus (Trottier et al., 2008; Lee et al., 2018; Li et al., 2018). In animals, vitamin A deficiency reduces the effectiveness of coronavirus vaccines and increases susceptibility to infections (Jee et al., 2013; Stachowska et al., 2020). For example, chickens lacking in vitamin A are more prone to infections similar to coronaviruses (Junaid et al., 2020).

The protective mechanism of vitamin A against pathogens involves its ability to enhance specific components of the body's innate immunity, which helps fight off viral infections (Trottier et al., 2009; Katze et al., 2008). Research by Yuan et al. highlighted the significant impact of Am580, an agonist of RAR-α, in combating MERS-CoV and SARS-CoV by disrupting lipogenic pathways regulated by SREBP (Yuan et al., 2019). Both SARS-CoV and MERS-CoV can suppress antiviral responses mediated by IFN-I, potentially delaying treatments (Yang et al., 2015; Hu et al., 2017). However, retinoids have been found to enhance IFN-I signaling, suggesting potential for combined therapies in both animal and cell models (Gudas, L. J, 2012).

Role of Vitamin B Complex

Vitamin B is essential for regulating the body's inflammatory response (Morris et al., 2010), serving as crucial cofactors in cellular reactions. These vitamins, including B2, B3, B6, and B12, help synthesize amino acids, the building blocks of antibodies and cytokines, and support the proliferation and maturation of lymphocytes, key players in the initial immune response (Maggini et al., 2018). Since B vitamins are water-soluble, they primarily function as coenzymes in vital bodily processes, each with its unique role in supporting immune function against infections (Zhang et al., 2018).

For example, vitamin B2, or riboflavin, is crucial for cellular energy metabolism (Spinas et al., 2015). Studies have suggested that both UV light and vitamin B2 can help reduce the levels of MERS-CoV in the body (Bashandy et al., 2018). Additionally, research indicates that vitamin B12 may inhibit the RNA-dependent RNA polymerase activity of the SARS-CoV-2 virus, which is essential for viral replication (Wu et al., 2020; Narayanan et al., 2020). Similarly, the active form of vitamin B6, pyridoxal 50-phosphate, has been found to inhibit the SARS-helicase enzyme, thus hampering viral replication (Tanner et al., 2005). Furthermore, vitamins B6, B12, and B9 (folic acid) have been shown to enhance natural killer cell activity, providing important antiviral defence. These findings suggest that B vitamins hold promise in mitigating complications related to COVID-19 infection (Tan et al., 2020).

Role of Vitamin C

Vitamin C is renowned for its antiviral properties, including its ability to boost interferon-alpha production, modulate cytokines, reduce inflammation, improve endothelial function, and restore mitochondrial function (Carr et al., 2017; Dey et al., 2018). Linus Pauling, a Nobel Prize winner, highlighted the beneficial effects of vitamin C on the common cold in the 1930s and 1970s (Heikkinen et al., 2003). Vitamin C supports the immune system in combating both bacterial and viral infections by promoting cell turnover and eliminating dead cells (Carr et al., 2017). Its antioxidant properties shield against oxidative stress-induced damage, with studies indicating enhanced resistance against avian coronavirus infections in chick embryos supplemented with vitamin C (Junaid et al., 2020). Additionally, vitamin C exhibits antihistamine effects, alleviating flu-like symptoms such as runny nose, congestion, sneezing, and inflamed sinuses (Field et al., 2002). Recent research suggests vitamin C consumption for managing lower respiratory tract infections, with supplementation emerging as a compelling therapeutic approach for COVID-19 (Matthay et al., 2020; Zhang et al., 2020). In the immune response against infections, vitamin C primarily functions as a potent antioxidant, serving as a cofactor for various enzymes involved in biosynthesis and gene regulation processes (Wintergerst et al., 2006).

Role of Vitamin D

Vitamin D serves a crucial role in the body as both a nutrient and a hormone, primarily synthesized in response to sunlight to support bone health. However, it also plays a critical role in immune function by promoting the growth and maturity of various cells, including immune cells (Baeke et al., 2010). Lack of sunlight exposure, especially common among older individuals who spend more time indoors, can lead to vitamin D deficiency (Holick and M. F, 2004), which is often seen in many middle-aged and older individuals with COVID-19. In calves, vitamin D deficiency is associated with increased susceptibility to bovine coronavirus infection (Nonnecke et al., 2014).

Recent studies highlight vitamin D's potent immunomodulatory effects in combating influenza and COVID-19. It's suggested that supplementation with oral vitamin D doses, along with other micronutrients, can strengthen the immune system against COVID-19 (Grant et al., 2020; Ebadi et al., 2020). Observational research further emphasizes a link between COVID-19 severity and serum levels of 25-hydroxyvitamin D, suggesting that maintaining adequate levels of vitamin D may help mitigate the severity of the disease (Panagiotou et al., 2020; Ahmad et al., 2024).

Role of Vitamin E

Vitamin E, a fat-soluble nutrient, plays a vital role in supporting the immune system by acting as a potent antioxidant. It helps mitigate oxidative stress by scavenging free radicals, which can damage cells (Lee and Han ., 2018 and Galmés et al., 2018). Research has shown that vitamin E deficiency can worsen myocardial injury caused by RNA viral infections like

coxsackievirus B3 in mice, as these viruses become more harmful under oxidative stress (Junaid et al., 2020). Similarly, calves deficient in vitamin E face an increased risk of bovine coronavirus infection (Nonnecke et al., 2014).

Vitamin E's antioxidant properties protect cell membranes from damage caused by free radicals. It also helps enhance the production of natural killer cells and interleukins, which are essential for immune function. These factors contribute to lymphocyte proliferation and a strong immune response against pathogens. These findings suggest that vitamin E may have therapeutic potential in combating COVID-19 (Gombart et al., 2020).

Role of Vitamin K

Vitamin K, a fat-soluble nutrient from the 2-methyl-1,4-naphthoquinone family, is found naturally in certain foods and is also available as a dietary supplement in two forms: K1 (phylloquinone) and K2 (comprising various menaquinones, MKs) (Hubicka et al., 2020). It acts as a co-factor and co-enzyme, playing a crucial role in hemostasis by synthesizing proteins and supporting various physiological functions (Janssen et al., 2020). During times of insufficient hepatic vitamin K, the body prioritizes coagulation factors over non-hepatic ones. Matrix Gla protein (MGP), a vitamin K-dependent protein, helps inhibit soft tissue mineralization and elastic fiber degradation. In patients with SARS-CoV-2, an increase in pulmonary MGP synthesis protects the extracellular matrix from inflammation-induced degradation, utilizing vitamin K from non-hepatic stores (McCann et al., 2009).

COVID-19 can lead to venous and arterial thromboembolic complications due to severe inflammation, hypoxia, immobilization, and disseminated intravascular coagulation (DIC). It may also induce blood clot formation and elastic fiber degradation in the lungs. Vitamin K1, responsible for activating hepatic coagulation factors, helps combat thrombotic complications in COVID-19 patients (Klok et al., 2020).

Role of Minerals

Sodium

Sodium plays a vital role in maintaining electrolyte balance and affects the expression of ACE2 in SARS-CoV-2 (Luo et al., 2020). Studies have shown that COVID-19 patients often have lower sodium levels, with meta-analyses confirming this trend. For instance, a study in the US found that the serum sodium concentration of COVID-19 patients was lower than normal, with a mean of 136.0 mmol/L compared to the usual level of 138.0 mmol/L (Habib et al., 2020). Moreover, research suggests that sodium levels tend to decrease as the severity of the disease increases, indicating that low sodium levels, or hyponatremia, could potentially serve as a biomarker for SARS-CoV-2 infection (Lippi et al., 2020).

Potassium

Low potassium levels, known as hypokalemia, can increase the risk of complications like acute respiratory distress syndrome (ARDS) and acute cardiac injury, which are common in COVID-19. Research suggests that SARS-CoV-2 binds to ACE2 receptors, leading to reduced expression. This decrease in ACE2 receptors results in higher levels of angiotensin-II, which contributes to hypokalemia (Alwaqfi et al., 2020). COVID-19 patients often have elevated plasma concentrations of angiotensin-II, which may lead to acute lung injury, similar to what is observed in animal models of SARS-CoV infection (Zemlin and Wiese, 2020).

Calcium

Calcium isn't just essential for bone health; it also helps fight viral infections by aiding in the expulsion of viruses from cells, offering protection against the common cold. Studies combining data show that critically ill COVID-19 patients often have lower calcium levels compared to those with milder forms of the disease. This suggests a link between serum calcium levels and disease severity. Similar to low sodium and potassium levels, low calcium levels, or hypocalcemia, could potentially indicate the severity of SARS-CoV-2 infection (Rodriguez-Morales et al., 2020).

Phosphorous

Phosphorus is crucial for synthesizing proteins needed for cell growth, maintenance, and repair (Vance, 2011). A retrospective analysis of clinical data from COVID-19 patients found lower phosphorus levels, indicating hypophosphatemia. This suggests a direct link between low phosphorus levels and the severity of COVID-19. Monitoring serum phosphorus levels in severe or critical COVID-19 cases could be important for predicting outcomes (Xue et al., 2020).

Magnesium

Magnesium, often underestimated, could be beneficial in handling the stress caused by the pandemic and the post-traumatic stress disorder affecting COVID-19 survivors, healthcare workers, and the general population. Furthermore, it plays a critical role in immune function by regulating various processes, such as immune cell adherence, immunoglobulin synthesis, Immunoglobulin M (IgM) lymphocyte binding, antibody-dependent cytolysis, and macrophage response to lymphokines (Ni et al., 2020).

Zinc

Several studies suggest that zinc possesses antiviral properties and plays a crucial role in immunity. It has been recognized for its ability to enhance immunity against H1N1 influenza (Sandstead et al., 2010). Similar to SARS-CoV,

the pathogenesis of SARS-CoV-2 relies on angiotensin-converting enzyme 2 (ACE2) for virus entry into host cells. Therefore, ACE2 is considered a promising target for COVID-19 treatment (Zhang et al., 2020). Evidence indicates that Zn2+ treatment leads to a decrease in ACE2 activity in rat lungs. Additionally, in vitro studies show that Zn2+ ions can inhibit SARS-coronavirus RNA polymerase, thereby suppressing viral replication (Te Velthuis et al., 2010), as summarized in Table 2.

Table 2: Role of immune boosters in combating Coronavirus

Immune booster	Role in Combating Coronavirus	Reference
Vit: A	Enhances immune function, supports innate immunity, and aids in the combat against viral infections like MERS-CoV and SARS-CoV by enhancing IFN-I signaling and disrupting lipogenic pathways.	(Huang et al., 2018 and Yuan et al., 2019)
Vit: B (complex)	Regulates inflammatory response, supports synthesis of antibodies and cytokines, and inhibits viral replication. Examples include B2 reducing MERS-CoV levels and B12 inhibiting SARS-CoV-2 RNA polymerase activity.	(Tan et al., 2020)
Vit: C	Boosts interferon-alpha production, modulates cytokines, reduces inflammation, improves endothelial function, and acts as an antioxidant, with potential therapeutic use in COVID-19.	(Shakoor et al., 2021)
Vit: D	Enhances immune cell growth and maturity, modulates immune responses, and is linked to reduced severity of COVID-19 infections through its immunomodulatory effects.	(Grant et al., 2020)
Vit: E	Acts as a potent antioxidant, supports immune cell proliferation, and enhances natural killer cell activity, potentially reducing the severity of COVID-19.	(Lee and Han, 2018)
Vit: K	Supports coagulation and prevents thrombotic complications in COVID-19 patients by maintaining hemostasis and protecting the extracellular matrix.	(Janssen et al., 2021)
Minerals	Sodium, Potassium, Calcium, Phosphorous, Magnesium, and Zinc play vital roles in maintaining electrolyte balance, reducing viral replication, and supporting immune functions. Sodium and potassium levels are indicators of disease severity.	(Martha et al., 2021 and Lippi et al., 2020)

Future Perspectives

Looking ahead, the future of vaccine research for Coronaviridae is promising, with several areas showing potential for improvement and innovation. One emerging trend is the development of next-generation vaccines that incorporate novel delivery systems and antigen design strategies to enhance efficacy and durability. For instance, researchers are exploring the use of self-amplifying RNA (saRNA) vaccines, which have the advantage of inducing stronger immune responses compared to conventional mRNA vaccines (Pardi et al., 2018). Additionally, advancements in structural biology and computational modeling are facilitating the design of more precisely targeted vaccines that can effectively neutralize diverse strains of coronaviruses, thereby reducing the risk of viral escape and future pandemics (Schäfer et al., 2016). Furthermore, the integration of adjuvants and immunomodulators into vaccine formulations holds promise for enhancing vaccine potency and duration of protection. These innovative approaches signify a shift towards more tailored and adaptable vaccine strategies that can better address the challenges posed by emerging infectious diseases like COVID-19 (Reed et al., 2013).

In addressing vaccine hesitancy and misinformation, a multifaceted approach is necessary to build trust, increase vaccine acceptance, and combat false narratives. Firstly, robust public health communication campaigns should be implemented to disseminate accurate information about vaccines, emphasizing their safety, efficacy, and importance in controlling infectious diseases (Betsch et al., 2018). Leveraging social media platforms and community influencers can help reach diverse populations and counteract misinformation spread online (Chou et al., 2018). Additionally, engaging with local communities and addressing their concerns through transparent and empathetic dialogue can help alleviate fears and build confidence in vaccination efforts (Gagneur et al., 2018). Furthermore, promoting vaccine literacy and critical thinking skills can empower individuals to discern credible sources of information and make informed decisions about their health. By fostering a culture of trust, transparency, and collaboration, stakeholders can work together to overcome vaccine hesitancy and ensure widespread uptake of vaccines, thereby contributing to global efforts to control infectious diseases (Kata, 2012).

Conclusion

In conclusion, the journey towards combating Coronaviridae through vaccines has been one of innovation, collaboration, and resilience. From the rapid development of vaccines to the ongoing efforts to address vaccine hesitancy and ensure equitable distribution, humanity has demonstrated its capacity to adapt and overcome even the most daunting challenges. As we look to the future, it is clear that continued investment in vaccine research, coupled with comprehensive strategies to promote vaccine acceptance and global cooperation, will be essential in safeguarding public health and preventing future pandemics. Together, by harnessing the power of science, empathy, and collective action, we can build a safer, healthier world for generations to come

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Chapter 32

Role of Vaccines in Controlling COVID-19 Pandemic

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ABSTRACT

This chapter thoroughly examines the critical role vaccines have played in the global effort to combat the unprecedented challenges posed by the SARS-CoV-2 virus. The chapter provides insights into the multifaceted impact of vaccination strategies on curbing transmission, reducing morbidity and mortality, and restoring societal normalcy by conducting an in-depth analysis of vaccine development, efficacy, distribution, and societal implications. It investigates the scientific underpinnings of vaccine development, encompassing diverse platforms such as mRNA, viral vector, protein subunit, and inactivated virus vaccines. It highlights the collaborative efforts of researchers, industry, and regulatory agencies to achieve accelerated timelines without compromising safety or efficacy. Furthermore, the chapter investigates the real-world effectiveness of COVID-19 vaccines in conferring immunity against symptomatic illness, severe disease, and transmission, as well as the challenges posed by emerging variants and vaccine hesitancy. It examines the complexities of vaccine distribution and deployment, emphasizing the importance of global equity, resource mobilization, and novel delivery mechanisms to ensure equitable access for all populations. Furthermore, it also investigates the broader societal implications of COVID-19 vaccination, such as ethical concerns, public health messaging, and the restoration of trust in science and public institutions. In conclusion, the chapter summarizes key insights and future perspectives, emphasizing vaccines' indispensable role as a cornerstone strategy in navigating the complexities of the ongoing pandemic and fostering global recovery and resilience.

KEYWORDS
COVID-19, Chemical Drugs, Control, Alternatives, Vaccine, prevention

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INTRODUCTION

The COVID-19 pandemic began in late 2019 when a new coronavirus, later named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), emerged in Wuhan, China. The disease (called coronavirus disease COVID-19), spread rapidly around the world, resulting in millions of confirmed cases and hundreds of thousands of deaths. The World Health Organization (WHO) officially declared COVID-19 a pandemic on March 11, 2020 (Baloch et al., 2020). The exact origin of SARS-CoV-2 remains unknown, with two main hypotheses: zoonotic transmission from animals to humans or a laboratory leak. The virus may have spread from bats to an intermediary species, possibly sold at Wuhan's Huanan Seafood Market, before infecting humans. SARS-CoV-2 spread quickly after being introduced into the human population, reaching more than 194 countries by mid-2022. The virus's high transmissibility and ease of person-to-person spread aided its rapid spread (Shaikh et al., 2020). SARS-CoV-2 enters cells primarily by binding to the ACE2 receptor. Once inside the cell, the virus uses the cell machinery to replicate itself, eventually killing the cell and releasing new viral particles. Symptoms range from mild to severe, with common ones being fever, cough, fatigue, shortness of breath and loss of smell or taste (Hao et al., 2022).

Several pneumonia cases were discovered in Wuhan, China, in November 2019. These were the first COVID-19 cases associated with the novel beta-coronavirus SARS-CoV-2. The genetic information became public on January 10, 2020, 54 days after the first case was reported. The first human vaccine doses were tested on March 13, 2020, 63 days after the SARS-CoV-2 sequence was published. By September 24, 2020, the SARS-CoV-2 vaccine landscape had 43 clinical trial

candidates and over 200 candidates (Zhou et al., 2019). The virus quickly spread around the globe. Many countries acted too late to implement preventive measures, resulting in a sudden increase in cases globally (Shereen et al., 2020). The virus SARS-CoV-2 was discovered to be phylogenetically similar to other bat-derived coronaviruses such as SARS-Cov-1 and MERS-CoV, confirming that bats are the virus's primary reservoir; however, the intermediate source of origin and transmission to humans is unknown (Chavez et al., 2021).

As of February 5, 2021, the SARS-CoV-2 virus had infected more than 105 million people and killed over 2.29 million people worldwide. As of February 5, 2021, the United States had over 26.7 million cases and 456,000 deaths, followed by India, which had over 10.8 million cases and 155,000 deaths (Shadin et al., 2021). COVID-19 has hurt people's health, lifestyles, and the global economy (Garcia and Cerda 2020). An extensive search for an effective drug against SARS-CoV-2 has not yielded any promising results. Hydroxychloroquine and Remdesivir have been advocated as desperate measures based on conflicting and inconclusive studies and have failed to combat the pandemic (Zhao et al., 2020). As the number of patients with COVID-19 increases, the detection, assessment and interpretation of the immune response to SARS-CoV-2 infection becomes more critical. More vaccine candidates are being developed; however, safe and effective COVID-19 vaccines are urgently needed to combat the increasing number of cases and deaths worldwide. These candidate vaccines must be developed quickly and available to all countries and populations affected by the pandemic. Vaccines can reduce disease incidence, prevent transmission, and reduce the social and economic impact of disease (Khuroo, 2020).

The virus enters cells primarily through the angiotensin-converting enzyme 2 (ACE2) receptor, causing symptoms ranging from mild to severe (Moghadas et al., 2021). Preventive measures such as vaccination, face coverings, social distancing, hand hygiene, and quarantine have been critical in limiting the virus's spread (Hafeez et al. 2020). On March 11, 2020, the World Health Organization declared COVID-19 a pandemic, affecting more than 194 countries (Bhatia and Abraham, 2021).

Significance of Vaccination in Controlling the Spread of the Virus

Vaccination has played a very vital role in reducing the extent that COVID-19 has reached by decreasing disease burden, hospitalization, and deaths. Vaccines offer personal protection and help achieve herd immunity, which is important in breaking the chains of transmission within communities (Baba et al., 2023). Indeed, studies have shown that vaccination significantly decreased the severity of illness and adverse outcomes, especially in vulnerable populations. Even though vaccines possess partial protectiveness against infection, their effectiveness is very high in preventing severe diseases and consequences (Moghadas et al., 2020).

Vaccination plays a major role in COVID-19 outbreaks, wherein vaccines are major preventive measures aimed at preventing further outbreaks. Indeed, vaccination is a mandate for developing immunity at the population level so that the virus does not spread on large scale (Okell et al., 2020). Vaccination alone will be inadequate but must be experienced with continued adherence to the NPIs of masking, hand hygiene, testing, contact tracing, and isolation of cases for their optimal effect (Hogan and Pardi 2022). The only way for a pandemic to be effectively controlled is through vaccination coupled with following the public health measures put in place. In other words, vaccination campaigns need a strong system of distribution and public health strategy for far-reaching, equitable vaccine access. It entails public trust, good communication, and sound continuous surveillance to monitor over time the effectiveness of the vaccines and to respond quickly and efficiently to emerging matters of concerns and hesitancy (Machado et al., 2022).

COVID-19 Vaccine Development

mRNA vaccine research was initiated in the early 1990s, with the use of altered mRNA, which can escape immune responses, realized in 2005 (Wu, 2020). A wealth of data has been obtained from decades of research on coronaviruses, HIV, and other viruses, guiding vaccine design strategies and blueprinting techniques for fabrication (Szabó et al., 2022). As the COVID-19 pandemic unfolded in late 2019, governments, organizations, and the private sector made a huge investment in research and development on vaccines. Previous knowledge in the field of vaccinology, coupled with new methodologies, facilitated the fast pace in which candidate vaccine development proceeded (Kuter et al., 2021). These investments led to the approval of several COVID-19 vaccines, facilitated by effective collaborations between academic institutions, pharmaceutical companies, and governments. Prominent among them are the Pfizer-BioNTech and Moderna vaccines, made with cutting-edge mRNA technology. With more accelerated processes and increased collaboration between regulators and developers, clinical trials of the COVID-19 vaccines have been conducted in record time. The U.S. Food and Drug Administration (FDA) authorized the use of two mRNA vaccines, Moderna and Pfizer-BioNTech, for emergency use (Le *et al*., 2020).

Types of COVID-19 Vaccines (e.g., mRNA, Viral Vector, Protein Subunit)

Certainly! COVID-19 vaccines are broadly classified into several types based on the technology used to create them. There are three major types: mRNA vaccines, viral vector vaccines, and protein subunit vaccines.

Vaccine Efficacy and Effectiveness

Two key parameters, vaccine efficacy, and effectiveness show an understanding of how well COVID-19 vaccines work in real-world settings in the prevention of several health outcomes (Evans and Jewell 2021). The CDC conducts observational

studies to assess VE related to infection (Rosenberg et al., 2022). According to recent studies, the mRNA vaccines developed against COVID-19 were found to be effective against severe illness and death during the time of the Omicron variant. At the same time, the effectiveness of the vaccines is likely to wane over time, particularly following the primary series, which therefore underlines the importance of booster doses in protection against this disease (Link-Gelles et al., 2023).

Table 1: Types of COVID-19 Vaccines with Mechanisms and Advantages

Sr. #	Vaccine type	Examples	Mechanism	Advantage	Reference
1.	Protein subunit vaccines.	Novavax (Nuvaxovid), Sanofi-GSK.	Use harmless pieces of the virus (e.g. spike protein).	Traditional technology, standard refrigeration.	(Marchese et al., 2022).
2.	mRNA vaccines	Pfizer-BioNTech (Comirnaty), Moderna (Spikevax).	mRNA is used for instructing cells to produce spike protein.	Rapid development, no live virus used, modifiable.	(Hermosilla et al., 2023).
3.	Viral vector vaccines.	Oxford-AstraZeneca, J and J's Janssen, Sputnik V.	Modified adenovirus delivers genetic material for spike protein.	1 dose option, robust immune response.	(Vanaparthy et al., 2021).

Long-term studies have shown that effectiveness against infections and hospitalization is reduced over time. This may be due to new variants emerging. Monitoring of effectiveness is important in guiding policy decisions on timing—for example, booster doses (Wu et al., 2023). Comparing COVID-19 vaccines, an mRNA vaccine from each of Pfizer-BioNTech and Moderna showed very high efficacy against symptomatic disease. The primary series, however, is inducible in protection that wanes over time, underscoring the need for updated vaccines and booster doses in order to keep up immunity against severe outcomes (Fiolet et al., 2022).

Table 2: Efficiency and Neutralization Activity of Major COVID-19 Vaccines against Variants

Sr. #	Vaccine Name	Efficiency against variants (%)	Neutralization Activity	Reference
1.	Pfizer-BioNTech	High (95%)	Effective	(Creecy *et al*., 2024).
2.	Moderna	Strong (94%)	Sustained	(Moghadas *et al*., 2020).
3.	AstraZeneca	Varied (82%)	Moderate	(Machado *et al*., 2022).
4.	Johnson and Johnson	Varies (71%)	Varies	(Livingston *et al*., 2021).

Efficacy and effectiveness of COVID-19 Vaccines

Efficiency and effectiveness are related but different concepts that describe the performance of COVID-19 vaccines (Olliaro et al., 2021). Efficacy refers to a vaccine's ability to prevent disease in controlled trial settings, with success typically measured in terms of symptomatic infection, severe disease, and mortality rates when compared to placebo groups (He et al., 2022). In contrast, effectiveness describes a vaccine's actual impact in real-world scenarios, where variables such as vaccine distribution, compliance, and emerging strains influence the outcome (Link-Gelles, 2023). Extensive analyses revealed that COVID-19 vaccines had varying levels of efficacy and effectiveness:

The overall efficacy was reported to be approximately 66.4%, 93.6%, and 79.7% against SARS-Cov-2 infection, severe COVID-19, and symptomatic COVID-19, respectively. One-dose booster immunization demonstrated 74.5% efficacy in preventing COVID-19 caused by the Delta variant (He et al., 2022). Pfizer-BioNTech and Moderna vaccines had higher efficacy rates, with 91.2% and 98.1%, respectively (Soheili et al., 2023). These findings suggest that COVID-19 vaccines are highly protective against severe diseases and mortality, even if they do not completely prevent asymptomatic infections (He et al., 2022).

Variant Impact and Boosters

As new SARS-CoV-2 variants emerge, vaccine efficacy may decrease over time. For example, the Delta variant posed a problem to the existing vaccines because protection levels were down and booster shots had to be encouraged to restore the protection levels (He et al., 2022). Continued monitoring of the effectiveness of the vaccines allows public health agencies to adjust policies for the best possible protection of vulnerable populations.

Longitudinal Perspective

Longitudinal vaccine efficacy monitoring brings out the trend of protection over time. Several recent studies do point to the fact that effectiveness against infection and hospitalization may wane with time given the emergence of new variants, although the evidence is still accumulating (Evans and Jewell 2021).

Variants of Concern and Impact on Vaccine Efficacy

The discovery of new SARS-CoV-2 variants has opened up a wide avenue of concern about how they are going to impact the effectiveness and efficiency of COVID-19 vaccines. From studies, it is indicated that various variants impact vaccine performance with regard to preventing infection, symptomatic illness, or serious consequences. COVID-19 vaccines have different efficacies against different variants. For instance, it was established that the first dose vaccination attained the highest overall effectiveness against the Gamma variant; on the other hand, effectiveness against all variants stood at

96% overall after the second dose. After the first dose, the efficacy against the Alpha variant was considerably higher than in the case of the other variants studied by Szabó et al. (2022).

This, therefore, tracked the efficacy of the vaccines over time, showing us the trend in protection levels. In connection, studies are showing that against infections and hospitalization, the efficacy of the vaccines may actually wane with time. This may be due to the variants that are now coming up. The message, therefore, is that there has to be continuous evaluation; maybe booster doses should be taken for protection. Recent studies have assessed the estimated vaccine effectiveness during the BA.5 and Omicron BA.4 periods. The findings indicate that vaccine effectiveness in protecting against medically attended COVID-19 illness decreased with increasing time since the last dose; however, estimated effectiveness was higher after receiving booster doses compared to a primary series alone (Hafeez et al. 2020).

Common and Rare Adverse Reactions Associated with COVID-19 Vaccination

Common side effects from COVID-19 vaccination include pain, redness, and swelling at the injection site. Fever, fatigue, headache, muscle pain, chills, nausea and joint pain. The majority of these side effects are mild and temporary, resolving in a few days without intervention (Mohseni Afshar et al., 2022).

Rare but notable adverse reactions to COVID-19 vaccination include anaphylaxis, Thrombosis with thrombocytopenia syndrome (TTS), Myocarditis and pericarditis, Vasculitis, hearing loss and tinnitus, and Immune-mediated disorders.

Anaphylaxis which occurs in less than 0.0003% of recipients. Symptoms include difficulty breathing, hives and a fast heartbeat. The treatment requires immediate medical attention. Thrombosis with thrombocytopenia syndrome (TTS), a rare blood clotting disorder associated with the JandJ/Janssen vaccine, affects approximately 3.8 cases per million vaccinated individuals (Yaamika et al., 2023). Myocarditis and pericarditis (inflammation of the heart muscle or sac surrounding the heart) are most commonly seen in young males following mRNA vaccination. Vasculitis is the inflammation of blood vessels, which includes capillary leak syndrome, leukocytoclastic vasculitis, and cutaneous vasculitis. Hearing loss and tinnitus, are rarely reported (Beatty et al., 2021). Immune-mediated disorders include acquired haemophilia A, immune-mediated thrombocytopenia and Guillain-Barré syndrome (Singh et al., 2022).

The Safety Profile of COVID-19 Vaccines

The safety profile of COVID-19 vaccines has been extensively studied, revealing a wide safety margin with mostly minor and self-limiting side effects. According to studies, 3 authorized COVID-19 vaccines in the United States, Pfizer-BioNTech, Moderna, and Johnson and Johnson's Janssen, have a low frequency of side effects, with most patients experiencing mild systemic reactions such as headache and fever (Singh et al., 2022). Serious side effects such as anaphylaxis and death are extremely rare, with rates as low as 0.0003% and 0.002%, respectively. Despite the overall favorable safety profile of COVID-19 vaccines, ongoing monitoring and surveillance are required to investigate any unexpected serious adverse effects. According to reports, the benefits of COVID-19 vaccination far outweigh the potential risks associated with known side effects, which are typically mild and brief (Kai et al., 2021). Indeed, their safety profiles have been reassuring. Indeed, through extensive data analyses and further surveillance efforts, close monitoring by regulators of the safety and efficacy of the vaccines is assured.

Challenges in Vaccine Distribution and Equitable Access

The difficult issues in the settings of COVID-19 vaccine distribution and access have been outstanding. Of many challenges, the low supply and heterogeneity in the vaccine distribution process can be said to be the most important, wherein high-income countries receive a disproportionately high number of vaccine doses in comparison with the struggling LMICs to get the required amount (Bae et al., 2020). Another challenge is the lack of domestic manufacturing capacity in LMICs, which favors reliance on imported vaccines at the cost of autonomy and flexibility in the distribution. The challenges in cold chain management are specifically in the cases of delivery of such vaccines which need special conditions for handling and storage (Ye et al., 2022).

Socioeconomic factors result in vaccine hesitancy and access problems, mostly in underserved populations. Persistent misconceptions about the safety and efficacy of vaccines continue to fan the embers of vaccination resistance, which have severely vexed herd immunity efforts globally. To design cost-effective public vaccination regimes, careful consideration needs to be given to timelines for vaccine efficacy and immunity. Global and local governance is in serious need of reform to drive accountability on equitable access and vaccination. These challenges underline the pressing need for coordinated efforts to enhance vaccine distribution and equity, more so in LMICs. Such initiatives, like COVAX or regional vaccine technology and manufacturing hubs, can help to solve these problems and turn the concept of more equal sharing of vaccines globally into reality (Privor-Dumm et al., 2023).

Role of Booster Doses and Ongoing Vaccination Efforts

Booster doses and continued COVID-19 vaccination efforts are very critical in the augmentation of immunity against the pandemic. Booster doses have, therefore, become necessary in boosting immunity due to the adaptation of viruses. Updated boosters of COVID-19 show improved immunogenicity over prior vaccines, underscoring the need for heightened vigilance with tailored strategies to improve booster vaccination coverage among adults. Booster uptake varies across demographic subgroups; targeted interventions are required to improve vaccination equity. The recommendation that all

adults, regardless of history of previous infection, be vaccinated with a booster dose underlined boosters' role in conferring maximum protection against COVID-19 (Lu et al., 2023).

Vaccination campaigns will reduce cases of COVID-19, hospitalization, and mortality. Indeed, they have been at the core of reducing disease severity and preventing infectivity, particularly in vulnerable populations with comorbidities. That notwithstanding, achieving wide-scale immunity through vaccination is important in the control of this pandemic; however, it requires persistent compliance with NPIs alongside vaccination efforts. At the same time, the speed of vaccine development and dissemination is unprecedented, reflecting global resolve in fighting COVID-19 through vaccination campaigns. The scale of mobilization entailed by public health resources and the application of thought-through vaccination strategies is thus, in particular, crucial to lessening disease burden and societal impact. Key among these findings is the contribution of booster doses and additional vaccination efforts toward slowing COVID-19 spread and mitigating public health repercussions. By paying attention to disparities in vaccine uptake, public health officials at all levels can help raise immunity levels toward a more effective pandemic response by implementing tailored strategies that increase coverage and underscore the role of vaccines in reducing disease severity (Moghadas et al., 2020).

Potential Impact of Vaccination on Future Control of the Pandemic

The COVID-19 vaccination campaign is a hefty potential for change both in the pandemic and protection of global public health. Vaccines are very outstanding tools in slowing down the infection and reducing its alarming consequences during nations' continuous battle with the crisis. Here is how COVID-19 vaccination might affect the control efforts of future pandemics:

First and foremost, vaccination opens the pathway to achieving herd immunity, which is defined as the point at which enough people have developed immunity to the virus, either by vaccination or previous infection, to disrupt its transmission cycle (Kassianos et al., 2022). As much as the achievement of herd immunity is quite elusive due to the complexities involved in human behavior and new viral variants cropping up, vaccination stands as a very decisive approach toward its realization. Second, vaccination reduces morbidity and mortality since cases of severe illness and deaths due to COVID-19 are significantly reduced. The strengthening of individual immunity, vaccines reduce loads on healthcare systems and thereby enable societies to return to pre-pandemic norms. Other than that, vaccination also reduces long-term complications from severe COVID-19, supporting the case for universal vaccination.

Thirdly, vaccination breaks transmission chains, slowing the virus's spread and providing room for gradual relaxation of NPIs. With increasing vaccination rates, NPIs will need modification in accordance with local epidemiology, releasing economies from a standstill and easily returning daily routines. Vaccination provides a platform for the development of novel COVID-19 therapies, particularly during the fourth phase. The study of the host-virus interaction has been very instrumental in developing anti-viral drugs and monoclonal antibodies that have the prowess to neutralize the virus and treat infected people. Globally, vaccination promotes global solidarity and cooperation; hence, people will display a united front against the pandemic. International organizations, like the WHO, work tirelessly to ensure that vaccines are rolled out and distributed equally between countries so that none is left behind in the war on COVID-19.

COVID-19 vaccination is one of the most powerful weapons in the fight against the pandemic. The vaccines have the potential to be a game-changer in the pandemic, shifting into a post-COVID period that is much safer and healthier: protection against severe diseases, reduction in transmission, and empowering the researchers to develop novel therapies (Harshani et al., 2022).

Conclusion:

It is propagated in this chapter that vaccination can play a very important role in every diversified strategy for the fight against SARS-CoV-2. Four key conclusions draw from an in-depth analysis of vaccine development, efficacy, distribution, and societal dimensions: both remarkable achievements and ongoing challenges in using vaccines to mitigate the pandemic's impact. The rapid development and deployment of the COVID-19 vaccines are an unprecedented scientific achievement, realized by a degree of collaboration, innovation, and regulatory agility to match. Real-world evidence shows vaccines reduce the burden of disease, hospitalizations, and deaths, pointing out hope where chanced uncertainty and despair had taken over the world amidst the pandemic. Therefore, it places major constraints on global vaccination disparities, second-generation variants, vaccine hesitancy, and calls for international cooperation, resource mobilization, and community engagement. Otherwise, the social dimensions of vaccination against COVID-19 stretch beyond the public health measures for responding to ethical concerns and public trust in society's road to recovery and resilience. As we come near the critical stage in pandemic responses, lessons learned from vaccines on board serve as a guiding framework for traversing these complex terrains that lie ahead. It highlights that it is not possible to forge a healthier and more resilient future without there being a continued commitment to vaccination efforts along with complementary public health measures.

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Chapter 33

Vaccine: The Only Way to Prevent FMD

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ABSTRACT

The evolution, types, efficacy, and impact on livestock of Foot-and-Mouth disease (FMD) vaccines are described in this chapter. In developing countries like Pakistan, FMD is an alarming disease of livestock and requires control measures because of its impact on the economy of a country. The importance of vaccines against FMD and challenges regarding the control of this disease has been described in detail in this chapter. FMD vaccines like live attenuated vaccines, capsid vaccines, recombinant proteins, and peptide vaccines are reviewed and their mechanism of action has been discussed. Moreover, the effectiveness of these vaccines against FMD is evaluated. For the control of FMD, the role of vaccine design, surveillance, and diagnosis are also explored. In short, this chapter reviews FMD vaccines, while addressing current status, future prospects, and the latest efforts to increase their efficacy and potency for livestock well-being and disease control. In this way, by controlling FMD, we can lead to economic progress.

KEYWORDS
FMD, Livestock, Control, Vaccine, Efficacy

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INTRODUCTION

Among the agriculture departments, the livestock sector plays an important role in the socio-economic progress of Pakistan. It is an emerging sector and occupies a unique position in the economy of Pakistan (Alexandersen et al., 2003; Aguilar and Rodriguez, 2007). Pakistan is an ideal place for livestock farming because of its diversity ranging from fertile lands to deserts. A significant contribution is being added by this sector to the GDP of Pakistan which is 11.89% and is 61% of the agriculture department's contribution. More than 8 million rural families in Pakistan have adopted livestock farming as a source of income. Cattle, buffaloes, sheep, goats, camels, and poultry are part of the livestock sector. Each species has its significance because of its unique nutritive value in the form of meat, milk, and eggs. Moreover, hides, leather, and manure are byproducts of this sector.

Our livestock sector is being affected by several life-threatening diseases such as FMD, hemorrhagic septicemia (HS), enterotoxemia, and brucellosis. All of these diseases are being prevented by vaccinations (Belsham et al., 2000; Arzt et al., 2014). Vaccines are a promising approach for the prevention of many infectious diseases in the livestock sector (Belsham et al., 1991; Bhat et al., 2013). In the past, COVID-19, polio, and smallpox were the major problems for human beings and all of these were controlled by vaccinations, thus emphasizing its importance in public health (Mort et al., 2005; Blanco et al., 2016). Vaccines are unique biological agents that play a role in the prevention of diseases by triggering the immune system (Barasa et al., 2008; Blanco et al., 2016). The impact of vaccines cannot be overlooked for public health. Vaccines are a major part of modern medicine and are a powerful tool for public health and disease prevention. As we are facing many health threats, vaccines are now essential weapons in our arsenals, describing that innovation and collective action can lead to a protected population (Golde et al., 2005; Arzt et al., 2010). The cloven-footed animals are affected worldwide by the FMD virus and the disease that has severe negative effects on a country's economy. RNA virus is its causative agent and SAT 1, SAT 2, SAT 3, Asia 1, O, C, and A are its serotypes. It occurs frequently, due to which livestock production in a country can be too low (Reid et al., 2002). Some other problems like the lack of development of veterinary infrastructure, absence of appropriate techniques for FMD diagnosis, unawareness among the farmers about FMD, expensive vaccines,

and unavailability of vaccines are the main reasons for its spread (Kivaria, 2003). The livelihood of several farmers is also affected. Since its outbreak in America in 1870, millions of animals have been sacrificed every year for the eradication of this disease (Sutmoller et al., 2003). The main reason behind this is mutations in the genome of its causative agent. Currently, in Europe and some other parts of the world, inactivated vaccines are a major tool for the prevention of FMD (Rodriguez and Gay, 2011).

Major Causes of FMD Spread in Asian/Developing Countries

The main reason behind its spread in developing countries is a lack of proper control measures. In these countries, the unchecked transport of animals and their products across borders is very common. Additionally, vaccines are not being developed in enough quantities (Kivaria, 2003). The lack of vaccine quality, lack of knowledge, and application of a single dose of vaccine without any booster application also limit its control.

Virus Distribution

The serotypes O, A, and Asia 1 are the most prevalent strains of FMD in Europe, Africa, and Asia. Serotype was first reported in the Philippines in 1995. Similarly, other serotypes SAT 1, SAT 2, and SAT 3 are also causing damage in African countries (Rweyemamu et al., 2008). The world reference laboratory is continuously trying to increase its reporting to provide the latest data. However, more is needed for such a disease which is present in all parts of the world.

Serotype O

This is the most prevalent serotype of FMDV worldwide and is commonly found in Asian countries like India, Bangladesh, and Pakistan. It is also present in some parts of the Europe, the Middle East, and Africa. Countries like China, North Korea, Afghanistan, Iran, and Taiwan are also being affected by this serotype (Sumption et al., 2008). For the prevention of FMD caused by this strain, the O_1 Manisa vaccine is being used. However, it does not provide cross-protection (Mahapatra and Parida, 2018). This vaccine protects more than 50% of animals from FMD after the vaccination of herds (Cox and Barnett, 2009).

Serotype A

This serotype also does not provide cross-protection and shows genetic diversity (Klein, 2009). This serotype is common in Thailand and Malaysia. The genetic recombination in this strain is its characteristic feature. Moreover, it is also prevalent in some regions of the world like Pakistan, India, Egypt, Turkey, and China. This serotype has been eradicated from South Korea. The vaccines for this serotype are Iran-05, A24 Cruzeiro, and A22 Iraq (Lee et al., 2015).

Serotype Asia 1

This serotype shows the least genetic variation when compared to other serotypes. This serotype affects the livestock in China, Pakistan, Turkey, Afghanistan, Bahrain, and Iran. The Asia 1/Shamir vaccine is the most suitable vaccine for the prevention of FMD Asia 1 outbreaks (Saeed et al., 2015).

Serotypes SAT 1,2, and 3

These serotypes are most common in African territories. SAT 1 is reported in South Africa, SAT 2 is reported in Tanzania, and SAT 3 is also reported in South Africa (Rweyemamu et al., 2008).

Serotype C

This is the latest discovered serotype of FMD. It is found in East Africa and South America. There is no report on its outbreak during the past few years (Paton et al., 2021). The use of serotype C for vaccine development may lead to its spread (Saeed et al., 2015). The summary of vaccines developed against FMD has been summarized in (Table 1).

Table 1: Overview of vaccines developed against common serotypes of FMD

Serotype	Vaccine	Reference
Serotype A	Iran-05, A24 Cruzeiro, A22 Iraq	(Lee et al., 2015)
Serotype O	O1 Manisa	(Mahapatra and Parida, 2018)
Serotype Asia 1	Asia 1/Shamir	(Saeed et al., 2015)
Serotype C	Not developed	(Saeed et al., 2015)

Types of FMD Vaccines

Recombinant Protein and Peptide Vaccines

Up to the 1970s, scientists were able to study the virus capsid structure. It was determined that a capsid protein known as VP1 is an antigen that is a candidate for vaccine development. So for the development of protein vaccines, VP1 is isolated from a virus and is induced for protection from FMD in cattle and pigs (Diaz-San Segundo et al., 2017).

Empty Capsid Vaccines

These vaccines have a few drawbacks such as the potential risk of virulence, incomplete inactivation, and interference

with the diagnostic tests. The empty capsid vaccines, as compared to other vaccines, are safer to use, eliminating the chances of viral replication within the host's body (Backer et al., 2012). The other benefit of these vaccines is that they trigger an immune response against a number of serotypes of FMD (Cao et al., 2016).

Live Attenuated Vaccines

The live attenuated vaccines provide a long-term and rapid immunity against FMD. The live attenuated vaccines against FMD are being developed by the traditional attenuation method of repeated viral passage in cell cultures. However, these live attenuated vaccines have a risk of development of disease and the animals may become infected with FMD (Niedbalski et al., 2019). Different types of vaccines developed against FMD are given in (Table 2).

Table 2: Types of FMD vaccines

Type			Description	Reference
Whole virus antigen	Inactivated vaccines	BEI inactivated vaccines	Used with various adjuvants	((Doel, 2003))
		New marked BEI inactivated vaccines	Obtained after induced mutations in NS proteins are used with different adjuvants	((Parida, 2009))
		DNA vaccines	Consists of cDNA that encodes the whole virus genome	(Cedillo-Barrón et al., 2001)
	Live vaccines	Leaderless vaccine	Attenuated virus triggering a neutralizing antibody response	(Diaz-San Segundo et al., 2017)
		Inter AUG mutant virus	Inoculated through aerosal route and attenuation is attained by mutagenesis in inter AUG region	(Piccone et al., 2010)
		Chimeric virus	Protection in pigs	(Uddowla et al., 2013)
		Deoptimized virus	High safety margin	(Diaz-San Segundo et al., 2016)
Subunit vaccines		VP1 epitope peptides	VP1 developed in *E. coli* protects both the cattle and pigs	(Diaz-San Segundo et al., 2017)
		B and T cell peptide epitopes	Full protection in pigs, but partial protection in cattle	(Cubillos et al., 2008)
		NS and VP1 proteins peptides	Partial protection in cattle	(Zhang et al., 2015)
		DNA vaccine	Partial protection in cattle	(Fowler et al., 2011)
		Empty capsid vaccines	Partial protection in cattle	(Li et al., 2008; Li et al., 2011; Li et al., 2012)
		Bacterial produced empty capsid vaccines	Full protection in cattle	(Lee et al., 2009; Xiao et al., 2016)
Live vectored vaccines		Vaccinia virus	Vaccinia virus delivers virus empty capsids	(Diaz-San Segundo et al., 2017)
		Adeno virus	Provides protection to cattle and pigs	(Grubman et al., 2012; Schutta et al., 2016)
		Avian pox virus	Protectiom in pigs only	(Zheng et al., 2006)
		Alphavirus	Protection in cattle only	(Gullberg et al., 2016)

The Role for Basic Research in the Rational Design of FMD vaccines

According to various researches, inactivated vaccines against FMD are available. These studies have explained the positive effects of these vaccines. One of the effects is protection from FMD, thus preventing livestock from infecting with FMD. However, these vaccines also have many shortcomings. As a result, molecular vaccines are developed to overcome their shortcomings. Although these vaccines have not filled all the gaps, many shortcomings have been resolved. These molecular vaccines are delivered with traditional adjuvants such as emulsions and cytokine-based adjuvants such as interferons. Other scientists have been able to develop FMD vaccines which are highly thermostable through their efforts. Another technique involves a change in the sequence of capsid proteins (Rodriguez and Grubman, 2009).

FMD Control Measures

FMD being an endemic disease is one of the fatal diseases of livestock worldwide. Proper diagnosis, surveillance, and mass vaccinations can lead to the eradication of this disease. Countries that have become FMD-free could achieve it through several control strategies such as culling of affected and in-contact animals. Furthermore, limiting the transport of animals across the borders was significant. In case of outbreaks, the mass vaccinations remain a single choice for further prevention of this disease (Vannie et al., 2007). Another major problem is that the available FMD vaccines are so expensive that they are not available to every farmer in the world. Moreover, these vaccines only protect for a short period and only prevent clinical infection, while subclinical infections remain in the livestock. So, it is a need to regularly immunize your

animals by vaccinations. For example, FMD has been controlled in Europe by regular vaccination (Sutmoller et al., 2003).

Diagnosis and Surveillance of FMD

As FMD outbreaks can spread worldwide, there exist severe economic threats for developed and developing countries. As the risk of FMD outbreaks remains present, there is a need for appropriate, proper, and rapid diagnostic tests. Moreover, the identification of specific serotypes is mandatory and challenging. However, FMD is initially diagnosed based on clinical signs. The diagnosis based on clinical signs can lead to a false diagnosis with other vesicular diseases. Therefore, laboratory tests remain a single option for the proper diagnosis of FMD in animals. Countries that are now able to diagnose respective serotypes are doing this through laboratory techniques. As a part of the FMD control strategy, we cannot ignore the importance of surveillance. The surveillance is directly related to diagnosis. The surveillance can be made possible by the combined efforts of governments, veterinarians, and the livestock owners (Grubman and Baxt, 2004).

Future Directions

As we are moving on our way to the prevention and control of FMD, there is still a need for further actions. By exploring novel strategies to develop effective vaccines against FMD, we will be able to develop cost-effective vaccines that will be available for every farmer in the world. There is a need to develop vaccines that provide more protection in fewer doses. Investigations should be carried out to study the breeds that are genetically resistant to FMD. There should be development of point-of-care diagnostic tests so that FMD can be treated in the subclinical stage of infection. There is a need to monitor FMD prevalence in the endemic areas of the world and for this, proper surveillance systems are required. The 'One Health' approach should be given importance to have betterment in the development of effective surveillance systems. Veterinarians and policy makers should give information to the farmers about FMD so that they can report it to health officials.

Conclusion

In conclusion, we can say that FMD is one of the fatal diseases of livestock and no region of the world is safe from its prevalence with few exceptions. It is a major threat to a farmer's livelihood, food security, and a country's economy. It hits the farming community in multiple ways i.e., disease in animals, sharp decline in milk yield and loss of young stock. The vaccines have remained a very successful approach in the prevention of this disease in regions like Europe and the UK, but unfortunately, it has not yet been eradicated in developing countries like Pakistan, India, Afghanistan, Iran, Egypt, etc. The diseases incidence despite regular vaccination has multifactorial reasons of failure. Proper diagnosis and surveillance strategies can help vaccines for the complete eradication of this disease, but yet vaccine has been a suitable option for the prevention and control of FMD. Extensive work both on research and extension ends is needed to control this disease in endemic regions of the world.

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Chapter 34

Role of Vaccine against Influenza Disease in Poultry

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ABSTRACT

Avian influenza virus (AIV) appears naturally in aquatic birds, affecting many species and spreading from birds to people. AIV subtypes (H5N1 and H7N9) have the capacity to infect people and cause acute influenza syndrome, posing a pandemic hazard. Subtype H5N1 is extremely pathogenic, but subtype H7N9 is significantly less harmful. A good understanding of disease etiology is critical for understanding the host's immune response, which in turn aids in the development of control and prevention techniques. The goal of this book chapter is to provide information on the disease's pathophysiology and clinical aspects. Furthermore, the innate and adaptive immune responses, as well as new findings on CD8+ T cell immunity against AIVs, are discussed in detail. In addition, the current state and progress in the development of AIV vaccines, as well as the hurdles, are addressed. The information stated will be useful in countering AIV transmission from birds to humans, hence preventing catastrophic outbreaks that could lead to pandemics globally.

KEYWORDS
Avian Influenza, Pandemics, Disease pathophysiology, Immunization, CD8+ T cell

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INTRODUCTION

Avian influenza (AI) is an umbrella term that describes the sickness produced by the numerous forms of influenza. A bird-infecting virus that occasionally causes viral disease in humans, but can be extremely severe if successfully transmitted to humans (Goneau et al., 2018). A significant number of confirmed cases require hospitalization and often times intensive care unit (ICU) care. The World Health Organization (WHO) reported that, from 2013 to 2022, bird flu outbreaks in animals and ailments in humans not only increased in number, but also emerged in a larger geographic area and among a broader range of animal groups (Charostad et al., 2023). Around 21,000 animal bird flu outbreaks and 2,000 human bird flu virus infections have been reported globally, resulting in the identification of 34 different subtypes of the bird flu virus. The most recent human case was reported in Vietnam in March 2024 (Fusaro et al., 2024).

Outbreaks

In the US, the H5N2 virus produced several epidemics in chickens and turkeys between 1983 and 1985, while the H5N8 virus infected ducks, chickens, and turkeys in Ireland (Kawaoka and Webster, 1985). Two viruses H5N1 and H5N2, caused outbreaks in chickens in Hong Kong and Italy in 1997 (Capua et al., 1999). Between 2003 and 2004 there were reports of H5N1 avian influenza epidemics in a number of Asian countries such as, South Korea, Japan, China, Vietnam, Thailand, Indonesia, Cambodia, and Laos (H. Chen et al., 2006). Since January 2005 and November 2022, highly pathogenic H5 avian influenza viruses have produced 8534 outbreaks worldwide, resulting in the loss of 389 million chickens. However, data on poultry lost during outbreaks before 2004 is not accessible (Zhu et al., 2022).

Australia reported outbreaks caused by at least three different subtypes of H7 including H7N7 (1975 and 1985), H7N3 (1992 and 1994), and H7N4 (1997) (Selleck et al., 1997). The H7N3 subtype caused outbreak in Pakistan (1995) and Canada (2004) (Naeem and Hussain, 1995). Data extracted from OIE-WAHIS acknowledges that from January 2005 to November 2022, multiple outbreaks of H7 viruses from multiple subtypes led to 106 outbreaks and the loss of over 33 million birds

globally. The outbreaks were reported in ten countries such as, Australia, Mexico, America, Canada, Pakistan, Korea, Chinese, Spain, Italy, and Germany. H7N3 caused 77 outbreaks in North America that resulted in the mortality of more than 29 million birds. European countries and Korea experienced 10 outbreaks of H7N7 while US and China experienced outbreaks of H7N9 (Shi et al., 2023).

The H9 subtype was first discovered in North America following an outbreak in turkeys in 1966 (Peacock et al., 2019). From 1999 to 2015, H9 subtype in Asia produced substantial disease outbreaks in chickens and other land-based poultry, including quail, pheasant, partridge, and other minor domestic poultry species. These viruses remain enzootic in chicken and are widely dispersed throughout Asia, the Middle East, and portions of Africa (Carnaccini and Perez, 2020).

Etiology

AI virus is a member of the Orthomyxoviridae family, which includes influenza (types) A, B, and C viruses. The most common cause of avian illnesses is influenza A virus (AbuBakar et al., 2023).

Avian Influenza Virus (AIV) is a pleomorphic virus that has filamentous encapsulation, helical symmetry, spherical or ovoid shape, and diameters ranges from 50 and 120 nm. The viral envelope includes the lipoprotein membranes that contain the nucleoprotein and nucleocapsid. AIV's genome is composed of enveloped, eight single-stranded, negative sense RNA segments (HA, NA, PB1, PB2, NP, M, PA, and NS). These segments code for ten different viral protein types, which include two matrix proteins, two NS proteins, HA, NA, PB1, PB2, another RNA polymerase complex and Nucleoprotein (Rehman et al., 2022).

The surface of the encapsulated AIV is coated with 500 different forms of glycoprotein projections or spikes. Among them most important are hemagglutinins (HA) and neuraminidase (NA), which are required for infection. There are eleven and eighteen antigenically distinct NA and HA correspondingly, resulting in 198 possible combinations (serotypes) of AIV (Iqbal et al., 2013).

Significance

AI is a very deadly illness that affects poultry birds, resulting in 80–100% morbidity and 30–100% mortality (Rehman et al., 2022). The Office of International des Epizooties (OIE) listed AI as a List A infection in 1997 (Ben Jebara and Shimshony, 2006). Of all respiratory viruses, the avian influenza virus stands out because of its highly diverse antigenic repertoire and segmented genome (Aamir et al., 2007). The global pandemic of avian influenza has enhanced the importance of the disease. The first reports of the AI subtype H5 were made in the United States, and subsequent outbreaks were noted in Pakistan, Australia, China, Jordan, and other countries. Later on, Subtype H9 of AIV becomes endemic and causes enormous economic losses to the poultry sector in Pakistan. Since 1997, avian influenza has also infected people in addition to wild and poultry birds (Nagy et al., 2024). The virus often causes no symptoms and is spread by migrating birds. Although all birds are prone to this virus, outbreaks often affect chickens and turkeys. The capacity of different strains to reassort, the frequency of genetic differences, and the potential for gene alterations provide obstacles in the development of influenza vaccines. The only ways to control the infection are through biosecurity and vaccination, but they are quite costly (Chowdhury et al., 2020).

Host Range

Several species are affected by different strains of the AI virus, including humans, sea mammals, birds, ducks, dogs, guinea pigs, horses, swine, and chickens (AbuBakar et al., 2023).

Epidemiology

Numerous avian influenza virus strains, including Highly Pathogenic Avian Influenza (HPAI) and Low Pathogenic Avian Influenza (LPAI), have been discovered in chicken farms around the globe. Variable clinical signs ranging in severity indicate an infection affecting the respiratory and gastrointestinal tracts (Rehman et al., 2022). A human may acquire an infection from poultry through direct contact with infected birds or contaminated surfaces. Conversely, eating raw poultry or poultry products, handling dead or diseased poultry, and defeathering are risk factors (Yang et al., 2022). It's possible that sparrows and quail will act as an intermediary host for the virus's maintenance and spread to poultry (Chen et al., 2006).

Disease Description

Based on pathogenicity and virulence, AI is categorized into two types: highly pathogenic avian influenza virus (HPAIV) and low pathogenic avian influenza virus (LPAIV).

- HPAI is a deadly strain that can quickly flare out in flocks, resulting in significant mortality rates.
- The constant presence of LPAIV in chicken flocks could have caused the virus to mutate into a pathogenic variant (Monne et al., 2014).

Clinical Signs

Clinical signs of AI consist of depression, diarrhea, abrupt reduction in egg production, reduced vocalization, shell-less or soft-shelled eggs, respiratory sounds, congestion, ruffled feathers, swelling of sinuses, head apathy, nasal and ocular

discharge, dyspnea, coughing, sneezing, redness of eyes, cyanosis of un-feathered skin, cyanosis of wattles and combs, nervous signs and incoordination (Nagy et al., 2024).

Postmortem Lesions

AI post-mortem lesions include facial and under-beak edema, a straw-colored fluid visible in subcutaneous regions after skin is removed, Engorged blood vessels, tracheal hemorrhages, muscular hemorrhages, pinpoint hemorrhages in the proventriculous, around the lining of the gizzard and throughout the intestine, muscle edema, breast bone hemorrhages, as well as fat deposit in abdomen, gizzard and heart (Lean et al., 2022).

Treatment

There is no therapy for AI, and most countries have a policy of culling infected birds. However, using broad-spectrum antibiotics, maintaining a healthy diet, and using excellent husbandry techniques will help to lessen the damage caused by tributary infections (Simancas-Racines et al., 2023).

Control

To eradicate AI in poultry birds, many preventative measures are employed. The primary objectives of control programs are management, prevention, and elimination (Mehta et al., 2018). This can be accomplished by adding crucial elements such as;

- Biosecurity procedures including inclusion and exclusion,
- Removal of contaminated poultry birds,
- Diagnose and monitor infection,
- Built host resistance and
- Reduced environmental contamination.

Using all of these methods, this devastating disease of poultry birds can not only be controlled but also eradicated (Tretyakova et al., 2013).

Vaccination

It is obvious that infections that can spread from animal pools continue to be a concern to global health. The properties of avian influenza viruses make them a worldwide threat (Nielsen et al., 2023). These viruses' split genomes and error-prone replication mechanisms could lead to the emergence of novel reassortant viruses due to the lack of immunological awareness within the human population. Furthermore, AIVs can infect humans, domestic animals, and aquatic birds, therefore there is a probability of reassortment and ultimately development of novel viruses (Kumar et al., 2017). One efficient way to prevent AIV infections from developing is by vaccination. Seasonal influenza vaccines are safe and can reduce the severity of annual influenza outbreaks. Currently, research is focused on developing new influenza vaccinations to prepare for potential pandemic outbreaks of the AIV in the future (Y. Chen et al., 2013). Understanding immune responses specific to influenza enables the discovery of new viral targets for the creation of vaccines. Most vaccines developed so far target hemagglutinin (HA), a protein on the virus surface. Because surface HA is susceptible to reassortments and antigenic drifts, immunizations need to be updated (Quiñones-Parra et al., 2014).

The restrictions of the conventional technique of virus cultivation in certain pathogen-free chicken embryos, and comparatively novel technique of culturing a virus in cells to generate a vaccine, have stimulated investigation into alternate approaches (Kerstetter et al., 2020). Recombinant HA-based vaccinations have been shown to be able to produce neutralizing antibodies against the avian influenza virus. However, antibodies generated against a particular influenza virus strain or subtype are unable to neutralize other strains or subtypes. Because of the virus's continual alterations, vaccinations required to be updated after a set period. In order to combat different influenza strains or subtypes, scientists are focusing their efforts on creating universal influenza vaccines (UIVs) with a wide spectrum of neutralization (Gomaa et al., 2020).

Types of Vaccines

In order to combat the pandemic influenza, a variety of inactivated viral vaccines (IVVs) and live-attenuated viral vaccines (LAVVs) are being developed lately. The technology behind these vaccines has been approved for execution of the present seasonal influenza vaccination program (Subbarao and Joseph, 2007). Preclinical research has indicated that live vectors of viruses that produce influenza virus proteins, DNA vaccines, and other platforms are being developed for vaccinations (Hoelscher et al., 2008).

The aforementioned section discussed various vaccines had been prepared and used to address different subtypes of AIV.

a. Live Attenuated Viral Vaccine (LAVV) for Influenza

Live attenuated viral vaccines (LAVVs) work on the basis that the internal protein genes of the vaccine donor virus have mutations that make the virus temperature-sensitive and attenuated. As a result, these vaccinations are given intranasally and are limited to reproducing within the upper respiratory tract (URT) (Pitisuttithum et al., 2017). The LAVVs can only multiply in a few specific ways in the colder URT, and cold adaptation stops the virus from infecting the

lungs. In the US, these viruses are licensed and in use (Baz et al., 2015). These are created by reassorting seasonal influenza viruses with cold-adapted viruses. Vaccinations against H5 infections, which can cause a potent CD8+ T cell response in ferrets and mice, have been created and tested in clinical trials using cold-adapted vaccinations (Mohn et al., 2017). Although LAVVs cannot elicit a strong antibody response, they can stimulate a long-term immunological memory, as demonstrated by the results of the Phase 1 studies (Pitisuttithum et al., 2017). This can be enhanced by delivering an inactivated vaccine 3-5 years later. Targeting intracellular proteins, these vaccines magnificently trigger T cell responses. It was found that these HA-specific T cells could produce a small amount of cross-reactivity when exposed to the HA of H5N1. Along with NA and HA gene segments extracted from a wild-type influenza, each LAVV is a reassortant virus composed of 6 internal protein genes from the vaccination donor virus (B. X. Wang and Fish, 2019). The development of LAVV, (a vaccine against the pandemic influenza virus), has the potential to trigger cellular and humoral immune responses in the mucosal and systemic regions. Even with its limitations, LAVV replication is sufficient to trigger T cell and systemic antibody responses that protect the host from developing new infections (Coelingh et al., 2014). The aim of both LAVV and Inactivated viral vaccine (IIV) formulations is to elicit immune response against HA and, to a lesser degree, NA. Despite the fact that LAVV vaccines with avian HAs have a greater ability to generate cross-reactive protection than IIVs, there are safety concerns regarding their use. This is because it might be argued that vaccination may allow for reassortment if the recipient also contracted a seasonal influenza virus that is circulating in the population at the same time. Furthermore, the benefits of LAVV vaccines are not universal throughout population groups (Peng et al., 2015; Rudenko and Isakova-Sivak, 2015).

b. Inactivated Viral Vaccine (IIV) for Influenza

There are two types of IIV formulations: sub-unit or split-virion vaccines and whole inactivated viral vaccines (WIVV), each of which has pros and cons. Owing to their crude manufacturing process and the subsequent stimulation of innate immune signaling pathways by leftover viral RNA, WIVV vaccinations frequently exhibit chemical inactivation and strong immunogenicity. Depending on the inactivation method used, the primary targets for neutralizing antibodies (HA and NA) may maintain their structural resilience after receiving a WIVV vaccination. WIVV vaccinations include internal antigens that have the potential to boost T cell responses that are cross-reactive to conserved viral proteins like nucleoprotein (Nypaver et al., 2021). However, as WIVV vaccines are more reactogenic than more thoroughly purified formulations, they have not been the vaccination of choice in recent years. The majority of immune responses to the hindmost vaccinations are focused on attacking HA because of production procedures that enrich for HA content (Nelson and Sant, 2019). On the other hand, it has been noted that whereas the initial immunization only results in a negligible or mild antibody response, a subsequent IIV boost can cause a robust antibody response. It is important to evaluate the different prime-boost strategies side by side and look into whether vaccinations containing DNA and adenoviruses also result in highly localized germinal center responses that are systemically recognized following the IIV boost (Ledgerwood et al., 2011; Gurwith et al., 2013).

c. Vector-Based Vaccines

Vector-based vaccinations appear to be a potential vaccine option. Most of viral vectors are referred to be "live" vaccines. These vaccines have excellent safety ratings since they are completely devoid of replication in people, even in those with impaired immune systems (Sayedahmed et al., 2020). One well-known potential vaccination vector is the modified vaccinia virus Ankara (MVA). Originally, this was produced as a smallpox vaccine with poor replication. Infected cells created endogenous antigens due to MVA, which effectively modified and presented antigens, hence inducing specific T and B cell responses. Furthermore, the procedure of creating the recombinant-MVA (rMVA) involves introducing genes that encode the required antigen into the viral genome (Pittman et al., 2019). Subsequently, many rMVA vaccine candidates, presenting various influenza viruses NP, HA, NA, PB2, M1 and M2, have been substantially studied based on ferret and mouse as well as on macaque immunization challenge studies (Sebastian and Lambe, 2018). With the utilization of rMVA vaccines that express the HA genes from multiple H5N1 viruses, the adequacy of these rMVA vaccinations was assessed in terms of their safety to reduce virus replication in animals exposed to viruses from different antigenic clades (de Vries and Rimmelzwaan, 2016; Sebastian and Lambe, 2018).

Additionally, there are several advantages of utilizing non-replicating adenoviral (Ad) vectors in the development of HPAIV vaccines. HPAIV antigens that are expressed in vivo following immunization can be incorporated into their genome since it is stable. Crucially, multiple human clinical trials have demonstrated the high safety and immunogenicity of Ad vaccines, which can be thermostabilized for stockpiling and preparation for pandemics (Kerstetter et al., 2020).

d. Universal Influenza Viral Vaccines (UIVVs)

The development of a UIVV has gained prominence as a scientific objective recently. Major knowledge gaps have been noted in a number of the published papers, and it was highlighted that innovative solutions to close these gaps must be funded (Erbelding et al., 2018). Diversifying the variety of vaccine compounds under investigation could be an approach for reducing reliance on egg-based production while increasing the scope and robustness of immunizations. Alternatively, other platforms may be employed to understand the immunological reactions required for universal effectiveness and to uncover novel correlates of protection, as different vaccine delivery systems induce immune responses with different

phenotypes. Many vaccine molecules, including mRNA, DNA, nanoparticles, conserved peptides, or VLP-based immunizations, are being assessed as UIVs could offer defenses against newly emerging pandemics (Freyn et al., 2020; Xu et al., 2020).

Aim of Production of Pandemic Vaccine

The current rise in cases of AIV transmission directly to humans and the continuing H5N1 influenza virus outbreak in humans and avian species across multiple countries demonstrate the serious risk that LPAIVs and HPAIVs pose to the well-being of humans. Given the difficulty in predicting which AIV subtype will cause an upcoming global health crisis, a suitable vaccine would elicit an immune reaction that protects the host against a wide range of influenza viruses from similar or distinct subtypes (de Jong and Hien, 2006). The HA and NA glycoproteins of influenza viruses are mutated genetically and antigenically to evade the immune system. Although neutralizing antibodies specific to the HA glycoprotein can quickly prevent influenza virus infection at mucosal or systemic sites of disease, cell-mediated immunity is still a major factor in the elimination of human influenza viruses (Chen et al., 2018).

While antibodies produced against the NA glycoprotein do not completely eradicate virulency, they do hinder the formation of new virus molecules, which needs the presence of viral NA proteins, and restrict the spread of the virus. Antibodies specific to NA can thereby decrease the severity of the sickness. Cytotoxic T lymphocytes (CTLs) are able to identify epitopes on the PB2, PA, and NP proteins of human influenza viruses. Therefore, in the event of a pandemic, cell-mediated immunity directed against the more conserved inner proteins could provide protection if a pathogenic virus with unique NA and/or HA glycoproteins infects people worldwide. The current generation of human influenza vaccinations is based on the idea of producing specific and protective antibodies against the HA glycoprotein of the strain that is more likely to spread disease (Altenburg et al., 2015).

Cross-reactive T cell induction is commonly noted in AIV-induced heterosubtypic immunity. The majority of cross-reactive T cells, especially CD8+ CTLs, work to maintain internal proteins, including nucleoproteins, matrix proteins, and polymerase complex proteins (Y. Wang et al., 2018). Recurrent AIV infection has additionally been shown to boost CD8+ T cells, which can cross-react with different subtype viruses, including H1N1, H5N1, and H7N9. UIVs' ability to activate cross-reactive T cell response remains an attractive approach (Zhao et al., 2018). Notably, safe and well-targeted vaccine delivery systems include DNA vaccines, LAVVs, virus-like particles and vector-based vaccines. VLP vaccines including HA, NA, and M1 have been shown to induce a significantly greater cell-mediated immune response in mice than the whole-inactivated vaccines. (Ren et al., 2015).

Challenges in Vaccine Formulation

- Predicting which strains will cause the next pandemic presents a huge hurdle in the development of an AIV vaccine. However, subtypes H5 and H7 remains in the news. Moreover, the continuous identification of novel AIVs makes the development of a pre-pandemic vaccine considerably more difficult. The wide spectrum of AIVs and the continual evolution of the antigen make the development of an effective vaccination even more challenging (de Vries et al., 2018).
- Traditional LAVV/IVV systems have limited immunogenicity, safety concerns, and a number of unique manufacturing challenges when used in the development of AI vaccines. Repeated vaccinations along with higher dosages of the vaccine are needed to address this problem (Rudenko et al., 2018). This will eliminate the possibility of "dose-sparing" and result in a shortage of vaccines. Additionally, several adjuvants are required to increase the immunogenicity of vaccinations (Ko and Kang, 2018).
- A major problem is that the production process takes a long time, which makes it hard to react fast in case of an outbreak. In the ideal situation, if viable seed stocks are identified and authorized by the WHO within a reasonable timeframe and these viruses grow to sufficient titers, manufacturing could be finished in five to six months. Pre-influenza season strain selection usually takes place 7-8 months in advance. Still, the process could take a lengthy period in unanticipated circumstances. A six- to eight-month production run is possible (de Vries and Rimmelzwaan, 2016).
- A challenge and potential risk associated with a large-scale drop in supply in the case of an HPAIV pandemic is an over-reliance on the production of embryonated eggs, which would reduce the availability of eggs required for manufacturing. Furthermore, because HPAIVs and the vaccine seed supplies they produce, have the potential to be embryo-lethal. Therefore, it is difficult to replicate viruses in eggs in order to create vaccine stocks. Moreover, professional staff and protocols are required to handle HPAIVs used for vaccine development in improved BSL-3 biocontainment facilities, which increases costs (Trombetta et al., 2019).

Conclusion

It is well understood that the host's immune response to an AIV infection is comprised of multiple complicated processes that work together to play critical roles in the host's defense. The pathogenicity of AIVs is determined by the interactions of host immune mechanisms with the virus. Severe infections cannot be controlled by inadequate or ineffective adaptive and /or innate immunity. An increased innate immune response and a cytokine storm are associated with AIV infections. The development of pandemic influenza vaccines has made significant progress, predominantly with the advent of oil-in-water adjuvants and the multi-component prime-boost approach that uses vector vaccines followed by protein vaccines, DNA, and LAVV. Compared to a highly strain-specific response seen with an unadjuvanted subunit

vaccination, these techniques increase the antibody response. Although our knowledge of the AIV–host interaction has greatly improved, further study is still required to completely comprehend the host immune system's dynamics upon detection of the developing AIVs. Filling these gaps will enable the development of novel antiviral medications as well as enhanced vaccines and immunization techniques.

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Chapter 35

Status of Vaccination against Hepatitis B Virus

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ABSTRACT

Hepatitis B virus (HBV) infects the liver and can cause chronic liver disease such as cirrhosis and hepatocellular cancer. HBV is a global public health concern that causes significant morbidity and mortality. Vaccines against Hepatitis B are the most cost-effective, safe, and effective methods available for controlling and preventing hepatitis B. Effective interventions that can stop infection and the spread of the disease are not being used, and HBV is still terribly underdiagnosed. HBV vaccination coverage at birth is still poor, especially in low-income nations or areas with high HBV prevalence. Even in high-income nations or areas, people suffering from HBV infection receive insufficient evaluation, care coordination, and treatment. The World Health Organization (WHO) wants to eradicate the spread of viral hepatitis as a global health problem by 2030, but if things continue as they are, it is predicted that the number of HBV-related deaths worldwide will rise by 39% annually between 2015 and 2030. We go over the present state and anticipated future trends of the worldwide HBV infection load in this review. We also suggest future directions and talk about gaps in the present care cascade.

KEYWORDS
Hepatitis B virus, Chronic HBV infection, Vaccination, Prevention, Immunization

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INTRODUCTION

Infection with the hepatitis B virus, also known as is regarded as a major cause of death and a health burden worldwide. The majority of the burden is attributed to the long-term effects of chronic infection, which usually include cirrhosis and hepatocellular cancer (Abdelgader et al., 2024). HBV assaults the liver at the levels of acute and chronic infection. The western Pacific and African regions show greater rates of HBV infection, with about 116 million and 81 million infected individuals, respectively, based on the World Health Organization (WHO). The best and most efficient way to stop HBV infection and the spread of HBV-related diseases is to be vaccinated cancer (Mangowi et al., 2024). The WHO has advised countries to incorporate the three dose main series for HBV into their national vaccination regimens since 1992.

Vaccination can prevent viral hepatitis type B, which occurs by the hepatitis B virus (HBV) and can be fatal. Newly infected individuals with HBV often have no symptoms and are unaware of their hepatitis condition for years. Only those newly diagnosed with HBV have symptoms. Symptoms may include excessive weariness, discomfort in the stomach, vomiting, and jaundice. Scientific data reveals that HBV does not directly cause cytopenia but causes liver damage through the cellular reaction to viral protein in infected hepatocytes (Ismail et al., 2024). Acute hepatitis B is often a short-term infection, with clinical signs and symptoms resolving within 1-3 months. The primary determinant of the clinical manifestation of acute illness and the progression of chronic infection is the age at which the HBV infection was first acquired. The chance of contracting a chronic HBV infection decreases with age; in comparison, 30%–50% percent of children infected before the age of six years and 1%–5% of adults get chronic infections, but 80%–90% of newborns infected within the first year of life do. When a baby is infected from birth, their immune system's ability to withstand viral antigens seems to be a major factor in the virus's persistence (Mukhamatsobirzoda et al., 2024).

Injecting drugs using shared syringes, needles, or other injection supplies can also spread HBV. Blood-borne pathogen transmission is frequently observed in healthcare facilities. Healthcare professionals (HCWs) are more likely to have HBV because of the nature of their profession, which involves contact with bodily fluids like blood (Chen 2024). Additionally, when they perform internships and provide healthcare services in healthcare institutions while pursuing their studies, health science students run the same hazards as HCWs. This high rate was caused by a number of factors, including poor HBV vaccination, non-adherence to recommended precautions, and ignorance of HBV.

The global eradication of viral hepatitis is now a feasible goal due to increases in vaccination coverage between 1990 and 2000 and the implementation of extremely effective preventive strategies and therapies. By 2030, the WHO set a high goal of 65% fewer deaths worldwide from hepatitis-related causes and 90% fewer new infections (Yao et al., 2024). The World Health Organization advises people to finish the three-dose vaccination program and get screened for hepatitis B surface antigen, particularly if they belong to high-risk groups like health care workers (HCW) and medical students. However, a prior study discovered that HCWs' low levels of preventative occupational practices are correlated with the high risk of HBV infection, underscoring the pressing need to shield HCWs from HBV infection (Mironova and Ghany 2024).

Transmission of HBV

Highly contagious, HBVs are transmitted through contact with infected bodily fluids or blood (saliva, semen, and vaginal fluid) on mucous membranes. HBV is most frequently passed from mother to kid at delivery and from infected to uninfected children throughout early childhood (horizontal transmission) in high endemic areas (Pocurull et al., 2024). The majority of HBV infections in low-endemicity areas affect adults in relatively well-defined risk groups, including those who are at risk from sexual contact, those who live with an infected individual, hemodialysis patients, those who are incarcerated, injecting drug users, people who are at risk from occupational exposure, people who are developmentally disabled and receiving long-term care, and visitors to areas with mild or high HBV endemicity. Currently, the only known source with human HBV genotype is humans, however, higher primates also have closely similar HBV genotypes (Hsu et al., 2023). Because of this, a thorough control approach may ultimately result in the elimination of HBV.

Diagnosis for HBV

Serological testing is necessary for a reliable diagnosis of hepatitis B since its clinical signs can be confused with those of other viral hepatitis causes. In order to determine if a person has an acute or chronic HBV infection, is immune to HBV due to a prior infection with HBV or vaccination, or is vulnerable to infection, this testing employs various (combinations of) serologic markers (Kumar et al., 2023). The presence of HBsAg in serum for at least six months, with or without subsequent HBeAg, is arbitrary in defining chronic infection. Anti-HBs are neutralizers that provide long-term protection against HBV infection when they are present in serum. In individuals who have developed immunity via immunization, the sole serological marker identified is anti HBs. Unlike those who have already contracted HBV, who have both anti-HBs and anti HBc IgG antibodies present at the same time (Lehmann et al., 2023).

Patients are also positive for serology for HBeAg throughout the early, highly replicative stage of HBV infection; some of these patients continue to be HBeAg positive over years. Although the detection of HBeAg suggests that the infected person's blood and bodily fluids are extremely contagious, all HBsAg-positive individuals should be treated as contagious (Wong et al., 2023). While HBsAg has been found in various bodily fluids, only vaginal fluid, serum, saliva, and semen have been shown to be infectious.

Treatment Options

For those suffering from acute hepatitis B, there is currently no recommended antiviral therapy because 95% of immunocompetent adults who are infected recover on their own. Individualized care is offered to those who have a persistent HBV infection (Lavanchy and Kane 2016). The major objectives of the care that is currently accessible are to keep patients comfortable, reduce their symptoms, and stop them from infecting other people. Notably, though, not every patient with persistent HBV requires treatment. Individuals receiving current treatment may benefit best from having active liver disease symptoms (Fabrizi et al., 2021). The Food and Drug Administration has approved oral antiviral medicines (entecavir, tenofovir, dipoxil fumarate, tenofoviralafenamide, and nonpreferredlipivudine, adefovirdipivoxil, and telbivudine) and interferon-α (standard and pegylated) for the treatment of chronic hepatitis B (Nayagam et al., 2016).

The current treatments for persistent hepatitis B are not curative, but they can lower the risk of liver cancer, delay or stop the advancement of cirrhosis, and enhance long-term survival as well as quality of life. Hence, the majority of patients who begin hepatitis B therapy must do so for the rest of their lives (Tang et al., 2017). Patient management becomes more challenging and complex due to the negative effects of the medicines and the need for frequent monitoring. Therefore, the first line of defense against hepatitis B is vaccination against the virus. When weighed against alternative interventions, vaccination is a more financially advantageous choice due to its cost-effectiveness and benefit-cost ratios (Kosinska et al., 2017).

Immunization

The hepatitis B vaccination is crucial since, globally, HBV infection continues to be the primary cause of cancer in the liver and results in high rates of morbidity and mortality (Das et al., 2019). Approximately 887220 people worldwide passed away from HBV infection in 2015. Additionally, according to estimates from the WHO, 257 million people had a chronic

HBV infection in 2015 (Kalra and Verma, 2020). The frequency and burden of this infection differ by location and subpopulation. Since 1982, commercially accessible hepatitis B vaccinations have been generated from plasma (Sheikh et al., 2018). These vaccines were created by American microbiologist Maurice Hilleman by extracting HBsAg subviral particles from the plasma of donors with chronic, asymptomatic HBV infection (Chang et al., 2017).

The highly purified particles found in the plasma were followed by several combinations of urea, pepsin, formalin, and heat to inactivate any remaining infectious particles. The first approved hepatitis B vaccinations were the result of successful trials using plasma-derived vaccines in several hundred million people (Liao et al., 2015). The initial HBV vaccinations were produced under the brands Hevac B (Institute Pasteur) and Heptavax B (Merck), and they were intended primarily for high-risk populations, which was the program's primary focus at the time. Public worries regarding the efficacy of the plasma derived vaccine continued and hindered its adoption in many populations, even though concerns regarding the vaccines' ability to prevent the spread of bloodborne pathogens, such as the HIV virus, have been shown to be unfounded (Nelson et al., 2016). Expensive vaccine prices and the absence of international vaccination regulations were two more obstacles to widespread vaccination (Spyrou et al., 2020). Recombinant HBsAg was used to create the first genetically altered hepatitis B vaccine in 1986. This led to the development of a second generation of HBV vaccines, which totally superseded the previous generation of vaccinations made from plasma (Howell et al., 2021).

Large-scale vaccine production was made possible by the invention of recombinant DNA technology, which expressed HBsAg in yeast and later in mammalian cells. The most popular type of recombinant vaccines, known as yeast-derived ones, are produced by genetically modifying *Saccharomyces cerevisiae* cells to express the HBsAg protein (Ward et al., 2018). When administered as a vaccine, HBsAg of HBV forms itself into virus-like particles (VLPs), providing adaptive antiviral immunity against HBV infections (Childs et al., 2018). Since they were the first vaccinations based on VLPs, recombinant vaccines for hepatitis B represent a significant turning point in the area of vaccination. Glycosylated pre-S1 and pre-S2 proteins are present in mammalian cell-derived vaccines together with the main HBsAg protein (Walayat et al., 2015). It has been demonstrated that these vaccinations, together with other newly discovered vaccine adjuvant formulations, are more immunogenic than the second-generation vaccinations because they cover more than just the HBsAg S epitope. Despite the fact that they are too expensive to be incorporated into the national immunization campaigns, they can protect immune compromised individuals and non-responders.

Hepatitis B Vaccine Development

Plasma Hepatitis B Vaccine

The inability of HBV to reproduce well in cell cultures suggests that an in vitro culture-based hepatitis B vaccination cannot be developed. The hepatitis B vaccine was developed through groundbreaking work by Dr. Krugman and associates (Gerlich, 2015). The infectivity of HBV carriers' plasma was demonstrated to be destroyed by boiling; the active vaccination of people with the boiled plasma produced antibodies against HBsAg (anti-HBs), and the vaccinated individuals were protected toward HBV challenge, demonstrated the effectiveness of HBIG in preventing HBV infection in humans (van Den Berg et al., 2015). These investigations showed that the hepatitis B vaccine may be developed using viral antigens that are naturally generated in HBV carriers.

Recombinant Hepatitis B Vaccine

Despite being safe and effective, the plasma hepatitis B vaccine relatively high cost has prevented it from being widely used (Chang et al., 2015). The implementation of this vaccine was further hampered by potential safety issues associated with plasma from carriers of HBV who might also be shared with HIV and other infections. Furthermore, there is a limited supply of human plasma contaminated with HBV, especially after the HBV vaccine when the incidence of HBsAg has declined. These elements prompted the hunt for substitute hepatitis B vaccinations (Cui et al., 2023).

The potential for use recombinant HBsAg as a hepatitis B vaccine was demonstrated by the effective cloning of the HBV S gene in bacteria. Amplification of the S gene was attempted in eukaryotic systems since the HBsAg generated in bacteria is unable to correctly assemble as particles akin to those in a normal infection in humans (Shirsat et al, 2024). *Saccharomyces cerevisiae,* the yeast that produces HBsAg, has the ability to combine into particles that resemble the 22 nm particles that humans make. Chimpanzees, mice, and monkeys were potently induced to develop an anti-HBs response by the hepatitis B vaccine, which consisted of HBsAg isolated from recombinant yeast cells. The chimpanzees who received the vaccination had complete protection against systemic exposure of homologue or heterologous human HBV (Mahmood et al., 2023).

Newly Licensed Hepatitis B Vaccine

In 2018, the HEPLISAV-B® vaccine, which targets people over the age of 18, received a license. Unlike the previous vaccination, which required three doses spread over six months, the new vaccine only needs two doses spaced one month apart (Conners et al., 2023). Before being approved, the HEPLISAV-B was known as HBsAg-1018 ISS. It consists of recombinant HBsAg mixed with a novel adjuvant that binds to Toll-like receptor 9 and stimulates B cells and plasmacytoid dendritic cells (Inoue and Tanaka 2020). The adjuvant is an oligodeoxynucleotide with immunostimulatory CpG motifs. The recently approved hepatitis B vaccination has the potential to produce early protection by eliciting a stronger anti-HBs response more quickly. The two-dose regimen spaced one month apart can improve immunization adherence. Even

though the novel vaccination did not cause any new negative effects in clinical studies, long-term safety data still need to be further investigated (Zhao et al., 2020).

Therapeutic Hepatitis B Vaccine

It is well recognized that both innate as well as adaptive humoral and cellular immune reactions are necessary for the host to eliminate HBV. Hepatocytes' ability to eliminate virions is primarily reliant on T-cell responses (Zeng et al., 2021). Therefore, a lot of work has gone into creating an effective hepatitis B vaccine employing the P, C, S, and pre-S genes of HBV together with a variety of protein-, protein-antibody complex-, peptide-, and DNA-based immunostimulants strategies to boost humoral and cellular immune responses (Locarnini et al., 2015). The therapeutic vaccines' clinical efficacy is, however, restricted, despite the fact that they induced particular humoral/cellular immune reactions in humans and demonstrated encouraging therapeutic effects in certain of those animals (Wang et al., 2021). Therefore, much more ground-breaking research is needed to create therapeutic vaccinations that are effective towards hepatitis B in humans.

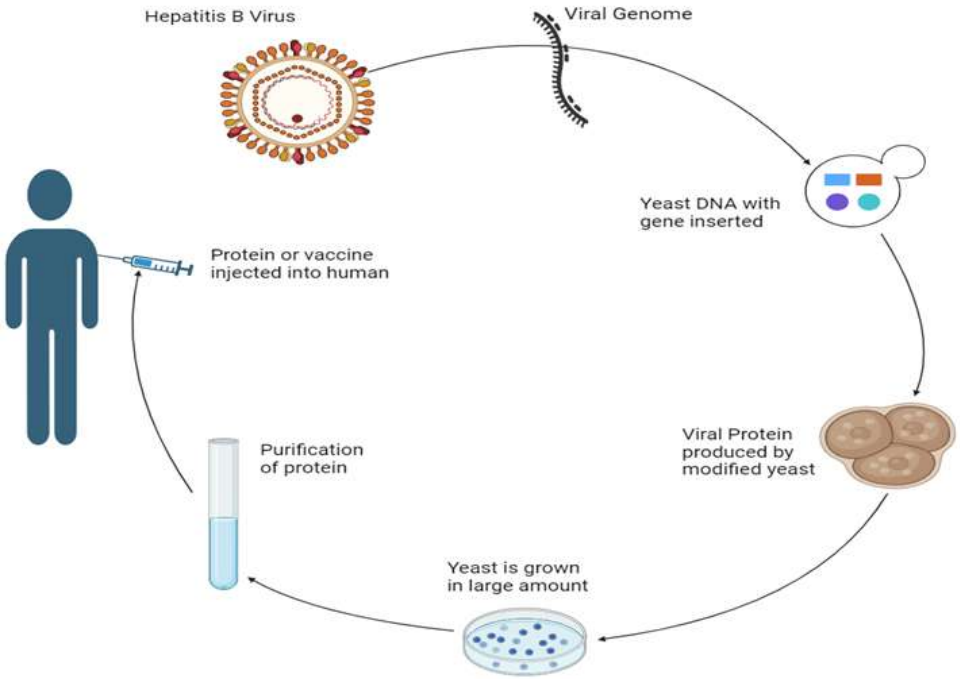

Fig. 1: Creation and application of a yeast-based HBV vaccine. (Retrieved from Biorender)

The most widely used hepatitis B rec-DNA vaccines are produced from yeast using genetically engineered *S. cerevisiae* yeast cells that express the HBsAg protein. A number of additional yeast species are employed in the manufacture of HBV vaccines. For example, the yeast *S. cerevisiae* is capable of producing HBsAg, which may combine to produce particles that match the 22 nm particles that humans make (Mahmood et al., 2023). Fig. 1 demonstrates the Different yeast-based technologies for vaccine development comprising pure protein immunogens, yeast display, complete recombinant yeast and virus like particulate.

Impact of Vaccine on Hepatitis B

Hepatitis B vaccination has had a significant influence on public health. The vaccine, which was created for the prevention of hepatitis B virus infection, has shown exceptional success in lowering the global burden of hepatitis B related disorders. A viral infection called hepatitis B can damage the liver and cause both acute and long-term liver disease (Xu et al., 2023). An important step in avoiding the spread of HBV infection was taken in 1981 when the hepatitis B vaccine was first authorized for use in the US. Since that time, it has grown to be a crucial part of regular immunization campaigns in numerous nations, helping to significantly lower the incidence and death of hepatitis B (Nayagam et al., 2023).

HBsAg is stimulated by the vaccine to create antibodies by the immune system. These antibodies shield the body from HBV infection over an extended period of time by stopping the virus replication and liver damage (Zhao et al., 2021). To achieve sufficient immunity, the vaccine is usually given in a sequence of doses, with one or two additional doses given after the initial dose. The effectiveness of the hepatitis B vaccine in avoiding HBV infection and its related consequences has been shown in numerous trials (Omame et al., 2023). Clinical studies have demonstrated that the immunization is quite successful in raising levels of protective antibodies in both adults and children, and that these levels of immunity continue for several years following vaccination.

The vaccination has been demonstrated to prevent infection in addition to the growth of cirrhosis of the liver and liver cancer, as also as the occurrence of both acute and long-term hepatitis B. Preventing the transfer of hepatitis B virus from mother to kid has been one of the vaccine's most important effects (Pantazica et al., 2023). Countries have prevented an

abundance of fresh infections in children by lowering the risk of vertical transmission through immunizing expectant mothers and babies. This tactic has been especially successful in high-prevalence regions where transmission from mother to child is a significant HBV transmission pathway (Cheng et al., 2022).

Globally, the incidence and prevalence of hepatitis B have significantly decreased as a result of the extensive adoption of hepatitis B vaccination programs. Chronic infection with hepatitis B has become far less common over the past couple of decades in nations like Taiwan and South Korea that have implemented universal vaccination programs. These positive outcomes show how vaccinations work to stop spreading of HBV and lower the overall disease burden in communities (Geta et al., 2024). The hepatitis B vaccine has improved public health indirectly alongside to its immediate impact on HBV infection. The vaccination has lessened the strain on healthcare systems by lowering the rate of hepatitis B, which has improved healthcare outcomes and reduced costs.

The hepatitis B vaccine is administered as a three-dose series via injection, usually in the arm. The WHO advises a vaccination schedule of 0, 1, and 6 months, however timetables may change depending on the national immunization program of a nation as shown in table 1. The lifelong risk of liver cancer can be decreased and protection against hepatitis B and hepatitis delta can be ensured by finishing the hepatitis B vaccine series, ideally starting at birth. The birth dose is crucial to a child's protection against hepatitis B since, in cases where they are not vaccinated, over 90% of newborns and up to 50% of young kids will have the infection for the rest of their lives. Be aware that different nations may have different immunization schedules for adults, children, and newborns as well as different vaccine brands and manufacturers.

Table 1: International vaccine schedule against Hepatitis B

Hepatitis B Vaccine Schedule					
Vaccine	Dose 1	Dose 2	Dose 3	Dose 4	References
3-Dose Vaccine	24 hours after birth	1 month	6 months	-	(Xu and Terrault 2024)
3-Dose Vaccine	At age of 1 year	1 year and 1 month	1 year and 6 months	-	(Kramvis et al., 2023)
4-Dose Vaccine	24 hours after birth	6 weeks	10 weeks	14 weeks	(Kusi et al., 2023)

Additionally, immunization campaigns have helped to avoid diseases like alcoholic liver disease and hepatitis C while also promoting liver health in general. In many regions of the world, hepatitis B vaccination protection is still below ideal levels, despite the vaccine's demonstrated efficacy and safety. Some populations face obstacles to vaccination uptake, including vaccine reluctance, low finances, and lack of availability of healthcare services (Block et al., 2021). In order to overcome these obstacles, initiatives are required to fortify the immunization system, raise vaccination knowledge, and enhance the availability of reasonably priced vaccinations.

In conclusion, by minimizing infection with HBV and its related problems, the hepatitis B vaccination has had a significant positive impact on global health. Countries have improved the wellness and good health of their citizens and considerably decreased the prevalence of hepatitis B through extensive immunization campaigns (Chien et al., 2022). To eradicate hepatitis B as a hazard to public health, however, more funding for immunization campaigns and initiatives to remove impediments to vaccination are necessary.

Side Effects of HBV Vaccine

It has been shown through numerous research and extensive real-world applications that hepatitis B vaccinations are extremely safe. The most commonly reported adverse effects in both adults and children are local reactions to the hepatitis B vaccine, which are typically mild and temporary (Udomkarnjananun et al., 2020). With a projected frequency of one instance in every 600,000 vaccine doses, anaphylaxis is the only significant adverse event that can happen after receiving the hepatitis B immunization. There is no link between the hepatitis B immunization and fever episodes, sepsis, neurological problems, or neonatal mortality in newborns (de Villiers et al., 2021). Thus far, there is insufficient data to determine whether the hepatitis B vaccine is linked to other documented severe side effects, such as transverse myelitis, arthritis, Guillain-Barré syndrome, spinal cord injury, retinal degeneration. Serious side effects following hepatitis B immunization are incredibly uncommon. It is impossible to determine if a serious adverse event that occurs after receiving the hepatitis B vaccine is the result of a real causal relationship or a coincidence (Pattyn et al., 2021). Thus, drawing firm conclusions from the case reports should be done with caution. The dissemination of equivocal findings by the press has a detrimental effect on the rollout of the hepatitis B vaccine. Nonetheless, it's important to keep an eye out for any possible health hazards related to the vaccinations, chronic fatigue, and autoimmune disorders (Machmud et al., 2021).

Conclusion

The state of hepatitis B vaccinations now represents a major advancement in preventive medicine. The hepatitis B virus, which causes hepatitis B, is a potentially fatal infection of the liver that can result in liver cancer, chronic liver disease, and other problems. However, the prevalence of new illnesses has drastically decreased globally since the introduction and widespread utilization of hepatitis B vaccinations. As vaccination technology has developed throughout time, more sophisticated formulations like recombinant DNA vaccines have been created, significantly enhancing vaccine efficacy and safety. The implementation of hepatitis B vaccination campaigns has had a significant effect on world public health. Furthermore, immunization campaigns aimed at teenagers and high-risk groups have made a significant contribution to

the fight against disease. Moving ahead, efforts to strengthen hepatitis B immunization programs will prioritize tackling these obstacles and inequities while striving for continuous innovation and progress. In summary, hepatitis B vaccinations have made significant progress in lowering the global burden of HBV infection. However, more work has to be done to accomplish global vaccine coverage, prevent HBV transfer, and ensure equal availability of vaccination for all. We can make progress toward a hepatitis B free future by building on previous triumphs and embracing continued innovation and collaboration.

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Chapter 36

Vaccine Hesitancy, Understanding Causes and Addressing Concerns

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ABSTRACT

Vaccines despite most effective medical treatments; vaccine hesitancy is still in top ten global health pressures declared by World Health Organization. Nowadays, it's thought that vaccine programmes could fail due to a lack of trust in vaccinations and this lack of trust is also not new; its present when first vaccine was make available. It is thought that vaccine hesitancy is the cause of declining vaccination rates and rising risks of vaccine-preventable disease outbreaks and epidemics. Factors that lead to vaccine hesitancy are grouped together in 3 domains. (1) Contextual factors refers to the impact of historical, socio cultural, environmental, fitness system, economic and political factors; (2) Individual and group influence refers to the impact of one's own perception of the vaccine or the social/peer environment; and (Immunivt) Vaccination-specific issues which include the cost, concerns regarding safety and efficacy and risk and benefits weightage. Vaccination effects are recorded on individual level, community level and even worldwide. According to estimation vaccines saves three hundred and seventy six million life years, ninty six million disability-adjusted life years (DALYs), and over six million deaths annually worldwide. So there are needs to make strategies to overcome vaccine hesitancy problem.

KEYWORDS
Vaccine hesitancy, Worldwide impacts, Political factors, Sociocultural factors, Complacency, Herd Immunity

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INTRODUCTION

Vaccines are among the most effective medical treatments now accessible. They are also among the fastest, least expensive , most well –proven remedies (Ozawa et al., 2016). Vaccines and Vaccination (the procedure of giving the vaccines) save lives, and the 21st century's longevity and sharp rise in life expectancy have been mostly attributed to the advantages of vaccinations (Andre et al., 2008). Vaccine based disease immunization continues to be in most effective public health initiatives of twenty first era. Additionally, tremendous progress has been achieved in immunising sizable populations against the most prevalent paediatric illnesses. Each year, Vaccination save millions of lives and have eradicated a number of illnesses both domestically and globally. The vaccine industry has fallen prey to its own success. But unfortunately, millions of individuals globally, meanwhile are still unvaccinated. The great accomplishment that has steadily raised life expectancy and improved population health around the world is currently in great difficulty, since its advancements has stalled for a few decades (Galles et al., 2021).

"A delay in acceptance or refusal of vaccines despite availability" is definition of vaccine hesitation (MacDonald, 2015). A significant obstacle to universal health care is vaccine reluctance both at the individual and community levels, as the unvaccinated population continuously contributes to the dynamic and costs of disease propagation (Causey et al., 2021). Vaccine hesitancy is in top ten global health pressures declared by World Health Organization due to its continuous occurrence (Nuwarda et al., 2022).

5Cs Model of Vaccine Hesitancy

The success of vaccine strategy is dependent upon wide acceptance of vaccines by people. Vaccine hesitancy is influenced by factors like confidence, Complacency, Constraints, Collective responsibility and calculations as described in figure 1. Confidence means trust in vaccine efficacy vaccine's safety and the trust on the system and the policy makers (MacDonald, 2015) . Complacency means perceived low risk of acquiring vaccine preventable diseases and little in general knowledge and awareness about vaccine roles (MacDonald, 2015). Constrains means availaibility of vaccines, accessability,

and affordability in low lost in addition with structural and physiological barriers by improving healthcare infrastructure to assure safe vaccine delivery and administration. Collective responsibility means in community people's willingness to protect others like in the case of herd immunity (Fine et al., 2011). Calculations is engagement in gathering extensive information about vaccine to build trust on it rather than hearing misinformation that ultimately leads to flawed decision making (Brewer et al., 2007).

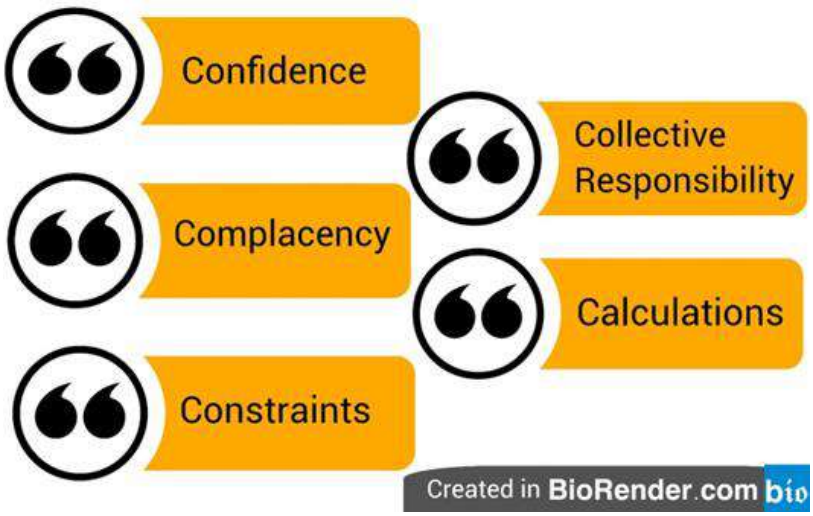


Fig. 1: Factors influencing vaccine hesitancy

History of Vaccine Hesitancy

A history of vaccine reluctance exists that began in France in 1763, when there was reason for mistrust of vaccinations. As time went on, people continued to hold onto this antiquated belief that vaccines inflict more damage than good and that vaccination is a curse, even in the face of technological advancements (Rothstein, 2015). Following are described some key happenings in relative to vaccine hesitancy.

Cow Mania (1798)

It would be more accurate to state that vaccination hesitancy has longstanding origins, dating back to the publishing of the Jenner vaccine discovery results. The local church said that it was directly against God's will to combine animal parts with human flesh. Others worried that the vaccination might result in "Cow-Mania," citing examples of an old woman who supposedly developed horns after receiving the shot and a youngster with an ox face (Nuwarda et al., 2022) .

1870's Anti Vaxx Movement across (EU) Europe

In London, the first Anti-Compulsory Vaccination League was established in 1867. Its goals included preserving individual autonomy and choice over vaccination in situations where vaccination was required. A number of such anti-vaccine movements later arose in various nations in the 1870s and 1880s (Grignolio, 2018; Nuwarda et al., 2022).

The Cutter incident of Polio Vaccine in 1955

In the US, one of the worst polio vaccine-related tragedies happened in 1955. Several batches that were made available to the general population contained the active polio virus, despite the obligatory safety testing being successful. Salk's vaccine, produced by a tiny, family-run business called Cutter Laboratories, was connected to more than 250 cases of polio. There were a few vaccination lots that were made public that included live, active polio virus instead of a fully inactivated virus. This vaccination, which included live polio viruses, was given to 120,000 children; 70,000 of them developed mild polio as a result, 200 of them suffered irreversible disabilities, and 10 of them passed away. The foundation for mistrust among the pharmaceutical sector was laid by this Cutter incident (Nuwarda et al., 2022).

1970s and 1980s

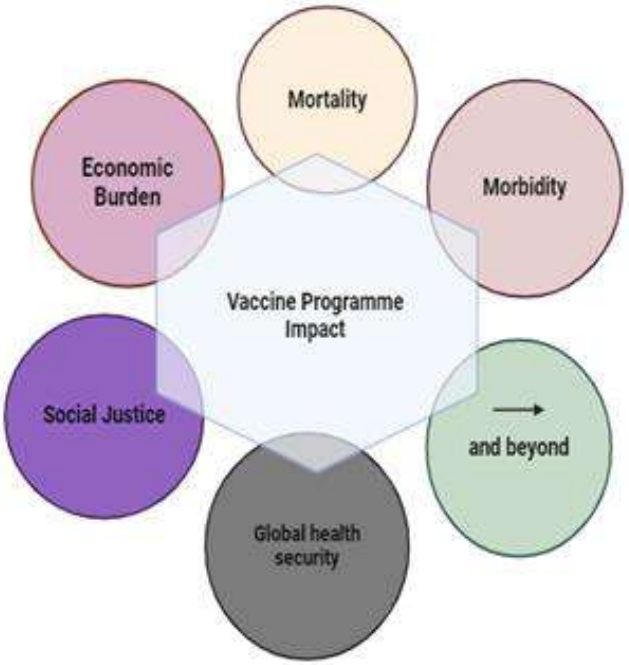
In 1982, after many controversies on diphtheria, pertussis and tetanus; a NBC documentary named A Shot in the Dark and Vaccine Roulette was released to change public's perception that was started to change due to controversy surrounding DPT vaccination programmes, which led to lower vaccination rates and legal actions against manufacturers (Baker, 2003; Fisher, 2011; Kulenkampff et al., 1974).

How do Vaccination Impact the World, Nations and Communities

As a reminder of how vaccines can help us get closer to a safe and healthy future, the COVID-19 pandemic has ushered in a new era of immunization and vaccination (Brendle, 2023). The creation of safe and efficient vaccinations against illnesses that cause major morbidity and death has been one of the most important scientific breakthroughs of the twenty-first century. Vaccination is undoubtedly one of the public health measures that has improved health outcomes

worldwide, along with access to clean drinking water and sanitary conditions. An estimated 6 million fatalities from vaccine-preventable diseases have been avoided annually thanks to vaccinations (Ehreth, 2003).

Fig. 2: Impacts of Vaccine programme



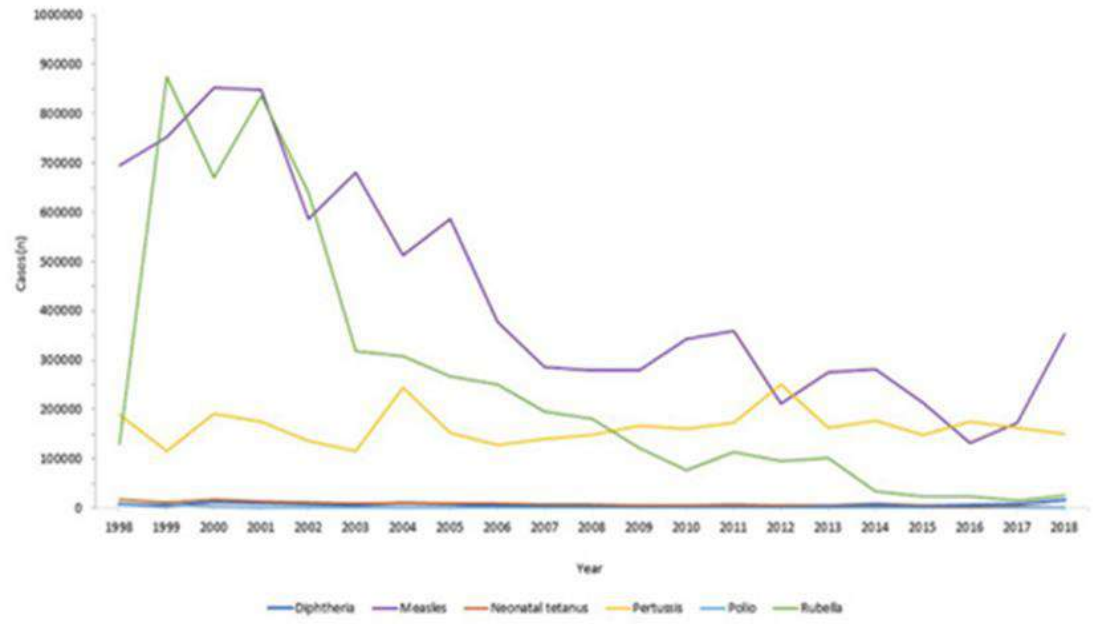


Fig. 3: Worldwide decrease in infectious illnesses. Data from the WHO over the past 20 years demonstrate the drop in rubella and congenital rubella syndrome as well as the control of diphtheria and tetanus worldwide (data not displayed). Information derived from the "Reported cases of vaccine-preventable diseases" dataset from the World Health Organization (World Health Organization, 2019c).

Impact of Vaccination in Community

The prevention of morbidity and death from major illnesses, which disproportionately affect children, has been the main effect of vaccinations as described in figure 2. Estimates shows that vaccine prevent six million deaths, 96 million disability adjusted life years (DALYs) and 386 million life years annually globally (J. J. V. Ehreth, 2003). Antiquated metrics for assessing the impact of vaccinations comprise vaccine efficacy, which measures the direct protection provided to a vaccinated group in ideal circumstances, such as trial settings, or vaccine effectiveness, which assesses the direct and indirect effects of vaccines on the population in an actual context (Wilder-Smith et al., 2017).

Herd Immunity

For the majority of vaccination recipients, personal, direct protection is the primary health benefit. The ability to create herd immunity is the added benefit of vaccination (as described in figure 3) from a community health perspective. When a significant enough percentage of the populace is immunized, the infectious agent's spread is stopped, safeguarding others who are not, who could include people who are too young, too weak, or too immunocompromised to benefit from vaccinations. As part of the standard EPI, very effective immunization programs have been put in place against encapsulated bacteria that are carried in the oropharynx asymptomatically but have the ability to penetrate and cause meningitis and septicemia in people of all ages. (Rodrigues and Plotkin, 2020).

Elimination of Infected Diseases

High levels of population immunity are necessary for the global eradication of infectious illnesses (described in more detail in figure 3) in order to prevent continuous transmission in our interconnected world (Andre et al., 2008). The World Health Assembly declared in 1980 that the smallpox disease had been completely eradicated through ring vaccination, thanks to Jenner's effective creation of the vaccine utilizing the vaccinia virus (Jenner, 2023; Strassburg, 1982).

Impacts of Vaccination on Nation

Until leaders give in to vaccine nationalism, which is a common occurrence, vaccines remain the best chance to contain the pandemic. The rapid development of safe and effective COVID-19 vaccines is a credit to modern scientific capabilities. It will also test the political will and moral resolve of the world community to put an end to this pandemic. As it is not feasible or practical to be able to predict the location or nature of the next emerging threat, investment of an estimated $4.5 billion/year in healthcare systems could help speed up responses to infectious epidemics by prompt identification of the agent and effective control measures to limit the spread and consequences of disease (Rasanathan and Evans, 2020). A number of wealthy countries have engaged in vaccine nationalism in the past. It is reasonable to prioritise the health of one's people, but the means by which this has been accomplished is morally and ethically repugnant.

Impacts of Vaccination on World

Boosting the Infrastructure for Social and Health Care

Global factors are also important, as seen by the EPI's creation in 1974, when all nations were required to supply these vaccinations, strengthening their infrastructure for primary and public health care in the process, which had benefits that extended beyond the vaccination program. Vaccination helps meet the Sustainable Development Goals by 2030 as well as the UN Millennium Development Goals. The Vaccine Alliance, or Gavi, has been a major supplier of money, vaccines, and assistance to nations where the gross national product per person was less than £1000 annually (Hinman and McKinlay, 2015). Long-lasting and advantageous collaborations can result from the creation of vaccination programs in low- and middle-income countries (LMIC) for use in other health and social care initiatives (Shearley, 1999).

Although the effects of vaccines are widespread and profound, they are not always easily measured, examined, or reported. Vaccine delivery to all children and vulnerable individuals around the world is still fraught with difficulties, particularly for those living in politically, socially, or geographically remote communities. Only sustained commitment and dedication on a global, national, and personal level will be able to overcome these obstacles (Rodrigues and Plotkin, 2020).

Factors Contributing to Vaccine Hesistancy

What’s behind vaccine hesitancy? Why are some people ‘vaccine hesitant’? We try to answer these questions or question like this by discussing the factors involved in Vaccine hesitancy. The factors of vaccine hesitancy are greatly affected by 3 main issues or determinants – complacency, confidence and convenience. .

The idea behind the child vaccine hesitancy is that ‘if I vaccinate my child and something bad happens’ like what would happen from almost any immunization or the symptoms that occur after immunization. The psychology behind that is that how they weight their role in that bad thing happening to their kid verses if they don’t take action and they get sick from that disease. According to Moral psychology, taking action allowing that harm to happen carries much more psychological weight than not taking action.

Here, we discuss all the factors influencing the person’s decision regarding vaccination. People's views and behaviors about vaccination are influenced by a wide range of factors, including cultural beliefs, misinformation, and concerns about safety and effectiveness. These factors are grouped together in 3 domains. Specifically, 1) Contextual factors refers to the impact of historical, sociocultural, environmental, health system, economic and political factors; 2) Individual and group influence refers to the impact of one's own perception of the vaccine or the social/peer environment; and 3) Vaccination-specific issues which include the cost, concerns regarding safety and efficacy and risk and benefits weightage as shown in Fig. 4.

Contextual Factors: Impacts Resulting from Past Events, Norms and Cultural Behaviors, Impacts of Health Sectors

Socio-economic Status (SES)/Level of Income

Socio-economic status is the first barrier that are lying under the heading of contextual factors. It affects the 3 factors that can contribute to the person decision for vaccine, which is knowledge about vaccine, its cost and the motivation. The

level of person's socio economic status determine the ability to receive the education that are necessary for understanding factors that improve the health status, that would raise awareness for the need of vaccination (Harapan et al., 2016). The cost include not only refers to the cost for vaccination but also the time and other factors that are required to achieve a good health. Also the type of work or job can also be a determinant, for example a person with high socioeconomic status such as a civil servant are more likely to have a positive attitude as compared to lower working class like farmers (Harapan et al., 2017; Harapan et al., 2016).

According to eight studies, high level of income is considered as the factor that contribute to vaccine acceptance as compared to residency in rural areas with low income levels. While five studies considered high household income as a factor that influence its acceptance (Efendi et al., 2020).

Level of Education

High level of education is a determining factor for vaccine acceptance, five studies proves that higher education impacts on person view and beliefs towards vaccine acceptance.

Exposure to Negative Media

Media play a massive role in vaccine hesitancy (Benninghoff et al., 2020). Religious authorities, scholars and public figures all can impact and emphasize the urgency of vaccination, raising awareness and establishing protocols etc. (Seale et al., 2015). Studies have shown that those who interact and follow such false material online are more likely to display vaccine hesitation. The information that circulates about vaccine in social media includes the unproven claims about the side effects of vaccine such as autism, fertility problems or other medical conditions without any scientific evidence.

Misleading information can also spread through social media platforms, such as conspiracy theories claiming that vaccines are a part of a government or corporate to control the population at the expense of public health (Rathje et al., 2022).

Historical Events

The impact that leave by past events are long lasting that they influence current attitudes towards vaccination. The one of finest example regarding historical event is MMR Controversy in late 1990s and early 2000s by Dr. Andrew Wakefield, connected the vaccine to autism which cause different population to become reluctant about getting vaccine

Vaccine and Vaccination Specific Issues

Costs

It is quite common we found some people hesitant towards some vaccines that has high cost and they are reluctant to take the vaccine as well as there are also other barriers such as vaccine time of delivery, location and inequalities (Efendi et al., 2020; Harapan et al., 2016).

Concerns Regarding Safety and Side Effects of Vaccine

This is the most important determinant of vaccine hesitancy. The conspiracy theories with the spread of other myths or misinformation lead to make people hesitant towards vaccine. There is a myth that the COVID-19 vaccine was developed hurriedly, hence its efficacy and safety cannot be guaranteed. However, research indicates that the two initial vaccines are both approximately 95% effective and did not cause any major or fatal side effects. There are so many reasons for the rapid development of Covid-19 vaccine.

Another myth is that the COVID – 19 vaccine can affect female fertility. The truth about the COVID – 19 vaccine is that it will not affect fertility, rather it stimulate the body to produce copies of the spike protein found on the coronavirus's surface. This "trains" the immune system of the body to fight viruses which has particular spike proteins (Mahase, 2020).

Risk – Benefits Weightage

This scientific barrier was found on the research of new and novel vaccines such as rotavirus vaccine, ebolavirus vaccine, Zika vaccine and vaccine for dengue. Because many people consider it risky to get a vaccine that has chances for potential side effects. People may compare the perceived risk with benefits of immunization. One study finds out that the main purpose of these vaccine to provide full immunization to population with or without minimum side effects. But population believe that there is no need to get vaccination if disease could be prevented with standard medical protocols (Padmawati et al., 2019; Widayanti et al., 2020).

Individual/Social Group Influences

Impacts of Family and Community Experience with Vaccine

Positive vaccination experiences of parents leads to a vaccine acceptances and they are more likely to vaccinate their Childs. Vaccinated parents feel the vaccine is generally safe. Fear for safety, morbidity, and mortality from unknown side effects is a reason. Parents who never vaccinate rely on friends and family for information and many fall a prey for false information. A reaction within normal bounds such as fever cause people to become reluctant towards vaccination.

Perspectives and Views

Individual views and beliefs on vaccinations, which are impacted by their level of awareness and understanding, are important for the acceptance of vaccines such as people often believe that the risks overshadow the benefits, as the vaccine contain toxic adjuvants which cause serious health impact such as autism an infertility .

Vaccination as a Social Norm vs Not Needed/Harmful

Some people hesitate due to concerns regarding the novelty of vaccine, inadequate testing as well as efficacy of vaccine. Misunderstanding can be caused by false beliefs that vaccine are unneeded invention and harmful (Callender; Callender and immunotherapeutics, 2016).

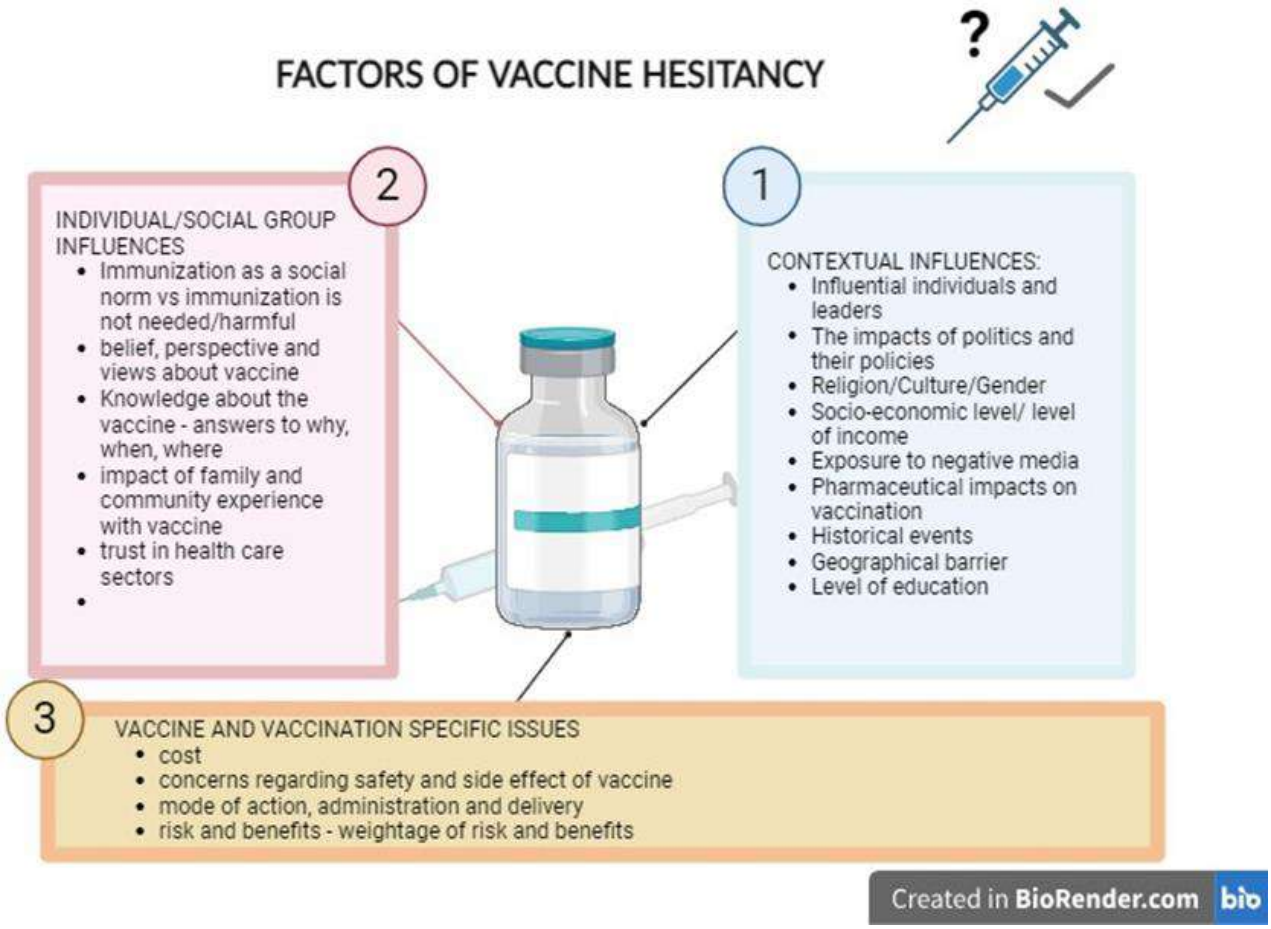


Fig. 4: Factors contributing to vaccine hesitancy

Effects of Vaccine Hesitancy

Because the illnesses that vaccines prevent are essentially unknown to the general public, many individuals no longer view vaccinations as required, despite their great effectiveness (Dubov and Phung, 2015). Vaccine reluctance is largely caused by parental worries about vaccine safety in general (McClure et al., 2017).

Many individuals are beginning to doubt vaccinations because of fear of purported toxins and additives in vaccines, which coincides with the increased public interest in so-called natural goods and therapies and raises questions about vaccine safety. Multiple needlestick injuries and receiving too many vaccinations for the immune system to manage properly are two more parental worries (Dempsey et al., 2011).

Immunization reluctance has far-reaching consequences. Community pediatricians who work with parents who are reluctant to get vaccines often report feeling more burned out and less satisfied with their jobs (McClure et al., 2017).

Some people's reluctance to get vaccines is influenced by their experiences of being socially excluded. The latter destroyed a sense of social cohesion, ruined trustworthy government-citizen ties, and created a plethora of socioeconomic obstacles to high-quality immunization services. Due to these experiences, a large number of marginalized individuals now reject vaccination as a form of agency, mistrust it, or choose not to get vaccinated because of the time and opportunity expenses involved (Cooper et al., 2019).

Internet searches for "vaccination" may turn up more anti-vaccination content than pro-vaccination content (Freed et al., 2010). Parents are left with conflicting information and unsure of whether websites, blogs, and articles on the Internet promote the risks associated with vaccinations (Gust et al., 2008).

There are significant dangers associated with vaccine hesitancy for the general public as well as for those who postpone or decline vaccination. Communities won't be able to achieve the coverage criteria required for herd immunity as a result. Vaccine hesitancy is a complicated and ever-changing social process that is influenced by several networks of meaning, logic, and power (Wiysonge et al., 2022).

The number of persons refusing to receive the required immunizations is rising day by day. Since the inception of vaccination, some members of the public have harbored doubts about the efficacy and safety of vaccines. However, data suggests that vaccination reluctance trends have been worse recently (Wiyeh et al., 2019).

Future Prospects

Addressing protection from immunization is intricate in light of the fact that there is no agreement about the wellspring of the issue or the best means for settling it. (Rittel and Webber, 1973)The inescapability of talk against vaccination has driven a few web-based entertainment stages to restrict the spread of content against vaccination (Fischer, 2019) However, this approach is positively an important stage in countering vaccination misdirection on the web, it is logical insufficient; numerous gathering went against to vaccine(s) have sorted out some way to move past these limitations. (Bradshaw et al., 2021). General wellbeing needs to take part in online entertainment conversations about immunization (MacDonald and Dubé, 2020). Correspondence procedures need to move past the information shortfall model to take on additional powerful methodologies (Rossen et al., 2016). Although, essentially imparting data about immunization wellbeing and adequacy to those who are vaccine hesitant is obviously inadequate to stem the development of hesitancy (Dubé et al., 2015). In the event that social disease adversely affects immunization acknowledgment, this cooperative local area approach can likewise be utilized to decidedly move the negative immunization talks and address antibody pundits (Buttenheim and Asch, 2016). Various drives that have prepared guardians who esteem vaccination and have furnished them with apparatuses to participate in certain exchange about vaccinations in their networks have shown promising outcomes (Attwell and Freeman, 2015; Schoeppe et al., 2017).

Conclusion

Vaccine not withstanding best clinical medicines; immunization aversion is still in top ten worldwide wellbeing pressures proclaimed by World Wellbeing Association. These days, it's believed that immunization projects could bomb because of an absence of confidence in immunizations and this absence of trust is likewise not new; its current when first antibody was make free. It is believed that immunization aversion is the reason for declining inoculation rates and increasing dangers of antibody preventable illness flare-ups and plagues.

As represented in the model, notwithstanding the elements influencing immunization acknowledgment at the singular level, a smart comprehension of antibody reluctance should be grounded in the specific verifiable, political and socio-social setting in which inoculation happens. Thought ought to likewise be given to more extensive effects on antibody aversion, for example, the job of baric wellbeing and immunization arrangements, correspondence and media and wellbeing experts.

The developing interest in immunization aversion has brought about the advancement of various apparatuses and procedures to upgrade vaccination acknowledgment. Numerous specialists have proposed ways of countering antibody aversion at the populace level, remembering straightforwardness for strategy pursuing choices with respect to inoculation programs, expert voiding training and data to general society and wellbeing ace videos about the thorough cycle that prompts endorsement of new immunizations and differentiated post-showcasing observation of immunization related occasions. Also, as focused by Larson and collaborate pinnacles, "extra accentuation ought to be put on paying attention to the worries and understanding the view of general society to illuminate risk correspondence and to consolidate public perspectives in arranging antibody arrangements and programmes."22 At long last, as their job is vital in supporting the outcome of immunization programs, more exploration is expected to comprehend the reason why some wellbeing experts, prepared in clinical sciences, actually have questions in regards to the security and adequacy of immunization.

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Chapter 37

Impact of Vaccination on Bacterial Meningitis

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ABSTRACT

Bacterial meningitis is a major source of illness and mortality in children. Children under 2 years old have an underdeveloped response to polysaccharide antigens, making them more vulnerable to encapsulated bacterium infections. Three bacteria i.e. *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitides* cause the majority of cases of acute bacterial meningitis. Since these vaccines have been around for the longest, high-income countries have been able to measure the incidence best. Conjugate vaccinations, which generate T cell memory, can provide immune protection to these children. The first of vaccines to be made available was the conjugate vaccination against *H. influenzae* type b (Hib). The incidence of all invasive Hib diseases, including meningitis, dramatically decreased after its introduction. This decrease was partly brought about by the vaccinations capacity to lower the organism's nasopharyngeal carriage and so create herd immunity. Since the introduction of the vaccine, cases of *H. influenzae* meningitis have been almost completely eradicated. Conjugate vaccines have been added to immunization schedules in developed nations to prevent bacterial meningitis, however, many underdeveloped nations cannot afford to use them due to their expensive cost. To deliver these incredibly effective vaccines to the places that need them, progress must be made.

KEYWORDS
Vaccination, Meningitis, Bacterial meningitis, Bacteria, Vaccine

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INTRODUCTION

Meningitis is a disease of the meninges and the subarachnoid space. It may also impact the brain cortex and parenchyma due to the cerebrospinal fluid's (CSF) tight anatomical interaction with the brain (Buonomo et al., 2024). The classic meningitis symptoms of headache, fever, and stiff neck are brought on by meningeal and subarachnoid space inflammation, which also results in pleocytosis in the CSF. The presence of the brain cortex and parenchyma as a result of vascular issues or direct inflammation can result in specific neurological abnormalities, loss of consciousness, and behavioral changes that are all considered indicators of encephalitis. Numerous infectious agents can cause acute meningitis, but it can also be a symptom of non-infectious diseases. The most serious type of this illness is bacterial meningitis, which is primarily acquired through the respiratory system but can also enter the digestive tract, as in the case of a listerial infection (Rybak et al., 2024). Meningitis may develop in the community on its own or as a side effect of invasive procedures or head trauma in a medical facility.

The tiny, pleiomorphic Gram-negative coccobacillus known as *Haemophilus influenzae* is unique to humans. Its growth requirements are very strict, it can only grow in culture mediums that are enriched with nicotinamide adenine dinucleotide (NAD) and hemin, such as chocolate agar. The two main categories of *H. influenzae* strains are capsulated and non-capsulated strains. The polysaccharide capsules of the capsulated strains are further classified into six classes according to their chemical makeup. The polysaccharide capsule of type b (Hib), the most virulent strain of *H. influenzae*, is primarily responsible for its virulence, as it is made up of polyribosylribitol phosphate. The nasopharynx is colonised by *H. influenzae* with the conjunctivae and genital tract being less affected (Alabdullah et al., 2024). *H. influenzae* and, to a lesser extent, *H. parainfluenzae* are the primary colonisers of the respiratory tract. Three to five percent of people have capsulated strains in the upper respiratory tract, while about eighty percent of people have NTHi strains in the nasopharynx. Direct contact with secretions or respiratory droplets is the two ways in which diseases are spread from one person to another. Hib was the most prevalent factor causing bacterial

meningitis in young children in the Iceland, United States, Sweden, the Netherlands, England, and Wales prior to the advent of Hib conjugate vaccinations. Children that are three months to three years old accounted for 75% of instances of Hib meningitis (Tapci et al., 2024). In high-income nations, the case fatality ratio for Hib meningitis was 5 to 10%.

The bacterial species *N. meningitidis*, often known as meningococcus, belongs to the Neisseriaceae family. In addition to being significant unique human pathogens that can cause meningitis and sepsis, meningococci are prevalent bacterial commensals of the nasopharynx. Only six of the 13 serogroups of *N. meningitidis* (A, B, C, W, X, and Y) cause the majority of invasive diseases that are fatal (SI et al., 2024). These serogroups are distinguished by distinct capsular polysaccharides. The pathogenic strains can arise and spread globally because they are part of a small number of genetically determined clonal complexes.

It is commonly known that invasive illness is an uncommon consequence of *N. meningitidis* carrier, but asymptomatic carriage is widespread. Although carriage is crucial to the development and spread of bacterial infections (Alfvén et al., 2024). As previously noted, infection without symptoms caused by both pathogenic and non-pathogenic *Neisseriae*, including *Neisseria lactamica*, may aid in the establishment of protection by generating a natural immunity against illness. For this reason, a lifetime of recurrent bouts of *N. lactamica* carriage and meningococcal is expected. It is unclear, though, how much cross-protection which is probably very temporary lowers the chance of infection and/or illness.

Determining the impact of vaccination regimens on the rising incidence of Invasive meningococcal disease (IMD) requires an in-depth awareness of the dynamics of meningococcal infection. The carriage rates and the disease to carriage ratio are particularly important parameters in order to understand how meningococcus circulates in human populations, evaluate changes in trends and the main causes of IMD outbreaks, and quantify the prospective effect of vaccination as a result of direct and indirect effects.

Epidemiology

Worldwide, bacterial meningitis is acknowledged as a contributing factor to both mortality and morbidity (Bhatti et al., 2024). However, factors such as age, location, and a significant amount of the causative agents can affect the rate of death and morbidity. The majority of patients are newborns and infants, particularly in developing nations. While bacterial meningitis is rare in affluent nations, it is regarded as a serious sickness in underdeveloped ones.

There are regional variations in the prevalence of bacterial meningitis worldwide. In the UK and Western Europe, the incidence is 1-2 cases per 100,000 people yearly, whereas in the Sahel region of Africa, it can approach 1000 cases per 100,000 people annually (Dione et al., 2022). Over the past few decades, there has been a significant decrease in incidence, which has been mostly attributed to the development and widespread use of conjugate vaccinations. A protein is linked to purified bacterial capsular polysaccharide in conjugate vaccines (Mandal 2021). This triggers a stronger and longer-lasting immunological reaction, particularly in young children. Majority of the incidence decline has been seen in children under the age of one.

There are an estimated 16 million cases of bacterial meningitis worldwide, and it is linked to high rates of morbidity and fatality in 2013, resulting in 1.6 million years of disability every year (Pinilla-Monsalve et al., 2023). The highest rate of meningitis illness is found in Africa. Before a vaccine was developed, the estimated annual rate of invasive diseases caused by infections with *S. pneumoniae* (pneumococcus) and *H. influenzae* type b (Hib) was 38 and 46, respectively, per 100,000 people in children under the age of five and *Neisseria meningitidis* infection was more than 1,000 per 100,000 per year among all ages (Parikh et al., 2020). Due to a lack of diagnostic techniques, it is unclear how common certain etiologies of community-acquired bacterial meningitis are in Africa.

Meningococcal disease epidemics in Africa are confined to a specific area known as the meningitis belt. From Senegal to Ethiopia, this sub-Saharan region is vulnerable to sporadic epidemics of meningococcal meningitis, with rates in the worst outbreaks approaching 1% of the population (Viviani 2022). Serogroup A *N. meningitidis* was typically the cause of epidemics, but a vaccination strategy against this bacterium that was started in 2010 has decreased its incidence (Leong et al., 2024). It has been demonstrated that some serotypes of the primary microorganisms that result in bacterial meningitis are more likely than others to cause severe illness. Meningococcus serogroups comprise the majority of illness cases, however a total of 13 serogroups have been discovered. Although at least 94 pneumococcal strains have been identified, the 10-valent and 13-valent vaccine formulations that are now on the market cover the serotypes that account for more than 70% of infections in most parts of the world (Oliveira et al., 2021).

In the Netherlands, following routine vaccination with a single dosage at 14 months together with a drive to catch up for kids and teenagers aged 1 to 18 years, which created herd protection for infants, there was a significant decline in MenC IMD among those who had received the vaccination (Parikh et al., 2020).

As a result of a single dose at 12 months and a catch-up vaccine for individuals under 20 years old, vaccination effectiveness ranged from 75% in Australia to 96.8% in Canada, where 82.1% of individuals between the ages of 2 months and 20 years received vaccinations (Soumahoro et al., 2021). Reductions in IMD incidence rates were noted in a number of other nations, including Brazil, Italy, and Canada. MCC vaccinations tend to offer significant levels of protection in the short term and lower serogroup carriage prevalence, leading to herd immunity, however, the length of protection varies with age, with older children experiencing longer protection than newborns (Ferreira et al., 2020).

Streptococcus pneumoniae

Pneumococcus is the most prevalent cause of bacterial meningitis in humans in various parts of the world. The polysaccharide capsule, which identifies more than 90 antigenically distinct serotypes of *S. pneumoniae*, serves as the target for all currently approved vaccines (Davies et al., 2022). For the past 15 years, pneumococcal conjugate vaccinations, or PCVs, have been administered. Targeting seven pneumococcal serotypes, PCV7 was recently licensed in the USA and Europe. More recently, PCV10 and PCV13, which cover ten and thirteen serotypes, respectively, were released. There are 23 serotypes covered by the PPV23 polysaccharide vaccine (Berman-Rosa et al., 2020). Conjugate vaccines were mostly used in children until recently. However, a recent placebo-controlled trial in adults 65 years of age and above revealed that PCV13 is effective against non-bacteraemic pneumonia, Pneumococcal pneumonia of the vaccination type and invasive pneumococcal illness with vaccination efficacies of 46%, 45%, and 75%, respectively (Heo et al., 2022).

There is evidence to suggest that pneumococcal vaccine (PCV) is more immunogenic than polysaccharide vaccine, yet the two vaccines are not comparable (Lucinde et al., 2021). Conjugate vaccinations also result in significant herd immunity, which protects others who are not vaccinated when a portion of the population is immunized. Both vaccinated and unprotected populations have significantly reduced disease rates due to vaccine serotypes, according to large research. Since the introduction of conjugate vaccinations, there have been reports of serotype replacement (El-Beyrouty et al., 2022). This is a rise in the prevalence of illness or asymptomatic miscarriage brought on by serotypes that are not immune to vaccinations. On the other hand, invasive pneumococcal disease incidence has decreased overall.

Neisseria meningitidis

There are thirteen serogroups of meningococci, of which five (A, B, C, W135, and Y) account for the majority of invasive illness cases. In Europe, serogroup B is the most prevalent strain, accounting for the majority of cases in Wales and England. Serogroup Y is the most prevalent in the USA and ranks second in several regions of Europe. Serogroup W135 is becoming more common in the UK and has been connected to a clone from South America. Because this clone is a member of the more lethal ST11 clonal complex, or cc11, disease induced by it is linked to a greater death rate (Bettencourt et al., 2022). Recent epidemics of meningococcal C illness among men who have sex with males have been linked to the same clonal complex.

The number of cases per 100,000 individuals decreased from 4.5 in 2001 to 0.6 in 2012, the incidence in the Netherlands has decreased.27 Other nations have observed comparable outcomes. In Africa, serogroup C illness first surfaced in 2015. Serogroup A has caused significant epidemics in the African meningitis belt, but, as a result of extensive immunization, there have been dramatic decreases in recent years (Mazamay et al., 2021). The goal of the WHO-Programme for Applied Technology in Health-led Meningitis Vaccine Project was to vaccinate 250 million individuals in Africa with the novel serogroup A conjugate vaccine (Parsodkar et al., 2021).

Haemophilus influenza

Prior to the widespread use of conjugate vaccinations, *H. influenzae* type B was a significant cause of meningitis, particularly in newborns and young children (Slack et al., 2020). Similar to meningococcal disease, H influenza type B has all but vanished in regions where vaccination is not routine, but it still poses a threat in other locations. Nonetheless, the prevalence of invasive Hemophilus illness brought on by non-type B strains has grown (Poplin et al., 2020). A portion of these infections are caused by different encapsulated forms of *H. influenzae*, including kind's e and f, although the majority are caused by non-typeable organisms.

Pathogenesis of Bacterial Meningitis

There are four primary phases in the bacterial meningitis pathogenesis, which include colonization, Entrance into the subarachnoid area, bloodstream invasion, and bloodstream survival (Barichello et al., 2023). Many elements of this pathogenesis are yet unknown. A mix of host and bacterial variables result in the ensuing infection and neurological harm. The pathophysiology of meningitis caused by *S. pneumoniae* and *N. meningitidis*. The upper respiratory tract mucous membranes are first colonized by a large number of meningitis-causing bacteria (Hasbun 2022). Via the bloodstream, bacteria may reach the subarachnoid area or by spreading diseases from nearby areas, such as the sinuses in the paranasal region (Hasbun 2022). Blood-borne pathogen invasion, subarachnoid space penetration is thought to occur primarily through a multi-step process that includes mucosal colonization, invasion, their survival, and proliferation of bacteria in the circulation, and ultimately breaking through the blood-brain barrier (Feagins et al., 2020).

Bacteria in the subarachnoid region produce bacterial lysis, which in turn activates the immune system. According to bacterial particles trigger an extra inflammatory response that results in continuous neutrophil migration across the blood-brain barrier and chemokine and cytokine production (Arshad et al. 2020). Chronic inflammation subsequently leads to vasculitis, increased intracranial pressure, cerebral edema, reduced cerebral perfusion, and metabolic disturbances, all of which worsen ischemia and neuronal damage as shown in (Fig. 1).To lessen complement-mediated bacterial clearance, a variety of bacterial surface substances, in addition to the capsule, target certain complement components. Through surface molecules such as fH-binding protein (fHbp), neisserial surface protein A (NspA), and porin B, *N.*

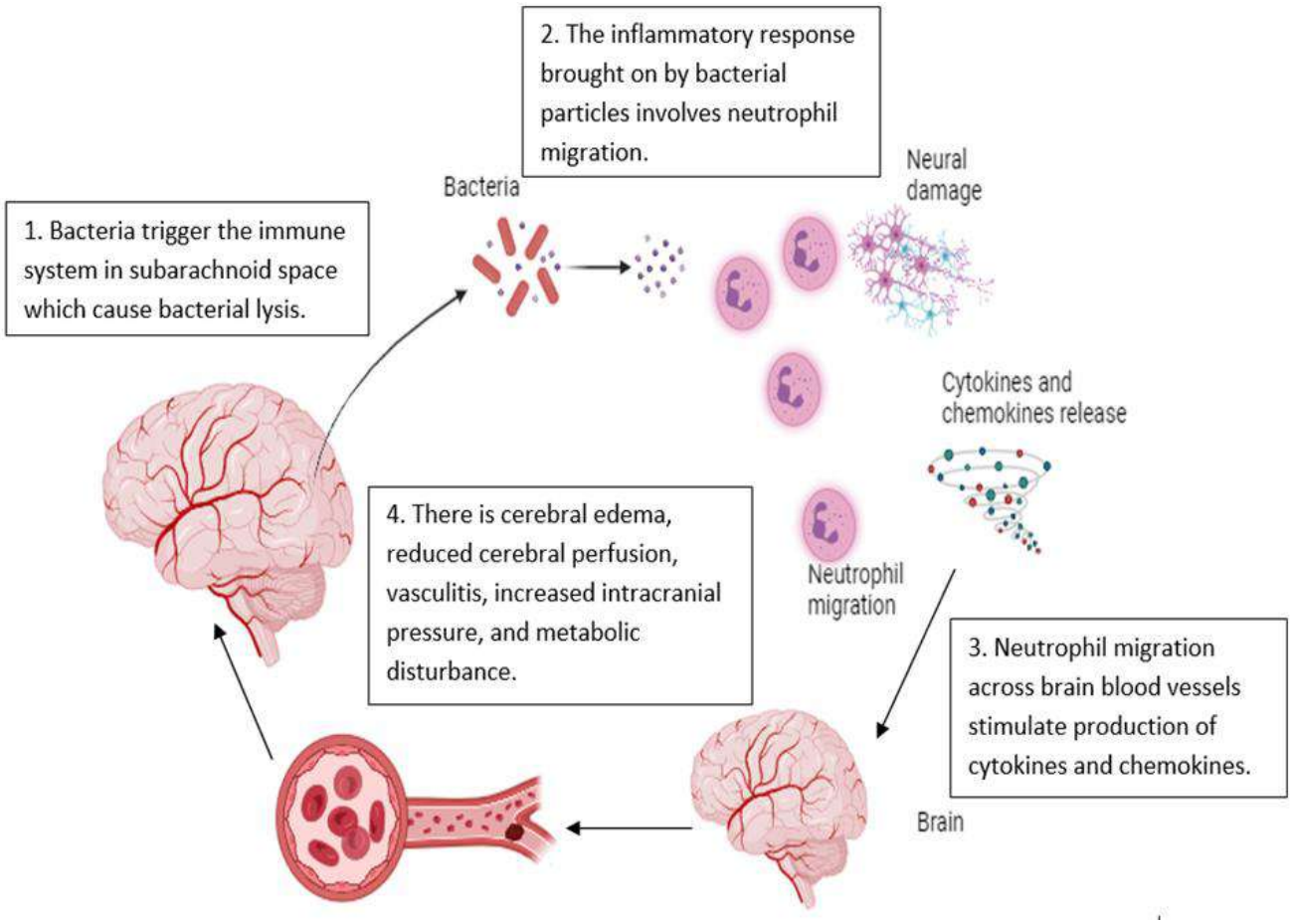

Fig. 1: Pathophysiology of Bacterial meningitis (Retrieved from Biorender)

meningitidis can directly connect to factor H (fH), the primary regulator of alternative complement activation. A "danger signal" for *N. meningitidis* has been identified as the rise in ambient temperature that happens when the bacteria move from the nasopharynx to the bloodstream (Farmen et al., 2021). This signal causes an increase in fHbp expression and capsular biosynthesis, which strengthens the bacteria defence against complement attack. Comparably, the surface proteins of *S. pneumoniae* engage with complement, deplete it, and block the complement cascade.

Effects of Vaccine

Vaccination is a safe way to build protection against a few of the most common causes of meningitis. Immunization protects those who are most susceptible to the illness, prevents it from spreading across communities, and eventually benefits the wider public. When compared to polysaccharide vaccinations, conjugate vaccines significantly boost serotype-specific T-cell-mediated immunity, decreasing both mucosal both invasive illness and carriage. Nevertheless, vaccination efficacy is restricted to serotypes that are included, and the introduction of non-vaccine and non-typeable serotypes has reduced vaccine efficacy. Licensed vaccinations against *N meningitidis* serogroup B are the only meningitis vaccines based on proteins that have been developed to date (Sulis et al., 2022).

Streptococcus pneumoniae

Since *S pneumoniae* is the most common cause of meningitis worldwide, immunization has been a major strategy for preventing the illness for over 40 years. Pneumococcal vaccinations contain the more than 90 capsular serotypes of pneumococcal bacteria that have been identified as posing the highest illness burden (Aravindkumar, 2022). Targeting 14 serotypes, pneumococcal polysaccharides (PPVs) comprised the initial pneumococcal vaccination, which was granted a licence in the United States in 1977 (Musher et al., 2022).

With the advent of the PCV vaccination in the USA in 2000, invasive pneumococcal illness in children was essentially eradicated, resulting in a reduction of over 90% (Deloria Knoll et al., 2021). This was due to the vaccine's inclusion of certain serotypes. In high-income nations, similar declines in meningitis and serotype-specific pneumococcal bacteremia have been noted in both adults and children. The introduction of PCV7 did not alter the frequency of pneumococcal meningitis in the UK, however, incidences decreased by 48 percent once the PCV13 vaccination was switched to in 2010. Since its release in 2010, PCV13 has protected against up to 80% of meningitis-causing strains globally, while coverage varies by area (Koelman et al., 2020).

Pneumococcal meningitis rates have decreased after PCV13 was added to the Expanded Programme on Immunization (EPI) schedule in 2012 in countries that are eligible for the Global Alliance for Vaccines and Immunization. Estimates of these reductions are beginning to emerge. Despite the outbreak of pandemic serotype 1 meningitis in Ghana, PCV13 may not be very effective although the prevalence of meningitis has decreased in children outside of the Sahel in low- and middle-income nations (Chapman et al., 2020). The expensive expense of PCV13 for nations that are not qualified for GAVI has restricted its global implementation. 2020 saw the WHO grant a license to the Serum Institute of India for a low-cost PCV10 vaccine. This vaccine contains ten serotypes that account for almost 80% of pneumococcal illness cases in children in low- and middle-income nations (Bilgin et al., 2022).

Neisseria meningitides

There is a great deal of variation in the incidence of meningococcal meningitis and the relevant serogroups throughout time and regionally. The five major disease-causing serogroups among the 12 meningococcal serogroups have a specific geographic distribution, with A, W, and X being the most common in the Sahel and throughout Africa. The most common serogroups are B followed by Y in Europe and Y followed by B in the USA. Serogroup W first surfaced in the UK in 2013 and was associated with the extremely virulent South American clone St11 (Bettencourt et al., 2022). The serogroup C monovalent vaccine was replaced by the quadrivalent ACWY vaccine as a result of this change in European vaccination strategy.

Vaccines against conjugate meningococci are very efficient in preventing diseases caused by particular serogroups of meningococci. One of the main goals of the WHO's plan to eradicate meningitis by 2030 is the vaccine against meningococcal illness (Venkatesan 2021). Previously very common throughout Europe, serogroup C has shown a sharp decrease in prevalence since a conjugate vaccination against it was introduced for children in 1999. According to a national surveillance study conducted in the Netherlands, herd protection contributed almost one-third of the meningococcal serogroup C vaccine's effects and did so for over ten years (Sundelin et al., 2023). The eradication of serogroup A created an ecological vacuum that has since been filled by other, more virulent clones, and meningococcal meningitis outbreaks persist in the Sahel.

Serogroup B became the most common cause of meningococcal meningitis and septicemia, causing outbreaks and severe disease with significant morbidity and mortality, as serogroup C declined in many areas (van Kassel et al., 2021). Meningococcal vaccines based on proteins were granted licenses based on the correlation of protection demonstrated by serological response rather than disease incidence. Since the serogroup B vaccination was released in September 2015, the number of invasive serogroup B cases in children in the UK has decreased by 75% between September 2015 and August 2018. According to reports, the bivalent protein vaccine MenB FHBP exhibits cross-protection (van de Beek et al., 2021).

***Haemophilus influenzae* type b**

Before the conjugate vaccine was included to the EPI schedule in 2006, *H influenzae* type B was a leading cause of meningitis in children globally, in addition to pneumonia, epiglottitis, and otitis media. The conjugate vaccine was initially made available in 1989, after the *H. influenzae* type b polysaccharide vaccine was first released in 1985 (Slack et al., 2021a). In the first year following the introduction of the vaccine, dramatic reductions in the incidence of pediatric meningitis of 75% to 95% were recorded in all countries.

Since *H. influenzae* type B meningitis was added to the EPI schedule, it has all but disappeared in nations where vaccination programmes have been put in place. Global reductions from 2000 to 2016 are estimated to have been 49% and during that same period, pediatric deaths from this pathogen are estimated to have decreased by 90% (McAllister et al., 2019). In areas with low immunization rates, *H. influenzae* type B continues to be a therapeutic concern. There is still a significant meningitis and other *H. influenzae* type B illness load in the Democratic Republic of the Congo, Nigeria, Pakistan, and India. Due to its association with antibiotic resistance, non-typeable *H. influenzae* type b has supplanted *H. influenzae* type b in many settings, which has consequences for the early antibiotic therapy of pediatric meningitis (Park et al., 2022). Different types of available vaccine against pathogens causing meningitis are listing in Table 1.

Table 1: Different types of vaccines against pathogens causing meningitis.

Bacteria	Vaccine type	Vaccine name	Commercial name	References
Haemophilus influenzae	Conjugate	Monovalent	Menitorix, Pediacel	(Slack et al.,2020)
Streptococcus pneumoniae	Conjugate	PCV-10	Pneumosil	(Janssens et al.,2023)
	Conjugate	PCV-10	Synflorex	(Berman-Rosa et al., 2020)
	Conjugate	PCV-7	Prevenar	(Davies et al., 2022)
	Conjugate	PCV-13	Prevenar 13	(Sempere et al., 2022)
	Polysaccharide	PPV-23	Pneumovax	(Niederman et al., 2021)
Neisseria meningitidis	Conjugate	MenA CWY	CRM197 diptheria toxoid	(McMillan et al., 2021)
	Conjugate	MenC	CRM197	(Pizza et al., 2020)
	Conjugate	Hib MenCY-TT	Tetanus toxoid	(Serra et al., 2021)
	Conjugate	Men A	Tetanus toxoid	(Ruiz García et al., 2021)
	Polysaccharide	MPSV4	NA	(He et al., 2022)

The frequencies at which the mentioned bacterial strains cause meningitis have significantly decreased as a result of the Hib and pneumococcal conjugate vaccine programs. Through herd protection, pneumococcal conjugate vaccinations decreased pneumococcal carriage, transmission, and meningitis in older non-vaccinated people as well as in vaccinated children. It is unclear whether the 10-valent and 13-valent vaccinations will have a similar effect to the widely used pneumococcal vaccine, which led to a rise in pneumococcal disease brought on by bacterial strains not protected by the vaccination (Slack et al., 2021b).

By employing preventative measures against these etiological agents, such as immunization against *N. meningitidis*, *S. pneumoniae*, and *H. influenzae* type B (Hib), bacterial meningitis can be decreased. The prevalence of bacterial meningitis has declined since the 1990s and 2000s with the advent of the pneumococcal and hib conjugate vaccines due to the deployment of additional preventive programmes that make use of these vaccines (Syrogiannopoulos et al., 2022). According to reports, the extensive use of the PCV and Hib vaccinations has together greatly decreased the number of cases of bacterial meningitis globally, among both vaccinated and unvaccinated individuals.

In July 2006, a recommendation was made for all children under two years old in Germany to receive a pneumococcal conjugate immunization. Originally, Prevenar® (PCV7) was used for immunization. Prevenar 13® (PCV13), which succeeded PCV7, was released in December 2009, while Synflorix® (PCV10) was used starting in April 2009 with the licensing of higher-valent vaccine formulations (Makenga et al., 2021). Health insurance companies cover the entire cost of vaccinations, and parents or pediatricians make the vaccine selection. A year following the immunization recommendation, PCV vaccination uptake was 80%.

Conclusion

The majority of cases of acute bacterial meningitis are caused by three bacteria: *Neisseria meningitidis*, Streptococcus *pneumoniae* and *Haemophilus influenzae*. Measuring the effectiveness of protein-polysaccharide conjugate vaccines is most reliable because over 90% of cases of *H. influenzae* meningitis occur in one serotype and one age group. Since these vaccines have been around for the longest, high-income countries have been able to measure the incidence best. Both meningococcal and pneumococcal meningitis have a wide age range and are caused by a variety of serotypes outbreaks and a lack of surveillance, particularly in low-income nations, make it difficult to assess their prevalence. Since the introduction of the vaccine, cases of *H influenzae* meningitis have been almost completely eradicated. With limited information from low-income settings, the prevalence of all-age pneumococcal meningitis has dropped by approximately 25%, despite over 90% decreases in disease linked to vaccine serotypes. Additionally, incidence of bacterial meningitis is declining in wealthy nations due to significant advancements in medicine, but in less developed nations, strict measures and regulations are needed to prevent it. Immunization campaigns have been found to be the most effective means of preventing bacterial meningitis globally.

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Chapter 38

Revolutionizing Parasitic Disease Control: The Role of mRNA Vaccines

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ABSTRACT

Parasitic diseases are the most neglected diseases worldwide. They can cause billions of dollars in loss to the world economy. Parasites can cause different diseases in animals as well as human beings. Contemporarily, no significant vaccination protocol against parasitic diseases is available worldwide to prevent these diseases prophylactically. Traditional vaccines have no promising results against them. The recent development of the mRNA vaccine against COVID-19 has paved new ways for research against these diseases. The efficacy, safety, and economy of mRNA vaccines as compared to other traditional vaccines have tremendous results. The scientific community is working on producing mRNA vaccines against these diseases. This Chapter focuses on parasitic diseases and their effect on human health, animal health, and safety. The development and production of mRNA vaccines against different parasitic diseases, mRNA vaccine comparison to other vaccines, Challenges in the production of mRNA vaccines, their availability, future strategies for mRNA vaccines, and the effect of such vaccines on global health in the future are some salient features of this chapter. In the end, it is concluded that mRNA vaccines are essential for the prevention and protection against parasitic diseases and there is a need for a collective response against these diseases.

KEYWORDS
Parasitic diseases, mRNA Vaccines, Global Health, Immunization, Disease Control

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INTRODUCTION

The development of messenger RNA (ribonucleic acid) vaccines is based on DNA and uses the cell's gene as a template. The gene is then transcribed and produced using the complementary base pairing concept (August et al., 2022). Traditional vaccinations are carried out by injecting weakened or inactivated microorganisms to produce immunity against the targeted microbe against which vaccination has been done. mRNA vaccines include a small portion of messenger RNA that contains the information for the production of certain proteins by the body. mRNA is isolated in the laboratory and packed in a lipid nanoparticle, which not only protects mRNA but also facilitates the entry of mRNA into the cells for the production of the desired protein. The vaccine containing mRNA and nanoparticles is injected into the muscles. Once the entry of mRNA into the cells, it tells the cell to make a protein that is displayed on the surface of the cell. This results in rapid immunological response. The immune system of the body considers these proteins as a foreign body and their defense mechanism is activated by generating antibodies to respond against foreign bodies and eliminate them. It resulted in the formation of memory cells that memorize this foreign body and whenever in the future, an actual pathogen attacks the body, a stronger defensive mechanism is launched in order to remove it. Moreover, these immune responses produce memory cells, allowing the immune system to rapidly respond in case of confronting the same pathogen as an infection in the future.

mRNA vaccines are easily adaptable and mRNA sequence can be easily produced by isolating the genetic sequence of a particular microbe or pathogen. In the same way, one can easily produce mRNA vaccines of new strains of microbe. The

vaccines based on mRNA are adaptable, and the sequence can be easily generated in the lab using the genetic information of the specific disease. Furthermore, mRNA vaccines can be easily generated for novel strains or variants of that particular illness. This was most apparent in the recent pandemic (COVID-19) when mRNA vaccines (i.e. Moderna, Pfizer-BioNTech) were produced and approved for urgent use in a time frame of less than one year(Chomicz et al., 2016). However, at present, mRNA vaccines necessitate a substantial cold chain supply, antigen delivery mechanism, possible immune system response variations, and a complicated process of manufacturing. The purpose of mRNA vaccines is to enhance stability, efficacy, and delivery mechanisms. Enhancing the temperature sensitivity and stability of mRNA vaccines has been a research emphasis. This resulted in easier storage and fast delivery of vaccines in areas where there are not enough cold chain facilities to transport and store vaccines. On the other hand self –amplifying messenger RNA vaccines are developed to generate multiple copies of mRNA in the cell. This results in the production of large amounts of proteins and an increased immune response. Moreover, research is underway to investigate the novel delivery process to achieve maximum efficacy. These techniques involve the use of nanoparticles and liposomes to transport messenger RNA to the particular target site. However, the development of combination vaccines having multiple messenger RNA sequences to produce immunity against many diseases having multiple strains is another research field. Although intramuscular injection is the current method of administering mRNA vaccines, research is being conducted to deliver the vaccine directly into the skin, which could result in a better immune response and allow for the use of lower vaccine doses (Aga et al., 2023).

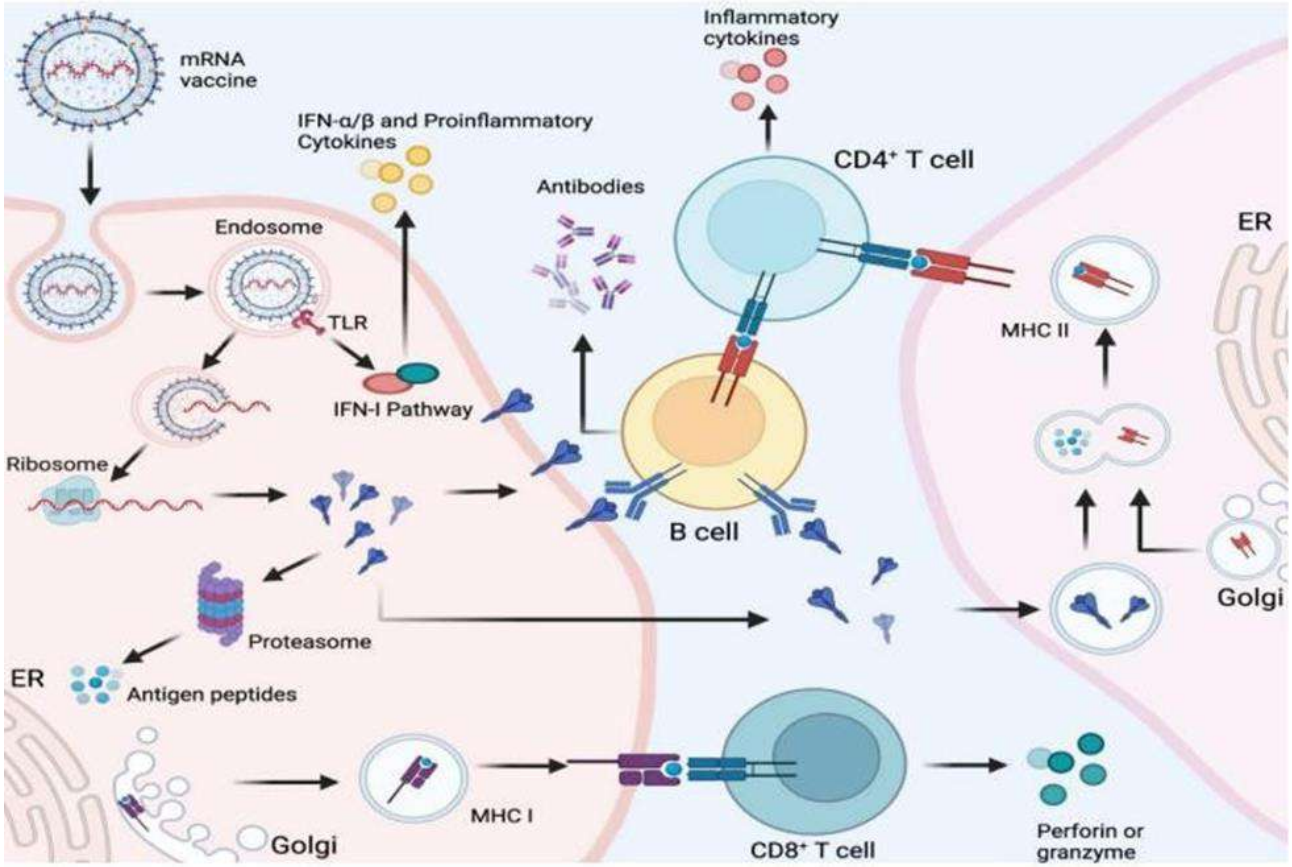

Fig. 1: Immune Responses (cellular immune response and humoral immune response) are produced as a result of injection of mRNA vaccines (Fang et al., 2022)

Parasitic Diseases: A Global Health Challenge

A host that has been infected with a certain parasite is said to have a parasitic infection. Microorganisms referred to as parasites depend on their hosts for survival. While many parasites have no impact on their host, certain parasitic infections can result in serious illnesses like malaria.

Parasitic diseases can be categorized into two classes, one which affects human health and others which affect animal health. When it comes to zoonotic parasites, on the other hand, which can cause disease and affect the health of both human beings as well as animals, it is difficult to distinguish between them. Echinococcosis is an excellent illustration of zoonosis. Adult Echinococcosis granulosus causes the infection in dogs, which is typically not very dangerous. However, the hydatid cyst, a metacestode of E. granulosus that causes echinococcosis in humans, is better described as hydatidosis. The primary intermediate hosts in the indirect life cycle of Echinococcus granulosus are ruminants. But just like the intermediate host of E. granulosus, man health can be affected either by engulfing eggs of the parasite or larvae. Sometimes these eggs develop into hydatid cysts in humans and reside in organs like the Eyes, Lungs, Liver, and sometimes the central nervous system (CNS), leading to the deadly illness known as hydatidosis (WHO, 2014).

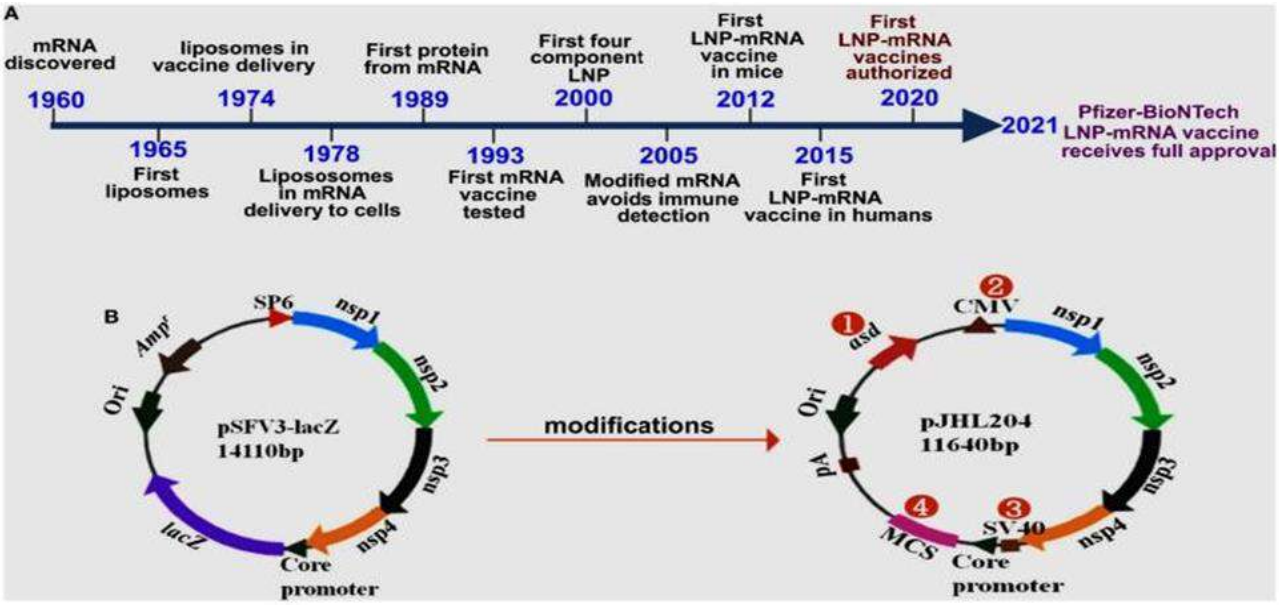

Fig. 2: Advances in Vaccine Development (Jawalagatti et al., 2022)

Impact on Global Health

Parasitic diseases are a challenge to both humans as well as animals worldwide. Human intestinal protozoa and helminth infections pose a serious threat to public health in some parts of the world (Chomicz et al., 2016). The rate of water-borne illnesses and social and economic situations are significantly correlated (Khan et al., 2021). The following are contributing causes to these infections: poverty, inappropriate eating behaviors, poor sanitation facilities, insufficient personal cleanliness, and lack of awareness (Khan et al., 2021).

It results in major economic as well as health losses worldwide. The lack of appropriate vaccines against them is a big issue. The prevalence rate of parasitic diseases worldwide is from 30-60% in humans. Worldwide, Intestinal parasite infection can affect over 3.5 billion people and every year 200,000 people succumb to death from this infection (Saab et al., 2004; Wakid, 2009). Much research are done regarding the prevalence of parasitic diseases in humans as well as animals. Some of the studies are shared here i.e. the percentage of intestinal parasite infection was 4.27% overall. Among them, 59.6 of men and 40.4% of women have been affected by it. The findings suggest that 5.1% of the participants were co-infected with two parasites, whereas 94.9% of the subjects only had one parasite. Compared to other age groups, the 0–9 age group had a greater rate of intestinal parasites. (17309032, n.d.).Similarly, another study on animals also showed shocking results, in all, intestinal parasites were discovered to be present in 21.5% of the dogs that were investigated. With a prevalence of 7.1%, Toxocara canis is found to be the most commonly present parasite. Following Toxocara canis is Toxascaris leonine with a prevalence of 5.5% then Cystoisospora species having a prevalence of 5.0%, Taenia spp. with a prevalence (of 3.9%, and at last is Dipylidium caninum with the prevalence of 2.8%. Intestinal parasite infections in dogs were more common in single-genus cases (18.7%) than in co-infected cases (2.8%). Intestinal parasite prevalence was higher in samples collected from the coastal region which is 25% as compared to samples collected from the rural areas (24.4%) and urban areas (20.6%). However, it was concluded statistically that a significant correlation exists between intestinal parasitic infection and the capture location.

Challenges in Controlling Parasitic Diseases

The main challenges in controlling parasitic diseases are; Changes related to Climate, the threat posed by vectors and vector-borne diseases (Sutherst, 2004; Medlock et al., 2012), increase in the number of emerging or reemerging parasitic infections (Taylor et al., 2001), developing and spreading anti-parasitic drug resistance at alarming speed (Jones et al., 2008), the prohibitively high cost of creating new anti-parasitic drugs.

Rapid Development Process of Vaccines

Coronavirus Infection (COVID-19) causative agent found to be severe acute respiratory syndrome coronavirus 2. COVID-19 cases were reported for the first time in December 2019 and soon it spread throughout the world in the shape of a worse ever seen pandemic.

As a response to this worldwide health crisis, a limited number of vaccines were approved for use in emergencies starting in November 2020. The most successful of these mRNA-based vaccines developed by Moderna, Inc. based in Cambridge, Massachusetts, USA, and BioNTech SE headquartered in Mainz, Germany/Pfizer Inc. headquartered in New York City, USA. The mRNA platform allowed vaccines to be developed quickly, but the need for ultra-cold storage has limited their application globally (Anand and Stahel, 2021).

Table 1: Comparison with Traditional Vaccines

	mRNA Vaccine	Traditional Vaccine	References
Manufacturing and development	Faster	Slower	(Sordo-Puga et al., 2022)
Effectiveness and safety	Safe and efficient for the great majority of Reliable Source of Individual	They can be quite successful, but not everyone can use them safely.	(Anand and Stahel, 2021)
Shelf life	Up to 6 months	May varies from 1-3 years	(Uddin and Roni, 2021; Sordo-Puga et al., 2022)
Length of immunity	They can be quite successful, but not everyone can use them safely.	Depends on the vaccine; some may provide permanent immunity, while others may need booster injections.	(Cybulska, 2022)

mRNA based vaccines have several benefits as compared to other traditional vaccines. The benefits include; Stable integration into the infectious elements or genome of the host cell rare, successful develop humoral immunity and cell-mediated immunity in the host, Tolerable by healthy organisms, mRNA vaccination has shown good results in treating various malignancies and preventing infectious illnesses, reducing costs and accelerating production through standardized processes in both human beings and animals (Kowalzik et al., 2021)

Advancements in mRNA Vaccines and Case Studies

Desired immunological qualities and an exceptional safety record are all combined in messenger RNA vaccines. mRNA vaccines are not limited by MHC haplotype and can provoke a balanced humoral immune response and cellular immunity depending on protein production. Moreover, mRNA is a safe vector because it only works as a transient carrier of information instead of interacting with the host genome. mRNA vaccines have higher flexibility as any desired protein can be obtained without modifying the process of production (Schlake et al., 2012).

Malaria

Malaria is among the most serious diseases in today's world. It can affect almost 40% of the world's population in the world, and it is found in tropical and subtropical areas. An estimated 200 million individuals get malaria annually in endemic areas. According to WHO report on Malaria 2016, 16% prevalence of malaria was recorded in children under the age of 5 year(WHO, 2016). As a result, malaria is among the most important tropical and pediatric illnesses worldwide. In recent years, the prevalence has been successfully reduced due to a variety of public health interventions, including vector control with mosquito nets, targeted insecticide applications, and the use of antimalarial prophylactic medications. However, more steps are required to properly protect children against malaria and lower the risk of infection. Efficient malaria immunization is a crucial component of these efforts. Research history however, demonstrates that creating a vaccine against malaria is a difficult task that is fraught with difficulties. As early as the 1960s, research was conducted on potential vaccinations. But the outcomes were pretty depressing, and the numerous vaccines did not live up to the hype. The European Medicines Agency did not give the vaccine, S/AS01 RTS a positive review until 2015. The trials of the vaccines were also conducted in Africa. On the contrary, fresh advances in vaccine research may potentially help with malaria research in the context of the recent pandemic (COVID-19). These comprised so-called mRNA vaccines, among other things. An immunological response brought on by an mRNA vaccination was initially reported in the early 1990s. Since then, studies and discussions on mRNA vaccines for potential prophylactic have taken place. Still, there was no real advancement in these vaccines until the Coronavirus (COVID-19) pandemic. Messenger RNA (mRNA) vaccinations against severe acute respiratory syndrome coronavirus 2 were created quickly and it has shown excellent efficacy against this virus in tests. It is hardly unexpected that businesses are concentrating on other illnesses and infections in light of this accomplishment. In addition to viral illnesses like AIDS or influenza, malaria ranks highly on this list. Many Pharmaceutical companies are investigating mRNA vaccines against malaria, notably the German firms BioNTech and CureVac. It's not a simple chore, though (Borkens, 2023; Kanoi et al., 2022; Tsoumani et al., 2023).

Leishmaniasis

Leishmania parasites cause various diseases through sand fly vectors. Vaccines have the potential for prevention, but factors have hindered clinical trials. Recent RNA vaccine maturation in COVID-19 suggests broader use of technology (Duthie et al., 2022). At the moment, Leishmaniasis can be prevented and managed only by controlling vectors and sensible treatment. But there aren't many medications on the market, and the ones that are either very expensive, have harmful side effects, or don't work since resistant strains of the disease have emerged. However, the vector control techniques are not as effective. Consequently, the world needs a safe, efficacious as well as reasonably priced Leishmaniasis vaccine. Even though a lot of academics have been focusing on this problem recently, only three vaccine ideas have advanced to the point of clinical trials. These are (i) a live, attenuated vaccination for humans in Uzbekistan; (ii) a dead vaccine for human immunotherapy in Brazil; and (iii) a second-generation vaccination in Brazil intended for prophylaxis in dogs.

Still, there are a minimum of six potential vaccines being developed. Expect that understanding of Leishmania species' full genome sequence will soon widen the scope of vaccine discovery and approaches that could deliver novel vaccines. (Srivastava et al., 2016; Mauel, 2002). The focus has shifted from developing a human VL vaccine to developing a CVL (Canine Visceral Leishmaniasis) vaccine. Currently, available vaccines against CVL (Canine Visceral Leishmaniasis) are ineffective. Strategies like using chimeric proteins and patent search can improve vaccine coverage. This work highlights immunological aspects and chimeric protein vaccine composition for CVL (Oliveira et al., 2024).

Trypanosomiasis

Tc24 is a flagellar protein generated from Trypanosoma cruzi. In mice, when the Tc24 protein combines with an adjuvant that can bind with TLR-4, then an immunological response of a balanced nature occurs. This results in increased antibody titers, IgG2a, and IFN-y levels. Immunization with Tc24 protein results in a lowering of parasitic load and increased chances of survival after being infected with acute infection. Messenger RNA vaccines have not been employed against T. cruzi even though some of them have developed better immune system responses than other protein vaccines. Heterologous vaccination regimen immunogenicity can be assessed by using Tc24 protein and messenger RNA-based vaccines. In mice, the vaccinated with a combination of mRNA/heterologous protein, messenger RNA/protein combination, Tc24 mRNA, and Lipid nanoparticles, Glucopyranosyl A (GLA) + C4-Tc24 protein were administered subcutaneously twice, three weeks apart, to mice (C57BL/6). Serum was taken to access IgG titers and isotypes, T-cell mediated specific responses were measured through the spleen. Two weeks following the last immunization, mice were put down to extract serum and spleen. It is concluded that in heterologous vaccination Tc24-C4 protein used for boosting and messenger RNA used for priming, induce a wide and potential specific immune response. This immune response is comparable to or better than homologous or heterologous vaccines (Poveda et al., 2023). The viability of creating a therapeutic CD vaccination based on mRNA that targets the two recognized T. cruzi vaccine antigens (ASP-2, an amastigote antigen, and Tc24, a flagellar antigen). The mRNA engineering procedures, lipid nanoparticle synthesis, and stability, assessment of their absorption by dendritic cells, and biodistribution in c57BL/J mice.

In a study, both monovalent as well as bivalent vaccination methods are used in an In vivo manner in mouse models to check the effectiveness and immunogenicity of these vaccines. This study suggests that ASP-2 in combination with Tc24 and mRNA demonstrated therapeutic advantage by decreasing parasitic population and inflammation of the heart. Therefore, our results emphasize the potential for generating multivalent vaccines and show the promise of messenger RNA (mRNA) vaccines as a treatment alternative to CD (Mancino et al., 2024). Research conducted on experimental models has indicated that the combined action of macrophages, Th 1 cytokines, lytic antibodies, T- T-cells, and CTLs (cytotoxic T lymphocytes) are necessary for the production of immunity against Trypanosomacruzi infection (Rios et al., 2019).

Challenges and Solutions in Developing mRNA Vaccines for Parasitic Diseases

Because mRNA vaccines are safer, more effective, and can be produced industrially, they have a great deal of potential to combat viral illnesses and cancer. The evolution of various mRNA types through sequence optimization has been observed in recent decades to address the drawbacks of high mRNA immunogenicity, instability, and inefficiency. Based on immunological studies, mRNA vaccines are combined with suitable adjuvants and different transport methods. Aside from sequence optimization, mRNA-delivering techniques can also help stabilize mRNAs and increase their effectiveness. Increasing the reactiveness of antigens provides an understanding of immunity produced by mRNA. Therefore, to address the problem, scientists used carrier-based mRNA vaccines, mRNA vaccines based on dendritic cells, and naked mRNA vaccinations (Wang et al., 2021). Several factors complicate the process of developing vaccinations: the variety of parasites' genetic makeup makes it more challenging to select antigens for subunit vaccines; a shortage of sources for extracting parasitic mRNA of parasites for the production of whole parasite vaccines and the lack of strong adjuvants that are specific to humans. Finding the immunogenic components specific to a certain disease is among the challenges addressed by the scientists working to develop messenger RNA subunit vaccines. Using immunoproteomic techniques is one way to accomplish this goal potentially. Cybulska describes the method to recognize Trichinella britovi immunogenic proteins by extracting the antibodies produced in flesh-based juice made from the carnivore host flesh infected naturally (Cybulska, 2022). Karabowicz et al. identify that Macrophage inhibitory factors (MIFs) produced by some nematodes also play a role in the control of immunological response produced in the host.

Parasite macrophage inhibitory factors (MIFs) influence leukocyte movement, cytokine release, and macrophage polarization by imitating the actions of the equivalent host molecule. Ancylostoma ceylanicum and Teladorsagia circumcincta, have also been used as vaccine antigens in animal immunization studies and have provided some level of protection against infection. MIFs derived from two parasitic nematodes i.e. Teladorsagia circumcincta and Ancylostoma ceylanicum are used as antigen for vaccines and have offered some degree of protection against infection (Karabowicz et al., 2022). Bąska and Norbury outline the role of the transcription factor Nuclear Factor Kappa B (NF- κB) in the body's reaction to parasites in another review. To help them survive in the host, these pathogens have developed a variety of ways to adjust the modulation level either up or down of NF-κB activity. Different facets of these systems were thoroughly explained about the types of host cells that were modulated and the parasite species (Bąska and Norbury, 2022). When developing vaccines, it is important to keep in mind that immunity plays an important part in the pathology that arises from an infection. Despite the lengthy history of the effectiveness of vaccination, still, there is still an unavailability of

vaccines against certain infectious diseases. Increased research into the best preventative and control measures for infectious diseases is necessary to close this gap. Any assistance in this field is much appreciated. Success factors include but are not limited to, identifying protective immunological systems, choosing the best vaccination antigen candidates, and determining the best routes and regimens. It is hoped that research on the host immune response will quicken the development of vaccinations and lead to the creation of new shots that will eventually be sold commercially.

It is necessary to focus on the creation of economical and sustainable production procedures. Despite being more efficient and secure than the vast majority of existing vaccines, the IVT reaction of mRNA relies on adding expensive and rare components. The subsequent processing of the vaccine is currently in stages of development and uses methods that are not efficient or cost-effective. These constraints can be removed by switching to continuous reduction in the process. It is suggested that a microfluidics technique comprising multimodal chromatography should be used to replace the need for numerous chromatographic processes and compartmentalize enzymatic reactions in conjunction with ISPR (in situ product removal), SDPR (substrate feed, and product recovery) modules. One potential solution for a sustainable, adaptable, and economical vaccine manufacturing technique that can support an on-demand response is the application of new production techniques that permit the repurposing and recirculation of compounds combined with high-throughput purification and clearly defined analytical methods in a continuous manufacturing process (Rosa et al., 2021).

Clinical Trials and Regulatory Approval

One of the vaccination technologies that is now expanding the fastest is the IVT mRNA platform. A consistent production technique can be used to make mRNA, just like other oligonucleotide-based vaccination technologies. This enables the screening of several vaccine candidates in an acceptable amount of time. Even while production costs are still high today, enhanced transcription and mRNA capping methods as well as greater competition among GMP RNA manufacturing facilities could soon lead to the realization of low costs for uniform production. Multivalent vaccinations may be created by combining various mRNA vaccine candidates. Even in the absence of extra adjuvants, mRNA vaccine produces innate immunity type-I that could result in CD8+ T cells immune response. Effective signal peptides can also be used to direct the immune response toward the creation of antibodies. The creation of vaccinations against parasite illnesses is greatly aided by these qualities, which are largely absent from traditional vaccine platforms. Compared to whole-cell vaccines, recombinant protein vaccines, or DNA vaccines, mRNA products are safer and less complex from a regulatory perspective. Now several mRNA vaccine candidates have made it into clinical trials, it will be fascinating to examine how the human immune system responds to them. Hopefully, mRNA vaccines will outperform DNA vaccines in terms of clinical outcomes due to their greater potency compared to DNA.

In the coming years, many new research findings about mRNA vaccine research should be released; many of these will likely be related to parasite illnesses (Beaumier et al., 2016; Barry et al., 2019). Despite decades of research on T. cruzi, still no vaccine have been developed to provide 100% immunity against the disease (Thran et al., 2017). On the characteristics of a protective vaccine, however, agreement exists. Specific T cells (CD8+) that target epitpes from both cryptic as well as immunodominant , T. cruzi antigens should ideally be induced by an anti-T. cruzi vaccine. Anti Trypnosoma cruzi vaccine induce specific T cells (CD8+) immune response against Immunodominant and subdominant T.cruzi antigen.

It is essential to have a balanced immune response when administering a therapeutic vaccine to prevent excessive host inflammation or autoimmunity (Jones et al., 2018). Messenger Vaccines found to be successful in screening targets against multiple antigens of T.cruzi in order to induceCD8+ T cell response, despite a certain learning curve and ongoing technological advancements (Versteeg et al., 2019).

Regulatory Landscape and Approval Processes

For the development of mRNA vaccines and their evaluation worldwide, the World Health Organization (WHO) is trying to develop an international consensus among member countries. In addition to giving nations technical support, this will expedite the global convergence of industrial and regulatory processes. WHO standards will support the Emergency Use Listing assessment and WHO Prequalification test of these vaccines as happened during the recent pandemic.

Currently, no guidelines are available for mRNA Vaccines. WHO expert committee on biological standardization (ECBS) has supported the upgradation and development of rules and regulations for messenger RNA Vaccines in the presence of up to date available scientific as well as clinical data. So the complete and updated document regarding the upgradation and development of rules and regulations for mRNA vaccines was presented before WHO ECBS in late 2021.The main aim of these amendments is to make sure the safety, quality, efficacy as well as distribution of these vaccines at global level and trust building in mRNA vaccines at international level. The World Health Organization is aiming to hold a talk between experts at international level regarding the scientific evidence available for mRNA vaccines, addressing the key issues related to mRNA vaccines and developing a concensus among experts on mRNA vaccines (Knezevic et al., 2021).

Potential Impact on Global Health In the Future

It will be necessary to compare the effects of vaccination to current methods of treatment, prevention, and other public health initiatives. While identifying gaps in these objectives, the application of mRNA technology should be coordinated with national and international health agendas, as well as those of non-governmental groups and the

pharmaceutical industry. These serve as a reminder that vaccination must be constructed with the long term in mind, even though they are not specifically designed for mRNA vaccines. In the rapidly evolving fields of prevention and therapy, this is essential.

Surprisingly, mRNA might also play an important part in the discovery of new technologies as well as the improvements in existing vaccination approaches. Success could also spur interest in RandD (research and development) for vaccines during discovery phase, to identify immunogens for subsequent vaccine development. In addition to fulfilling a need, an mRNA vaccine may also play a significant role in pandemic preparedness and response. Designing an assessment system requires first identifying pertinent indicators for evaluating the utility of mRNA technology for virally induced malignancies and infectious illnesses. These should take into account the global and regional burden of disease, the viability of biological and product development, and implementation and access issues. They should also be consistent with or complementary to the ongoing work in this field. Three high-level factors should be taken into consideration when assessing the benefits of using messenger RNA technology regarding mRNA vaccines ⅰ) Vaccine features ⅱ) Vaccine feasibility ⅲ) and the Burden of illness (WHO SCIENCE COUNCIL, 2023).

Future Perspective

mRNA vaccines have shown safe and durable immune responses during clinical and pre-clinical experiments in both people and animals (Zhang et al., 2019). By optimizing the 5′-UTR (5′-untranslated region) of mRNA vaccines, the transitional yield can be increased. Currently, lipid-based nanoparticles and lipoplexes are the most common methods for delivering mRNA. Polymers and hybrid nanoparticles of lipids and polymers hold considerable potential regarding affordability, safety, and efficiency. Although they have advantages, their instability at high temperatures makes distribution and packaging challenging. Compared to DNA vaccines, they are less effective and safer than inactivated vaccines.

The development and research on messenger RNA vaccines require an additional understanding of their mode of action and efficacy. The memory cell's size and immune responses against specific antigens including memory B cell and memory T cell responses should both rise in the future. The broader application of mRNA for treating and preventing viral infections can be enhanced by further developments in mRNA formulation and delivery utilizing various nanomaterials. So, much of the research work is needed in this field to develop safer, more reliable, and more effective vaccines against these parasitic diseases.

Conclusion

It has been concluded that mRNA vaccines against parasitic diseases are the need of the day. The development of these vaccines against parasites should be encouraged by the world to provide good health and improve the standard of living for both humans and animals. Due to the safety, potency, efficacy, and fewer expenses to make them, mRNA vaccines are replacing traditional vaccines. Between a huge supply of potent vaccines and the emergence of pandemic infectious illnesses, only mRNA vaccines can bridge the gaps between demand and supply.

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Chapter 39

Vaccination and Alternate Control Strategies against *Clostridium botulinum* and *Clostridium difficile*

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ABSTRACT

Vaccines have an important role in public health and disease prevention. In this study, we will see the different types of vaccines and mechanisms of action and also learn about some prophylactic and therapeutic vaccines. We'll also read about the characteristics of *Clostridium botulinum* and *Clostridium difficile* and their toxins respectively. We also learn about their treatment options. While we have effective vaccines against various bacterial threats like tuberculosis, cholera, and botulism, researchers are constantly innovating. New recombinant vaccines offer safer alternatives, and exciting developments are underway for challenging bacteria like *Clostridium difficile*, responsible for severe hospital-acquired diarrhea. In short, the fight against bacterial infections continues with promising advancements in vaccine technology. Other methods of control like the use of antibiotics, Fecal microbiota transplantation, and some others have also been discussed. The promise of vaccinations to control these infections and the necessity for more study and the development of safe and effective vaccines are emphasized in the paper's conclusion.

KEYWORDS
Vaccine, *Clostridium difficile*, *Clostridium botulinum* alternative Control

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INTRODUCTION

Clostridium botulinum bacteria are gram-positive, rod-shaped, anaerobic bacteria. It can form spores (Meurens et al., 2023) spores can be oval (Kanaan and Tarek, 2020). *Clostridium* is the largest genus of bacteria and is divided into many phylogenetic groups and has a lot of diversity. It is primarily pathogenic in humans and some are saprophytic (Corsalini et al., 2021). *Clostridium botulinum* belongs to the genus *Clostridium* due to its common ability to synthesize the BoNT protein (Munir et al., 2023). *Clostridium botulinum* is divided into 7 species (A) which are based on the type of neurotoxin they produce (Kanaan and Tarek, 2020).

Similarly, *C. difficile*, a gram-positive, anaerobic, spore-forming bacterium, (Chandrasekaran and Lacy, 2017) spreads through feces and thrives in various environments, including humans, animals, and even our surroundings. It highlights the potential spread through contact with carriers, infected individuals, contaminated areas, and even some animals (Czepiel et al., 2019). It is the primary global cause of pseudomembranous colitis and diarrhea (Napolitano and Edmiston, 2017) linked to nosocomial antibiotics (Regenbogen et al., 2020).

Toxins Produced by *Botulinum* Genus

The primary disease caused by the bacteria is called botulism (Brunt et al., 2020) and it can be of four forms: foodborne, wound, infant, and adult colonization (Maslanka et al., 2013) depending on its forms it can enter the individual in many ways (Munir et al., 2023). Adults can develop a form of botulism "hidden botulism", similar to infants, due to digestive tract issues (potentially from prior antibiotics or surgery). This causes similar symptoms to infant botulism

(Grenda et al., 2017). It is a group of 4 different bacteria that are composed of different strains (Abdolmohammadi Khiav et al., 2021). It can either contaminate food or penetrate the wounds. Some clostridial species (C. botulinum, C. difficile, C. perfringens, C. tetani) are normally present in the body but may cause adverse effects when suitable conditions (anaerobic environment) it is a severe neurological condition that causes flaccid paralysis, (Meurens et al., 2023) in humans, and can happen from eating improperly prepared food (not cooked, preserved, or refrigerated well enough) that contains bacteria spores or the toxin they produce. In adult intestinal botulism C. botulinum bacteria grows in the intestines and makes the toxin there, causing illness. (Zaragoza et al., 2019).

Wound botulism is caused by toxins released by bacteria present in wounds. Infant botulism occurs by botulinum spores ingested by infants and bacteria residing in the intestines and releasing toxins. (Gaware et al., 2011). This disease is characterized by descending paralysis and also involves symptoms like ptosis, blurred vision, dry or sore throat. If botulism occurs by ingesting toxins then diarrhea, abdominal pain, and vomiting may also be seen. (Byrne and Smith, 2000).Although the mechanism of action of the neurotoxins is the same (Yan et al., 2020). BoNT is the major potent biological toxin having a structure similar to prototoxin having two chains bound by disulfide bonds. It cleaves into active toxins of heavy and light chains (Munir et al., 2023). Some accessory proteins are also present which are involved in protecting neurotoxin from low pH and also facilitating its absorption in the gastrointestinal tract. (Carr et al., 2021). These toxins are fatal enough to affect a large population even if a small number of bacteria are present. It can be transmitted from one person to another and due to limited antitoxins supply intensive care is required to prevent disease (Cenciarell et al., 2019).

Botulinum toxins can affect veterinary species too, food food-borne botulinum being more common in animals. (Sundeen and Barbieri, 2017).

C. *difficile* toxins damage intestinal cells, causing inflammation and fluid loss leading to diarrhoea. Debate exists around the specific roles of different toxin types (TcdA vs TcdB) in causing disease (Schaeffler and Breitrueck, 2018). *C. difficile* from animals can contaminate meat and survive cooking, potentially spreading through air and manure, posing a risk to human health through farm and food processing (Lim, et al., 2020).

C. difficile releases toxins mainly TcdA and TcdB (Chandrasekaran and Lacy, 2017) that mess with cells' internal processes, disrupting functions like barrier integrity and triggering inflammation (Aktories et al., 2017). The specific receptors they bind to, the unique way they create holes in membranes, and how they move across these holes remain mysteries. (Orrel et al., 2017). A third toxin, CDT, disrupts another critical structure (actin) leading to further cellular disarray (Aktories et al., 2017). These poisons cause diarrhea, tissue damage, and inflammation (Kordus et al., 2022).

Mechanism of Toxin Binding

Botulism toxins produced by botulinum bacteria act by cleaving the SNARE proteins that are involved in the release of a neurotransmitter, acetylcholine, that causes a neurological disorder (Brunt et al., 2020) as shown in Fig. 1. BoNT toxins, when ingested, form complexes with NAPs in the gastrointestinal tract to protect themselves from stomach acid and proteolytic conditions. These complexes, containing BoNT and a non-toxic non-hemagglutinin protein, facilitate its passage through the intestinal barrier. Once in the bloodstream, BoNT targets motor neurons, with a high potency due to its neuronal specificity and catalytic activity (von Berg, 2020). BoNTs consist of a 150 kDa protein with a 50 kDa light chain and a 100 kDa heavy chain (Dressler and Adib, 2005).

The toxin acts when the HC facilitates the LC's attachment to specific receptors on presynaptic membranes; the LC then cleaves proteins involved in synaptic vesicle fusion; and the HC helps the LC translocate into the cytoplasm (von Berg, 2020). Because BoNT specifically targets motor neurons, this cleavage prevents the release of neurotransmitters, resulting in muscle paralysis at neuromuscular junctions (Lin et al., 2020).

***Clostridium*-associated Diarrhoea**

Clostridium difficile is of great concern in patients with inflammatory bowel disease (Balram et al., 2019). Previously mostly a hospital-acquired infection, *C. difficile* is becoming more and more problematic in the community (Hung et al., 2021). This bacteria is a major contributor to diarrhea-related hospital mortality and produces severe diarrhea that can range from moderate to life-threatening (Balram et al., 2019). Gut bacteria play a major role in the colonization of this bacteria. The presence of certain bacteria may help prevent its colonization (Crobach et al., 2018). Its pathogenesis involves disrupting host microbes and that leads to the release of its toxins after colonizing in the colon (Dotson et al., 2018) as shown in Fig. 2. Bile acid shifts favoring primary forms like cholate and taurocholate, along with reduced competition from commensals, trigger C. difficile spore germination and create a growth-friendly environment (Schaeffler and Breitrueck, 2018).

Vaccines

Vaccines are a great public health achievement and have played a key role in eliminating large numbers of diseases (Vetter et al., 2018). Vaccination has also reduced the risks of death by many pathogenic agents (Orenstein et al., 2017). Vaccines aim to develop immunity against various pathogenic organisms that cause lethal effects in humans and animals. They provide an immune response against pathogens and activate the innate immune system without any memory. It is followed by the activation of an adaptive immune system that has memory for future infections (Vetter et al., 2018).

Primarily they are composed of antigens derived from the pathogens. Additionally, other compounds like stabilizers, preservatives, excipients, etc might also be present (Ghattas et al., 2021).

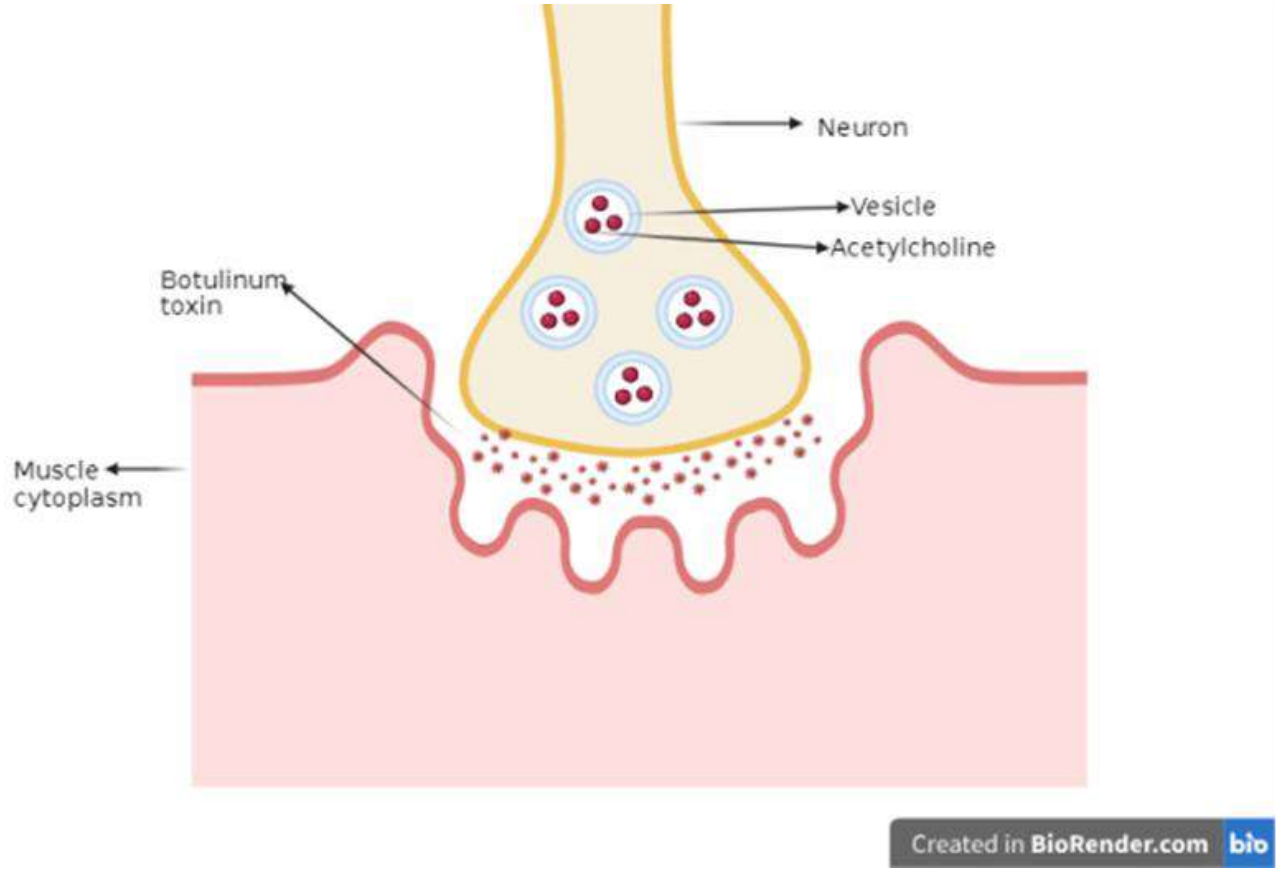

Fig. 1: Botulinum toxin blocking Ach transmission causing Botulism. Created with BioRender.com

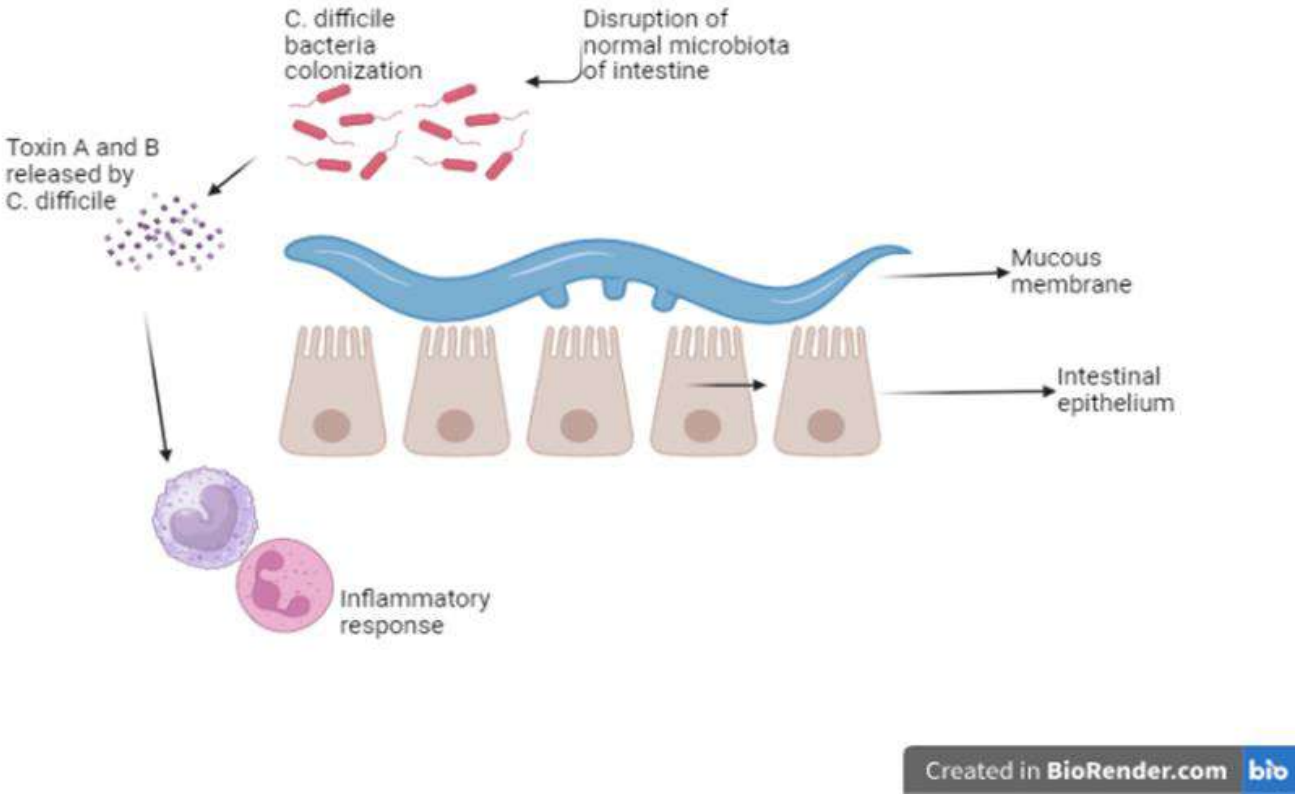

Fig. 2: Colonization of bacteria releasing toxins that affects the intestine. Created with BioRender.com

Vaccines also provide community protection by preventing the spread of the diseases. Hence vaccines are the most efficient and cost-effective tools for disease prevention (Orenstein et al., 2017). The development of vaccines began in China when smallpox was being tried to be prevented. Edward Jenner (Barakat, 2021) investigated that pus from cowpox lesions when transmitted to humans can help to provide immunity against smallpox in susceptible hosts (Lombard et al., 2007). Later vaccines against anthrax disease were made by Louis Pasteur. Vaccines have evolved and new technology has made their production more sophisticated (Tenorio et al., 2022). In general, vaccines have been made against various infectious bacteria, viruses and rarely parasites to reduce the risk of diseases in humans and animals. Vaccines against bacteria and viruses are available commercially.

Vaccines against Bacterial Infections

Vaccines have been present against many bacterial infections protecting humans against fatal diseases. Traditional vaccines protect against diseases like influenza (De Gregorio and Rappuoli, 2012). There have been many successful vaccines against bacterial causative agents as described in table 2. Some pathogens are there against which vaccines are not made and the use of adjuvants is enhanced, also work is done to strengthen the vaccines to be used against the complex pathogens (Osterloh, 2022).

Vaccines against some Common Bacterial Infections

Vaccine	Causative agent	Vaccine trade name	Route of administration	Type of vaccine	References
Tuberculosis vaccine	*Mycobacterium tuberculosis*	BCG vaccine	Percutaneous	Live attenuated	(Ghattas et al., 2021)
Cholera vaccine	*Vibrio cholerae*	Vaxchora®	Oral	Live attenuated	(Ghattas et al., 2021)
Meningococcal group B vaccine	*Neisseria gonorrhoeae*	Trumenba®	intramuscular	Synthetic peptide vaccine	(Ghattas et al., 2021)
Pneumococcal 13 valent conjugate vaccine	*Streptococcus pneumoniae*	Prevnar 13®	Intramuscular	Conjugated polysaccharide vaccine	(Ghattas et al., 2021)
Diphtheria vaccine	*Corynebacterium diphtheriae*	Infanrix®	Intramuscular	Inactivated vaccine	(Ghattas et al., 2021)
Tetanus toxoid vaccine	*Clostridium tetani*	Infanrix	Intramuscular	Inactivated vaccine	(Ghattas et al., 2021)
Typhoid Vi polysaccharide vaccine	*Salmonella typhi*	Typhim Vi®	Intramuscular	Polysaccharide vaccine	(Ghattas et al., 2021)
Haemophilus B vaccine	*Haemophilus influenzae*	Liquid pedvaxHIB®	Intramuscular	Polysaccharide conjugated vaccine	(Ghattas et al., 2021)

Vaccination against *Clostridium*

Currently available *C. difficile* vaccine options are injectable and solely target toxins. The modified, non-toxigenic *C. difficile* strain that expresses a fusion protein of toxin fragments is the basis for the oral vaccination (Wang et al., 2022). While vaccines based on CD spore components are promising, they are still in early development. Initial trials using BclA1 showed limited success, while vaccines targeting CdeM, CdeC, and CotA proteins offer the potential for further development and broader protection (Principi et al., 2020).

Toxoid Vaccine

It has been demonstrated that protective vaccination against botulism using botulinum neurotoxin toxoid, which is made via formalin inactivation similarly to TeNT and DT toxoids, is efficacious. The three most generally utilised and financially accessible BoNT/An items are Dysport® (abobotulinumtoxinA, Ipsen, Paris, France), Botox® (onabotulinumtoxinA, Allergan, Dublin, Ireland), and Xeomin® (incobotulinumtoxinA, Merz, Frankfurt am Principal, Germany (Fonfria and Maignel et al., 2018). Traditional toxoid vaccines, while effective, are slow to produce, require inactivation (potentially leaving formaldehyde traces), and have a lengthy manufacturing process (Abdolmohammadi Khiav and Zahmatkesh, 2022). The structure of botulinum toxins allows scientists to modify them, creating safer and more effective versions that can target different areas of the body beyond just nerves (Rasetti-Escargueil and Popoff, 2020). Toxoid vaccines are available against *C. difficile* and show promise in neutralising toxins, they don't prevent colonisation, sporulation, or shedding, potentially increasing asymptomatic carriers. Other vaccine types like monoclonal antibodies, polysaccharide-based, and anti-adhesion vaccines are under development (Henderson et al., 2017).

BoNT Vaccine against *Clostridial* Genus

Pentavalent Toxoid Vaccine

An injection that protects against five different forms of botulinum toxin is called the MDPH pentavalent botulinum toxoid vaccination. It is delivered subcutaneously on a predetermined schedule, with annual boosters. In comparison to another product, the vaccination includes less formaldehyde but still contains preservatives and aluminium phosphate. The

vaccine has been used in multiple lots, the main lot being Lot PBP-003, which has been in use since 2005 (LA Smith, 2009). Pentavalent BoNT toxoid vaccine offered some protection but it has waning efficacy over time, declining potency against specific toxin types and high prevalence of local side effects (Gupta and Pellett, 2023).

Heptavalent Botulinum Antitoxin

The licensed antitoxin that is presently on the market in the US is called heptavalent botulinum antitoxin (BAT®) (Sobel and Rao, 2018). Inoculation with the pathogen or bacterin-pathogen or hereditarily changed or different immunizations is a defensive way against clostridial contamination. Worldwide, several experimental or commercial vaccines have been developed. Albeit regular immunizations including pathogen immunizations are vital, the new age of antibodies is a compelling option in contrast to customary antibodies (Abdolmohammadi Khiav and Zahmatkesh, 2022).

1- Recombinant Vaccines

Researchers have developed a new approach to designing vaccines against botulinum neurotoxins (BoNTs). Use of full toxins can prove to be dangerous so they make use of nontoxic pieces of the toxin called recombinant domains. These domains are still able to trigger the immune system to produce protective antibodies against the full toxin. This new strategy has the potential to lead to safer and more effective botulism vaccines. (Shi et al 2022). Notably, specific antigens (Hc and HN-L) provided excellent protection and complete immunoprotection (Shi et al., 2019). Combining different antigens did not affect the immune response, suggesting that efficacy depends on the specific antigen properties rather than their combination. In other words, choosing the right antigen is crucial for achieving strong and specific immune protection. (Liu et al., 2020). Furthermore, BoNT itself can elicit an immune response. BoNT B produces a stronger immune response than BoNT (Carr et al., 2021). BoNT were bio-engineered to produce an immune response in humans (Rasetti-Escargueil and Popoff, 2020).

2- Botulinum toxin C vaccine (Hc)

BoNT Hc vaccines are highly effective against specific toxins, are safe and easy to produce for large-scale production, and only work against the specific toxin they are designed for (Webb et al., 2017).

By 1993, sequences encoding BoNT serotypes A–G had been identified. All serotypes of P. pastoris expressed codon-optimized Hc genes. With this approach, the disadvantages of E. coli as a host organism are avoided. P. pastoris allows for controlled glycosylation patterns, which can be crucial for proper antigen function. While E. coli can hyper-glycosylate BoNT(Hc) proteins, which is undesirable, P. pastoris can achieve more precise modification. Intracellular expression: By expressing BoNT(Hc) proteins inside the P. pastoris cells instead of secreting them, P. pastoris completely avoids the undesirable glycosylation that occurs in the secretory pathway. The Hc genes for BoNT serotypes are protected against fatal botulinum toxin in murine and preclinical models (LA Smith, 2009).

3- Bivalent Vaccines

A viable option for preventing infections with BoNT A and B is this bivalent vaccination. AlhydrogelTM and other adjuvants may be crucial for stimulating the immune system's response to the vaccination. Although the vaccine is still in the research and development stage, early results are promising (Webb et al., 2020). Polyvalent and improved bivalent vaccines were also developed (Abdolmohammadi and Zahmatkesh, 2022). The most broadly utilized immunizations are bivalent *(C. botulinum* C and D) immunizations that are figured out with formaldehyde-inactivated poisons obtained from the maturation of *C. botulinum* (Mbhele et al., 2023). A bivalent (BoNT/C-D) pathogen immunisation has been likewise ready and administered in homegrown creatures, particularly in domestic animals. (Abdolmohammadi Khiav and Zahmatkesh, 2022).

4- Monovalent Vaccine

A purified monovalent type F toxoid is available for the bacteria given I/M and S/c in humans. A monovalent (B) pathogen immunisation is being administered in horse crowds (Abdolmohammadi Khiav and Zahmatkesh, 2022).

Vrec Vaccines

One study investigated the effectiveness of different antigens in generating immune responses against a toxin. Although each antigen had a unique defence mechanism, they all elicited strong humoral immune responses (Liu et al., 2020). Vrec200 may be considered the most cost-effective vaccine for large-scale production, Vrec400 was the best vaccine among those tested because it induced higher levels of antibodies and maintained higher levels of antibodies for the longest time (Otaka et al., 2020). Massive numbers of army soldiers have received human botulism vaccinations to protect them from potential bio-terrorism strikes (Roja and Chellapandi, 2024).

Antibodies against *C. difficile*

The key to preventing diarrheal sickness is having antibodies to the toxins from *Clostridium difficile*; TcdA antibodies shield against the first infection, while TcdB antibodies lessen its severity. Immunizations against both poisons show promise in avoiding disease symptoms (Riley et al., 2019). There are several *C. difficile* immunization techniques, divided into groups according to their target (toxins or surface proteins) and mode of administration (injection or mucosal)

(Bruxelle et al., 2018). *C. difficile* antibodies offer protection against symptomatic infection but not colonization, while breast milk components, in addition to IgA, might offer potential defense against toxin A (Schaeffler and Breitrueck,2018). Targeting cell surface antigens aims to prevent bacterial colonization, ultimately blocking toxin production and spore spread (Pizarro-Guajardo et al., 2019).

Bezlotoxumab for Recurrent *C. difficile* Infection

In recurrent *Clostridium difficile* infections Bezlotoxumab may be used as a treatment for recurrent infection. It is an antibody that targets the *C. difficile* toxin and prevents recurrent infections (Jarmo et al., 2020).

Bacterial Flagellins

Bacterial flagellins are essential for inducing both innate and adaptive immunity in mucosa-associated lymphoid tissue and at the systemic level (Bruxelle et al., 2017)

Non Toxigenic Membrane Fraction

Nontoxigenic *C. difficile* membrane fraction (ntCDMF) induced the highest serum IgG levels when delivered subcutaneously, compared to oral, intrarectal, and nasal routes (Senoh et al., 2018).

Multi Protein Vaccine

A multi-protein *C. difficile* vaccine is available and predicts that the vaccine is stable and interacts with immune cells (Basak et al., 2021). Pre colonization with one kind of *C. difficile* furnishes security from challenge with a more destructive strain (Leslie et al., 2021).

Bezlotoxumab, which neutralises toxin B, is the only monoclonal antibody effective in preventing recurrent CDI, but has potential side effects like heart failure and needs further characterization for wider use. Oral hyper immune way is promising yet information in people is restricted (Principi et al., 2020).

Vaccination against CDI is estimated to reduce infection in vaccinated patients, with most of the prevented cases among the vaccinated population itself. This suggests that vaccination can be an effective strategy to control CDI (Toth et al., 2020).

Other Control Measures

1. **Chemical Control (antibiotic)**

Fidaxomicin (FDX) and metronidazole (MNZ) demonstrated potential in combating *C. botulinum,* their outcomes fluctuated. FDX strangely increased the generation of toxins while decreasing the viability of a particular strain. On the other hand, MNZ stopped the growth of toxins while quickly eliminating all tested strains. However, more research is necessary before making a clinical suggestion to ensure safety and achieve effective MNZ concentrations in vivo. (Yutani et al., 2021)

C. botulinum being affected by antibiotics shows increasing resistance against tetracyclines and cephalosporins. High resistance against nitroimidazoles and gentamicin is also seen. (Banawas, 2022). All strains mainly show resistance against amoxicillin and trimethoprim. Resistance to nalidixic acid was also reported (Koluman et al., 2013).

Clostridium difficile

There are several antimicrobials which are seen to be effective against the clostridium difficile infection. These include cadazolid, surotomycin, and ridinilazole, alongside established options like ramoplanin, fusidic acid, nitazoxanide, and rifampin (Petrosillo et al., 2018). When there is an increased risk of CDI antibiotics such as ampicillin, amoxicillin, cephalosporins, clindamycin, and fluoroquinolones, are used while Metronidazole, vancomycin, and fidaxomicin are antibiotics that are commonly used (Peng et al., 2017).

Antibiotics are effective against bacteria but due to several defense mechanisms like resistance genes, changing target sites and forming biofilms can result in acquiring resistance against the antibiotics (Spigaglia et al, 2018). The CD has efflux pumps and some innate properties that render antibiotics ineffective against the bacteria (Wickramage et al., 2021). *C. difficile* has been known to develop resistance against most commonly used antibiotics by its defence mechanisms against antibiotics (Peng et al., 2017). Fluoroquinolones and clindamycin demonstrated the highest levels of resistance among high-risk antimicrobials for the development of CDI. A percent of the isolates were resistant to tetracycline (Sholeh et al., 2020)

There are different strategies for controlling the foodborne microbe *C. botulinum*. (Munir et al., 2023)

1. **Heating**

Heating is effective but has some drawbacks. More up to date advances like high-pressure handling and beat electric fields offer elective ways of controlling this danger while saving food quality (Munir et al., 2023). Traditional food preservation methods like heat, acid, and added solutes can damage food or not be suitable for all products (Assal et al., 2023).

2. **Microfiltration and other Strategies**

Microfiltration and UV-C show promise for spore removal, but their effectiveness against *C. botulinum* (Assal et al., 2023). Ionising radiation, high hydrostatic pressure combined with heat can be used to control C. botulinum in food. Combining HPP with heat shows the best results for spore inactivation. Treatments like pulsed electric fields (PEF), intense light pulses (ILP), and cold plasma (CP) for controlling *C. botulinum* hazards in the food industry (Munir et al., 2023).

3. **Probiotics**

The addition of sorbate can effectively prevent the growth and toxin production of harmful *C. botulinum* bacteria in processed cheese, meat, and laboratory media. This suggests its potential as a control measure for this foodborne pathogen (Glass et al., 2017). Some probiotics were also effective for controlling *C. botulinum* due to their antioxidant and antimicrobial benefits (Hamad et al., 2022). Refrigeration works well against some *Clostridium botulinum*, which can grow at 3°C and requires cooking at 90°C for 10 min for inactivation. (Golden et al., 2017). Naturally probiotics and their derivatives can be used to prevent *C. botulinum* growth and toxin production, potentially contributing to food safety strategies (Alizadeh et al., 2020). Healthy gut may prevent the colonisation of bacteria and its potential toxin release so probiotics can be effective to enhance the gut microflora and prevent spore germination (Harris et al., 2020).

4. **Incineration of Carcass**

To prevent the spread of botulinum spores from the animals, the spore containing carcasses need to be incinerated or eliminated to prevent the spread of disease (Gutiérrez-Arnal and Marín, 2024).

5. **Bacteriocins**

Bacteriocins are also beneficial in preventing the colonization of clostridial species, especially clostridium botulinum. Furthermore, improved nutrient uptake can also help prevent the disease (Harris et al., 2020).

Clostridium difficile

1. **Fecal Enemas**

Historically, stool transfer was used for treating diarrheal diseases and it was applied for CDIs in 1958 for the first time. Some antibiotics like Vancomycin and Metronidazole are used against CDI (Khanna and Gerding, 2019) but can't stop the recurrence of CDI, so fecal material transfer (FMT) by endoscopy (duodenum or ileocolonic) and enema reduced the recurrence of CDIs. But it is not a first-line treatment (Aljarallah, 2017).

2. **Adsorbents**

Different toxin-binding agents are used as an alternative strategy to treat difficile infections. Tolevamer was used to treat hamster's *C. difficile* colitis. Oligosaccharides like Synsorb 90 were used to treat toxin A-induced enteritis in rat ileum, encouraging results were obtained but it is not used now (Roshan et al., 2018). This is a potential treatment for C. difficile involving the use of various "binders" like resins, sugars, and polymers to capture the bacteria's toxins in the gut before they cause harm (McFarland, 2005).

3. **Probiotics**

Lactoferrin (a milk protein) is being used as an alternate strategy for slowing *C. difficile* growth and ultimately reducing the toxin production rate of *C. difficile* (Dieterle et al., 2019). *Saccharomyces boulardii* used as probiotic control of CDI, needs more research, however, some trial results suggest that it may be useful in preventing subsequent CDI. Bio-K+ (Lactobacillus acidophilus CL1285, L. casei LBC80R, L. rhamnosus CLR2) compete with *C. difficile* for space and resources and have good efficacy and safety against CDI (Goldstein et al., 2017).

4. **Phage Therapy**

Phage therapy has also been considered as a control strategy for CDI but due to the temperate nature of all the *C. difficile* phages, the CDI problem wasn't mitigated by the use of pages (Heuler et al., 2021).

Conclusion

Vaccines play an important role in preventing *Clostridium botulinum* and *C. difficil*e infections. *Clostridium difficile* is associated with nosocomial CDI diarrhoea and *C. botulinum* is associated with botulism that involves nervous disorders. The vaccines do their action by controlling toxin spread but there are limitations such as they do not cover all serotypes, safety not known yet, many vaccines are not yet licensed, and are expensive too. New-generation vaccines, such as tetravalent and heptovalent ones, are required for more effective control and elimination of the hazardous effects of *C. Botulinum* and *C. difficile*. Antibiotic therapy, probiotics and fecal microbiota transplantation are examples of alternative control techniques that offer more options for treating bacterial infections. Recurrence and antibiotic resistance, however, continue to be major challenges in the management of Clostridium infections. There is a need for a strong vaccination protocol and elimination of spores or preventing those spores from germination. Maintenance of hygiene and using vaccination along with secondary control strategies can play a significant role in reducing clostridium infections and preventing their re-occurrence.

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Chapter 40

Advancements in Vaccination Strategies for Aquaculture: Protecting Fish Health and Sustainability

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ABSTRACT

Aquaculture is experiencing exponential global growth compared to all other sectors involved in animal food production. Aquaculture has encountered significant economic challenges worldwide as an outcome of infectious diseases of diverse origins, including viral, bacterial, and other types.

The occurrence and progression of fish diseases are a significant obstacle to fish production. Despite the detection and treatment of infections using various therapeutic and preventative methods, vaccinations with broad-spectrum activity are crucial for preventing diseases in aquaculture. Aquaculture vaccines have effectively decreased the utilization of antibiotics in fish farming. Nevertheless, the efficacy of treatments such as antibiotics and probiotics appears to diminish when novel mutant strains emerge and disease-causing microorganisms acquire resistance to routinely employed drugs. The vaccine excels in terms of efficacy, safety, and convenience. Additionally, it can acquire superior economic advantages, rendering it the most appropriate approach for managing fish infections. Significant constraints in the progress of fish vaccine advancements include limited knowledge of fish immunology, a large number of unlicensed vaccines, high costs, and the stress associated with administration. Thus, vaccinations developed utilizing modern molecular techniques can be considered an efficient means of treating disease-causing infections in aquatic organisms. The advancement of molecular biology allows for the utilization of specific pathogen components and novel adjuvants to increase immune protection. This study explores traditional and modern fish disease vaccines, highlighting advancements in technology and recommending future research directions. It emphasizes the need for novel vaccination strategies, aquaculture expansion, and strong coordination between pharmaceutical companies and academic research.

KEYWORDS
Aquaculture, Vaccination, Immunology, Disease, Antibiotics, Probiotics

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INTRODUCTION

Fish diseases continue to be a significant economic concern in commercial aquaculture on a global scale, despite the existence of numerous innovative therapeutic approaches. Antibiotics and chemotherapy are used to cure diseases, but they have some obvious side effects including safety concerns and issues related to drug resistance. Vaccination is a crucial tool in preventing viral and bacterial diseases in fish, contributing to the social, economic, and environmental sustainability of aquaculture worldwide. Since the 1940s, numerous vaccines have significantly reduced the effects of certain fish diseases. Vaccinations have replaced antibiotics in certain regions of the globe, and millions of fish receive them annually. For example, in Norwegian Salmon farming, the use of antibiotics has significantly decreased, making vaccination the most sustainable and cost-effective method for treating infectious diseases in fish (Sneeringer et al., 2019).

Vaccination is the most suitable approach to disease prevention and pathogen control from an ethical, environmental, and economic aspect. Since vaccination is thought to be a cost-effective means of preventing certain life-threatening diseases, it is playing an important role in aquaculture. The selection of diseases to be vaccinated against, as well as the selection of vaccine type, vaccination technique, time of administration, and revaccination use, are all considered components of a vaccination strategy. However, to develop new vaccines and enhance the available suboptimal vaccines against bacterial and viral diseases, much research is required. Special consideration should be given to vaccine safety, efficacy, and the administration route (Adam, 2019).

Novel vaccination techniques must be affordable, simple to use, and less traumatic for animals to eradicate livestock diseases and improve food security in a world that is promptly evolving as a result of climate change. Immunization against disease is the best pathogen management strategy in the aquaculture sector for ethical, environmental, and economic reasons. Vaccines derived from inactivated or isolated pathogen components or dead pathogens are less

effective at eliciting weak immune responses. Immersion is more applicable than injection methods for vaccination (Bogwald and Dalmo, 2019). Despite increased use of chemical disinfectants and antimicrobials in aquaculture, fish diseases persist due to dense animal populations and poor conditions, necessitating continued vaccination strategies. Vaccines typically consist of an adjuvant, a carrier, and a specific antigen that triggers specific immune responses after vaccination.

History

The term "vaccination" was first used by Edward Jenner, who used cowpox infectious material to immunize a boy against smallpox. After that, he named the procedure "vaccine inoculation," and then "vaccination." As a compliment to Jenner's achievement, Louis Pasteur later proposed that the term "vaccination" be used to refer to the prophylactic inoculation of microorganisms. The term "vaccinology" is relatively novel. In 1977, Salk and Salk developed vaccinology, which explains the multidisciplinary aspect of disease prevention. This approach is founded on the idea that microorganisms trigger the immune system to prevent infectious diseases in both individuals and populations (Salk and Salk, 1977).

Vaccinology encompasses various disciplines, including immunology, and has been extensively studied over the past 150 years. Scientists from various fields, including pathology, microbiology, ichthyology, anatomy, biology, physiology, hematology, and fish diseases, have contributed to this field. Prior to World War II, experiments by R. Gudding and W.B. Van Muiswinkel on humoral antibodies in vaccinated fish were published (Gudding and Muiswinkel, 2013). Fish were also included in research on developmental and comparative immunology to understand the immune system's evolutionary development. The research involved various fish species and environmental variables like temperature (Muiswinkel, 2008).

Adjuvants are crucial in fish vaccinology and immunology, with Freund's adjuvant and aluminum hydroxide being the most effective. Oral immunization is considered the only effective method for disease prevention. Research on terrestrial animals and humans has influenced the goals and path of fish vaccinology and immunology. Spray vaccination, based on poultry experience, has not been extensively applied in the field. Another effective method is to immerse fish in a diluted vaccine solution for a brief period. Bath vaccine relies on the lateral line, skin, and gills to uptake antigens. Injections are the most efficient mode of administration, but they cannot be used for juvenile or small fish and are labor-intensive. Future advancements in immersion, oral, and delivery methods will address these issues (Muiswinke and Nakao, 2014).

Basis of Fish Vaccination

Fish immune systems are designed to defend against bacteria, viruses and other foreign antigens, or proteins. Therefore, it's critical to determine the immune system's morphological and functional maturity before applying any vaccination strategies. Although the field of fish immunology is relatively new compared to human and veterinary immunology, the methods used are comparable (Muktar et al., 2016).

Innate Immune System

Fish have innate systems that include physical barriers like skin and mucous membranes, specialized cells like natural killer cells and macrophages, and specific soluble chemicals like complement and interferon. These systems are initially defense against external invaders, with antibodies produced locally by mucosa-associated lymphoid tissues. Fish's immune system consists of non-specific cells called granulocytes, cytotoxic cells, and monocytes or tissue macrophages. Bony fish complement systems have the duplication and diversification of many components, and recent research has demonstrated that fish have functional homologues of mammalian cytokines (Muktar et al., 2016).

The Adaptive Immune System

There are three classes of fish: Agnatha (fish without jaws), Chrondrichthyes (cartilaginous fish), and Osteichthyes (bony fish). They exhibit vertebrate adaptive immunoglobulins, immune T-cell responses, cytokines, and histocompatibility complex molecules. The immune system of fish is different from higher vertebrates, involving humoral and cell-mediated responses. Fish contain the majority of generative and secondary lymphoid organs in mammals. The anterior kidney of teleost fish is believed to produce histo-compatibility complex molecules, leading to the development of B and T cell in the thymus. B-lymphocytes are generated and mature in the kidney, while progenitor T-cells migrate to the thymus for maturation (Du et al., 2022).

Administration Methods

Three fundamental techniques are used in aquaculture to give vaccines: oral (with food), immersion and intramuscular (IM) or intraperitoneal (IP) injection.

Oral Vaccination

The oral route is the most common immunization method due to cost, convenience, reduced stress, and suitability for all fish sizes. Oral nano vaccines are more successful in increasing host survival. Other oral delivery methods include artemia, plant-expressed vaccines, biofilms, attenuated bacterial delivery, and microalgae (Dadar et al., 2017).

Injection Vaccination

Introducing a tiny amount of a recognized antigen into the fish is known as injection vaccination. Immunization via injection is the most efficient and offers long-term protection. Most injection vaccinations are multivalent, including mixtures of dead viruses, viral proteins or bacterins. The aquaculture of Salmon and Trout is the primary application for injection vaccination. Nevertheless, this approach has a number of drawbacks, including managing stress, injecting, requiring a lot of labor and being expensive (Dhar et al., 2014) (Table 1).

Immersion Vaccination

The most common vaccine method is immersion immunization, which can be applied via spray, hypersonic infiltration (HI) or direct immersion (DI). Additionally it was found that HI protects *Cyprinus carpio* against *Aeromonas salmonicida* more effectively than DI. Bath immunization requires a considerable amount of the vaccine, but does not cause handling stress (Bogwald and Dalmo, 2019).

Table 1: Comparison of delivery routes of fish vaccines (Ji et al., 2020).

	Injection		Immersion	Oral
Vaccine Type	Intraperitoneal injection: Inactivated, Live attenuated, subunit, Live vector vaccines	Intramuscular injection: DNA Vaccines	Inactivated vaccines, Live attenuated vaccines	DNA Vaccines
Advantages	Prevents multiple diseases. Enhances immune effect with adjuvant. Required antigen dose is small and accurate.		Suitable for all sizes of fish. Easy operation.	Avoid stress. Suitable for all sizes of fish. Facilitate large-scale use.
Disadvantages	Labor intensive Time-consuming. Unable to immunize small fish. Easy to damage fish body.		Large antigen quantity needed. Produce strong stress Poor immune effect.	Large antigen requirement. Antigen loss in gastrointestinal tract.

Types of Vaccines for Fish Pathogens

Bacterins

Bacterial vaccines in aquaculture are typically inactivated, obtained from a specific strain's broth culture. Bacterins activate humoral immune responses, which are associated with antibodies. Some vaccines, like those for Salmonids against *Aeromonas salmonicida subsp*. *Salmonicida*, can be obtained through aqueous formulations, while others require injection of oil adjuvant bacterins for adequate protection (Mondal and Thomas, 2022)

Live Attenuated Vaccines

Live attenuated vaccinations are a type of vaccine that uses living microbes that have lost their ability to cause serious illness. They can be beneficial for aquaculture as they require a prolonged period for the antigen to spread effectively and stimulate the immune system's cellular branch. Some live vaccines, such as *Edwardsiella tarda*, *E. ictaluri*, and *Aeromonas salmonicida*, have undergone experimental testing. However, before they can be used in the field, concerns about safety, stability, reversion to virulence and the potential for the disease to disseminate to non-target animals must be addressed. The only approved vaccination for ESC in Catfish in the USA is an attenuated live *E. ictaluri* vaccine (Zheng et al., 2021).

DNA Vaccines

A type of vaccine known as DNA vaccine that creates an immune-stimulating section of a pathogen in animals, providing an internal source of vaccine material. They are superior to traditional vaccines due to their unique immune response in mammals, including cytotoxic cells, antibodies, and T-helper cells. However, the safety of fish, the environment, and consumers must be considered before using DNA vaccines in aquaculture businesses. The DNA sequence contains the genetic information for a single gene found in a microorganism, preventing virulence reversion, which is crucial for environmental safety. DNA vaccination has been proven to produce robust and protective immunity against various viral diseases in fish (Reyes et al., 2017).

Polyvalent Vaccines

Polyvalent vaccines are the best for protecting fish species against various illnesses, covering primary serotypes in specific geographic regions. They are equally effective in Salmonids and Turbot, providing equal or better protection. However, antigen competition may arise, and caution must be exercised in the development of polyvalent vaccinations, especially when injectable forms are involved (Sahoo et al., 2021) (Fig. 1).

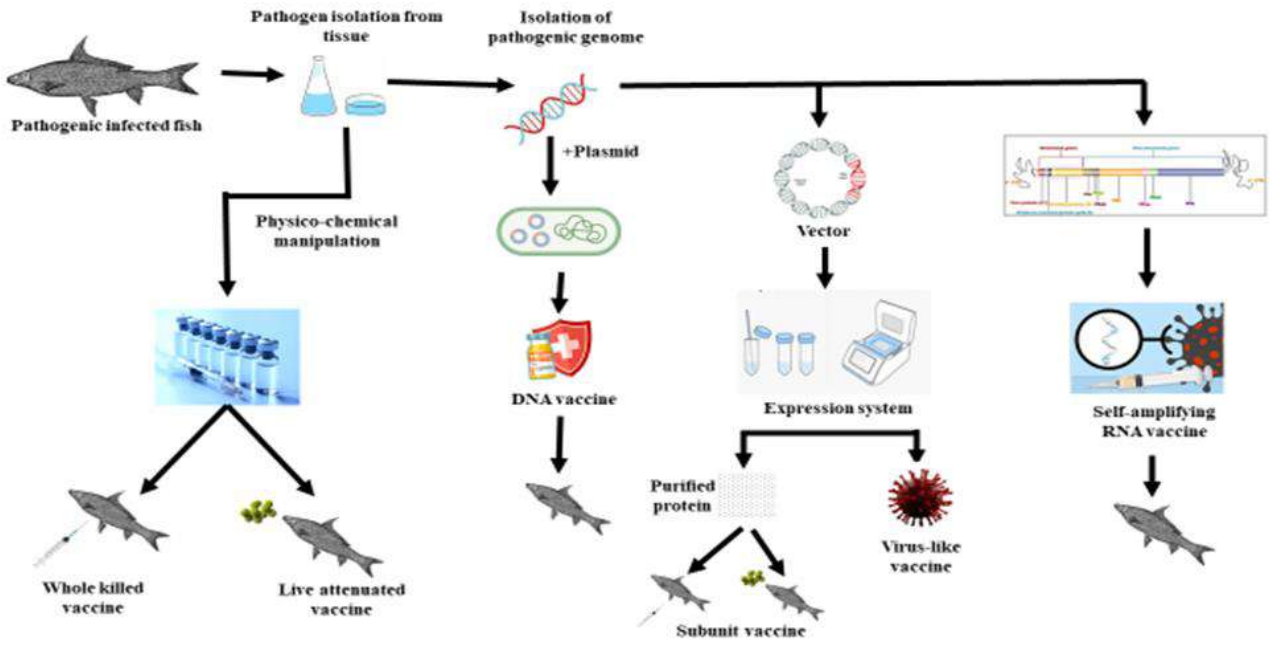

Fig. 1: Various approaches for fish vaccine development (Ma et al., 2019)

Current Status of Fish Vaccines and its Applications

Bacterial Vaccines

Vaccination is crucial in commercial fish farming, particularly salmon cultivation. Vaccines for European Seabass, Seabream, Japanese Amberjack, Yellowtail Tilapia, Atlantic cod, Channel Catfish, Salmon, and Trout are commercially available. Vaccines produced by experimental methods using inactivated bacterial infections have demonstrated efficacy (Muktar et al., 2016).

Bacterial Kidney Disease (BKD)

Bacterial kidney disease is caused by *Renibacterium salmoninarum*, a fastidious, aerobic, and non-spore-producing bacterium. Immunization experiments using live, synthesized, or classical bacterins have been recorded. However, because of the pathogen's intracellular structure, vertical transfer, and possible immunosuppressive role of protein p57, the effectiveness of immunizations in field conditions is uncertain. Novartis' commercial aquatic live vaccination, "Renogen," has been licensed to prevent BKD spread (Delghandi et al., 2020).

Rainbow Trout Fry Syndrome (RTFS)

Microbial cold water disease (BCWD) and peduncle sickness in Salmonids are linked to *Flavobacterium psychrophilum* since 1948. However, early life stages of fish are unfeasible for this method. Recent trials on Juvenile Rainbow Trout showed oil adjuvanted i.p vaccinations as the only effective protection. Despite the absence of licenced vaccinations for this sickness, certain countries are utilising autogenous bacterins derived from single farm isolates (Takeuchi et al., 2021).

Furunculosis

Furunculosis, caused by *Aeromonas salmonicida subsp*. *Salmonicida*, affects fish of all life stages, from fry to brood stock. It is often caused by sudden water temperature increases and changes in fish physiology, such as breeding or mollification. Despite the development of several furunculosis bacterins since 1980, salmonids can still be infected orally, subcutaneously, or by immersion (Braden et al., 2019).

Vibriosis

Vibriosis, a significant bacterial illness affecting marine fish globally, is a major concern. Many commercialized vaccines, primarily for injectable or bath administration, contain only O_1 or a combination of O_1 and O_2a serotypes (Mohamad et al., 2021).

Yersiniosis (Enteric Red Mouth Disease)

Enteric Red Mouth Disease, caused by the bacterium *Yersinia ruckeri*, predominantly impacts Rainbow Trout in freshwater environments and Atlantic Salmon in marine habitats. The disease is caused by an anaerobic, unable to move, Gram-negative rod. An extensively used commercial vaccine, consisting of formalin-inactivated whole cultured cells of Y. Ruckeri serovar I, Biotype 1 (Hagerman strain), is currently accessible (Yang et al., 2021).

Pseudomonadiasis

Pseudomonas anguilliseptica is thought to be the most important pathogen for farmed fish among the *Pseudomonas* species (*P. chlororaphis*, *P. anguilliseptica*, *P. putida*, *P. fluorescens* and *P. plecoglossicida*) obtained from infected fish. Recently, bacterins including both primary serotypes were produced and introduced to aqueous and non-mineral oil. These bacterins were then tested in experimental tests involving Gilt-head Sea Bream and Turbot, and their effectiveness was established.

Viral Vaccines

Despite extensive research, few viral vaccines are approved for fish virus. Currently, most fish virus vaccines are synthetic proteins or inactive viruses. There are no approved live virulent or DNA vaccines, however, controlled field trials are currently underway in Canada for a singular DNA vaccine that targets infectious hematopoietic necrosis (IHN). Synthetic subunit proteins or inactivated viruses are the foundation of the majority of viral vaccines for aquaculture. It is challenging to develop inactive viral vaccines that are cost-effective, due to high doses and ineffectiveness unless administered by injection. Live viral vaccines have shown positive results in fish testing (Gong et al., 2021).

Infectious Pancreatic Necrosis

The aquatic Birna virus, linked to chicken infectious bursal disease, causes infectious pancreatic necrosis in fish. It affects younger fish, but carriers may have a carrier status, making controlling the illness difficult. In the UK, a vaccination against Atlantic salmon is licensed for provisional marketing (PMA), highlighting the potential risks associated with this virus in fish raising (Hua et al., 2021).

Salmon Pancreas Disease Virus

Salmon pancreas disease virus, which causes pancreatic disease, shares a close relationship with sleeping sickness in Rainbow Trout. Despite biosecurity measures, Trout growers are still at risk. A salmon pancreas disease vaccine is available through a premarket approval (PMA), but must be administered individually, unlike other combined Salmon vaccines that require a single injection (Aida et al., 2021).

Fish Vaccines against Parasites

Fish parasites are prevalent in both wild and farmed populations, causing issues in aquaculture. Fish have defense mechanisms against parasites, and those that survive natural infections have greater immunity or resistance. However, cultivating parasites for vaccination is expensive and relies on a population of hosts instead of using cell cultures. The use of natural hosts for production is also expensive and problematic for safety documentation. Therefore, the most practical approach to developing commercial parasite vaccines is likely to involve the identification and synthesis of protective antigens, at least for low-cost vaccines (Shivam et al., 2021).

Factors Affecting Efficacy

The effectiveness of a vaccine is influenced by a number of factors, either directly or indirectly. When creating vaccines for laboratory and field trials, it is necessary to consider aspects like as adjuvants, challenge models, stress, immunization doses and duration, temperature, and fish size (Rathore, 2022).

Influence of Adjuvants

During the initial stages of vaccine development, mineral oil was commonly used as an adjuvant. Fish vaccines are developed using various adjuvants and immuno stimulants, including oil emulsions, aluminum salts, FCA, FFIA, montanide, saponins, immune stimulating complexes, nano/microparticles, toll-like receptor ligands, cytokines, derivatized polysaccharides, and bacterial derivatives. Despite their effectiveness, for Atlantic Cod (*Gadus morhua*) they pose some major negative effects, indicating the need for future research to identify the most effective adjuvants (Dalmo et al., 2016).

Challenge Models

Vaccination in fish is difficult because of their watery habitat. Developing a reproducible challenge model for aquatic vaccines entails considerations such as the particular strain of pathogenic bacteria and the techniques used for delivering the challenge, and challenge dose optimization. Injection challenges are used to evaluate the impact of *Aeromonas spp.* vaccinations for Tilapia, providing a more accurate understanding of natural events. The Risk-Performance Stimulation (RPS) method is widely used for evaluating vaccine efficacy, but it only provides survival rates against death and does not provide information on vaccine protection mechanisms. Lower doses of bacteria improve protection and decrease mortality (Monir et al., 2020).

Protection Duration and Vaccine Dosages

Tilapia, with a 4-6 month culture lifetime, require long-term protection from MAS vaccines. The effectiveness of the vaccine is directly correlated with the quantity of vaccinations administered with booster vaccinations resulting in higher antibody titers and host survival. Factors such as total biomass, fish age, vaccine exposure duration, salinity, and pH of the

immersion water also impact the fish's ability to digest the antigen (Liu et al., 2016). Optimizing antigen doses for oral and immersion immunization methods can be challenging, however, this can be accomplished by evaluating the correlation between the injectable immunization dose and the level of protection, as intraperitoneal injected fish quickly absorb and develop adaptive immune responses (Ismail et al., 2017).

Body Temperature

Fish, being cx74old-blooded organism, have a body temperature equal to their environment. The vaccination period depends on the species and temperature of the fish, with warm-water species experiencing a quicker immunological response. Warm-water species like Seabass, with ideal temperatures of 22°C, can develop antibodies one week after immunization. Antibodies against Atlantic salmon, which have an optimal temperature range of 10-12°C, typically appear 4-6 weeks after vaccination (Dubey et al., 2016).

Stress

Apart from temperature, immunological suppression can be induced and vaccination efficacy limited by stress resulting from natural or artificial variables such photoperiod, seasonal variations, saltwater; toxic materials, crowding, handling, and transportation. Farmed fish are unable to escape similar stressors that wild fish are not, and chronic as well as acute stress can make them vulnerable to various pathogenic bacteria and opportunistic ones (Monir et al., 2020).

Reverse Vaccinology

Reversing vaccinology, a new biotechnology, has gained attention for its potential in vaccine development against various pathogenic organisms, although the successful and effective development of such a vaccine typically takes years. The development of vaccines has decreased from 5-10 years to 1-2 years due to this approach of vaccination. The software uses bioinformatics to predict immune sequences, with sections predicted to express as recombinant proteins (Dadar et al., 2017) (Fig. 2).

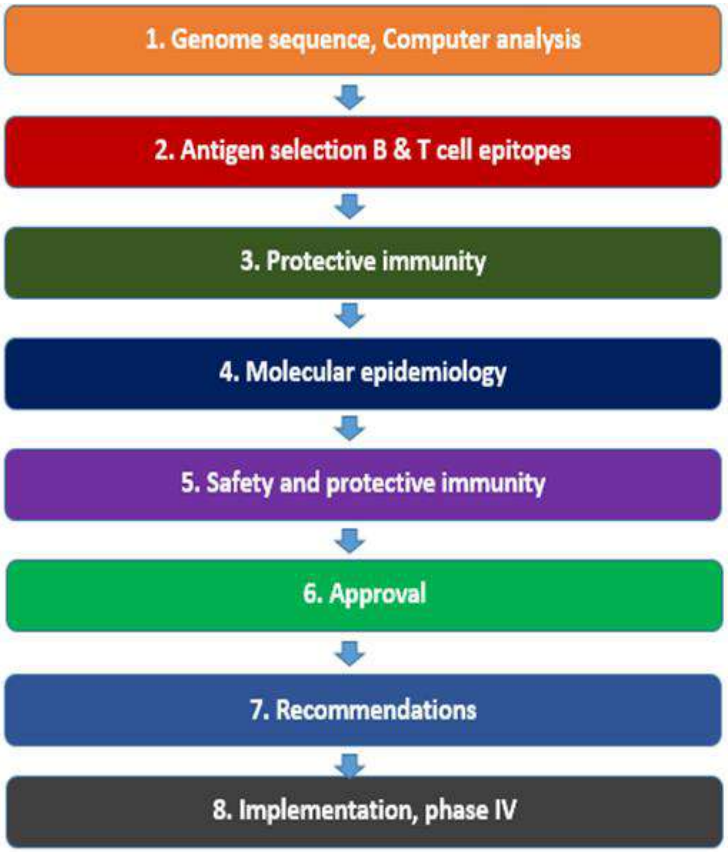

Fig. 2: Steps involved during Reverse Vaccinology (Sette and Rappuoli, 2018)

Photobacterium damselae subsp. *Piscicida* is one of the marine species to which this technique has recently been applied. Numerous investigations into reverse vaccination have revealed serious side effects. Software has been used to build vaccines against *Flavobacterium columnare*, a significant internal pathogenic fish virus that causes columnaris, and *Edwardsiella tarda*, a pathogen that causes edwardsiellosis (Mahendran et al., 2016).

Reverse vaccine research of the Lumpfish, *Cyclopterus lumpus*, from *Pasteurella atlantica* has the ability of avoiding pasteurellosis in aquaculture, according to a study report by Ellul et al. (2022). In order to prevent these disease crises, the most effective gene contender are given priority in the production of vaccines, according to their research and functional evaluation.

Limitations in Fish Vaccine Development

To produce some kind of long-lasting defense against a particular disease is the main objective of vaccination. It has been disputed whether the fish's immunological memory or ongoing sensitization from an antigen depot is responsible for the oil-adjuvant shot vaccinations' efficient long-term immunity. The potential for parental immunization to safeguard offspring is further limited by apparent insufficient maternal resistance in fish (Adams, 2019).

Research on the true processes behind vaccine protection in fish is limited due to the lack of isotypes matching mammalian IgA, IgE, or IgG, and the less pronounced humoral immune system response in fish. Immunologic assays are often used to measure the presence and titer of specific antibodies, but T-cell response assays are still in their early stages. The varying immune systems of different fish species limit research opportunities on pathogen-induced immunity and immunity induced by vaccines (Chellapandian et al., 2023).

Commercial fish vaccines face affordability challenges due to their high cost and the need for a low antigen dose. Fish require higher doses than terrestrial species, making it difficult to develop affordable deactivated viral vaccines. Over the past decade, commercial fish vaccine formulations have shifted to combinations of two to five vaccines. This level of complexity is further complicated by the fact that not all antigens elicit an insulating immune response, and antigens fluctuate in their immune dominance, and the fish's immune system has a specific and restricted ability to respond to specific antigenic molecules (Kossack et al., 2020).

Many fish species are too delicate to withstand vaccination pressure or may experience severe adverse reactions. The most prevalent method is oral vaccination, which is characterized by its simplicity and stress-free nature. Pharmaceutical corporations have conducted most research on fish vaccines, but there are few published studies on the subject. The main disease issues in different species may manifest as larvae or fry, as the animal reaches a suitable size has a fully formed defense mechanism (Cao et al., 2019).

Recent Molecular Advances for Development of Vaccines

There is evidence that preventive measures in fish such as grass carp activate receptors for viral nucleic acids, high mobility group box proteins (HMGBs), toll-like receptors (TLRs), retinoic acid inducible gene-I (RIG-I) like receptors (RLRs), and PRRs. According to recent genetic research, they can be altered to produce a suitable amount of protection and are important in stimulating the fish immune response to fight viral infections (Rao and Su, 2015). Additionally, toll-like receptors are distinctively capable of recognizing pathogen-associated molecular patterns (PAMPs) in microorganisms, which activates their immunological signaling cascades and strengthens the natural immunity. Additionally, they are said to be crucial for the development of adaptive immunity.

Accordingly, TLR activators and additives may be added to a vaccine composition for fish and other aquatic organisms to produce an efficacious vaccination. The invention of vaccines, such as designer cell lines, immunomics-based vaccinations, marker vaccines, structural vaccinology (SV) and dendritic cells, is additional recent innovation in the field of molecular biology (Singh et al., 2015). When it comes to vaccine development, adjunction with better adjuvants may be crucial when it comes to immunizations that fail to elicit robust responses from the immune system. This is because enhanced adjuvants can raise the amount of protection to the intended extent (Effio and Hubbuch, 2015). The conventional method of vaccine creation, which involves the utilisation of live attenuated or entire inactivated viruses, is being substituted by a more sophisticated molecular technique. This novel methodology employs computational design and sophisticated synthetic techniques to produce precisely formulated conjugate vaccines (Figure 3).

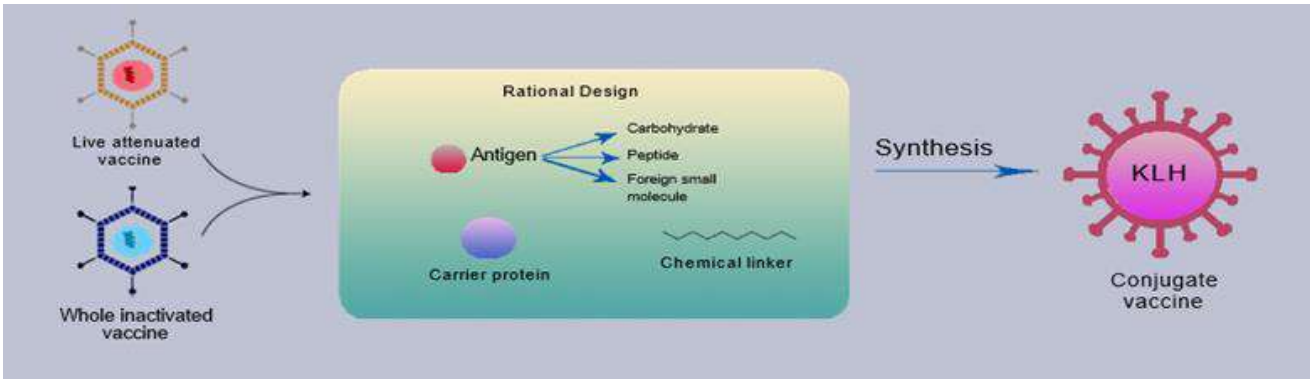

Fig. 3: Progress in the molecular development of artificial vaccines (Jones, 2015)

Safety of Fish Vaccines

Fish vaccine safety is a concern due to their low immune system stimulation capacity, potentially leading to serious diseases and reduced fish output. To ensure safety, vaccines must meet safety standards, including ten times the recommended dose. Deactivated or dead vaccines are suitable for aquatic organisms due to their pathogenic content. Improved live vaccinations are the only concern, as they contain pathogenic organisms (Dadar et al., 2016). DNA vaccines

are effective due to their minimal need for immune-stimulating pathogens. They offer benefits such as co-administering multiple vaccinations, cost-effective production methods, durability in preservation due to plasmid DNA's chemical stability, and quick DNA sequence alteration for targeting novel disease variants. They also provide immunity against spreading diseases, combat attenuated live vaccines, and correct protein folding orientation of pathogen antigens, which are not always generated using recombinant proteins (Jansen et al., 2017).

Adjuvant treatment is not necessary for enhancing immune system reactions and immunity mediated by cells. They are not dependent on oil adjuvants, which have been linked to specific adverse effects. In 2005, the Canadian Food Inspection Agency granted approval for the use of Apex-IHN®, a DNA vaccine, to combat IHN. These vaccinations are effective in stimulating immunity against viral fish diseases such as VHS in trout and have been approved in the United States. Elanco Animal Health has successfully produced Clynav, a DNA recombinant vaccine, which has received approval in the EU and Norway for its effectiveness against pancreatic illness in Salmonids. (Aida et al., 2021). Elanco Animal Health has developed a DNA recombinant vaccine, Clynav, to protect Pacific and Atlantic Salmons and Rainbow Trouts from VHS and enhance both the natural and acquired immune responses in other fish species, while also preventing salmonids from pancreatic disease in Norway and the EU (Jansen et al., 2017; Aida et al., 2021)

Correlates of Vaccine Protection Efficacy

Exploring in vitro correlates for vaccination protection efficacy presents novel research prospects in vaccine delivery methods. Current in vivo challenge procedures are considered non-economic and unethical, necessitating alternative, quantitative, and non-lethal immunological techniques to assess the protective potential of new vaccines without sacrificing immunized fish. In aquaculture, three distinct correlates of vaccination protection, antigen dosage, measuring the level of antibodies and xamining the manifestation of surrogate indicators of defensive immunity can serve as significant benchmarks.

Antibody Titer

The correlation of antibodies as a marker of protective immunity in fish has not been proven yet. Passive immunization can assess the protective effects of antibodies, with studies showing a positive relationship between post-challenge protection and antibody titers. However, verifying the protective threshold for a vaccine remains a challenge. The primary issue is that different fish in the same vaccination group exhibit varied behavioral patterns, leading to differences in antibody titer values. Additionally, the absence of immunoassays to measure IgT levels on mucosal surfaces on vaccination response is another issue. Appropriate diagnostic instruments for evaluating IgT titers expressed on mucosal surfaces are necessary to further correlate the antibody response with vaccination efficacy (Khunrang et al., 2023)

Surrogate Markers of Protective Immunity: Expression Analysis

Surrogate markers of protective immunity are genes that function as biomarkers for vaccine protection against immune-eliciting antigens. The MHC-I route mediates DNA vaccination, while MHC-II molecules usually mediate vaccine antigens to T-cells. Both responses may be initiated concurrently, with one taking precedence as the immune response matures. A protective immunity for fish vaccines is also suggested by surrogate biomarkers, as cellular immune responses are crucial. Research suggests developing surrogate indicators for fish vaccines could be a new avenue for future investigation (Dalmo, 2018).

Antigen Dose

There is a clear correlation between the amount of antigen in vaccination formulations and the level of protection it provides to fish. Numerous studies have demonstrated that vaccines' protection is directly influenced by a difference of log10 in antigen concentration. For instance, Red-Spotted Grouper exhibited higher protection against the nervous necrosis virus challenge when administered inactivated whole-cell vaccines with an antigen dose exceeding 107.5 (Yamashita et al., 2009).

Antigen dose is also a crucial factor determining protective efficacy in various fish species. These studies demonstrate that it is possible to establish a cutoff antigen dose that correlates with protection, which can be used as benchmarks to evaluate all subsequent vaccine batches. Protective vaccines are those that contain an antigen dose that is equal to or greater than the protective antigen dose, while those that are below the dose are considered suboptimal (Dubey, 2016).

Conclusion and Future Outlook

Fish vaccinations have become a reliable and affordable method for managing infectious diseases in aquaculture over the past two decades. These vaccines can significantly reduce losses caused by certain diseases, reducing the need for antibiotics. However, there are several health-related issues that must be addressed to ensure sustainable fish output and improve fish health.

The production of fish vaccines is expensive and time-consuming, making it necessary to identify and monitor diseases causing large-scale mortality through low-cost technology. Advancements in vaccine manufacturing and development have been made, but most research is still in its early stages. Issues that hinder the development of cost-effective, multivalent vaccination strategies remain unaddressed. Fish production costs are lower than other aquatic organisms, so it is preferable

to use affordable vaccines to combat fish pathogens. Multivalent salmonid vaccines and supplementary antigens are needed to combat viral infections such as VHSV, IHSV, ISAV, and PDV. Reassembling the tertiary structure and refining the new generation mechanism that produces protein glycosylation are also necessary.

Adjuvants are necessary to boost the efficacy of vaccine formulation in inactivated or killed vaccines to achieve sufficient and long-lasting protection. Presently available injectable vaccines offer defense against infections caused by bacteria like furunculosis, vibriosis, and winter ulcers. High-value fish species, such as Salmonids, are given priority for contemporary multivalent fish vaccines that contain antigens from multiple diseases. However, the development of effective antiviral vaccines can pose a challenge. Future fish vaccines should include current vaccines without an adjuvant platform to improve antiviral responses after vaccination. Genetically modified organisms can be employed to overcome safety issues associated with live attenuated microorganisms returning to virulence and instability.

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Chapter 41

Precision Vaccinology: Maximal Protection and Minimal Side Effects

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ABSTRACT

Vaccinology is an empirical subject to date with a traditional concept where vaccination works on a public health paradigm of the "same dose for everyone for a similar disease." Recent advances in computational biology shifted the vaccine development approaches towards predictable methodologies that enable better design and maximum effectiveness. It is referred to as "precision vaccinology," which aims to improve healthcare quality by tailoring the treatment process according to the unique characteristics of each patient. There is a significant diversity in the immune response to vaccination, and many factors influence it. These include gender, age, genetics, health issues, pre-existing antibodies, gut flora, and nutritional and environmental factors. Furthermore, vaccine parameters such as type, side effects, adjuvant and dose, schedule, and route of administration are also critical in immunization. Understanding all of these factors and how they influence vaccine response helps develop a precise vaccine with more efficacy and the most negligible side effects. From design to administration, numerous innovative insights and technologies exist in the development of precision vaccines. However, there are still many studies of the varied immune responses across sex, age, immunological status, and special conditions (pregnancy, cancer, etc.) that are chiefly required in the field of vaccinology.

KEYWORDS
P4 Medicine , Precision Vaccinology, Vaccine Development, Vaccine Efficacy, Immune Response, Next-Generation Vaccines

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INTRODUCTION

Infectious diseases are among the top ten public health challenges (Organization, 2019). According to the World Health Organization (WHO), over seventeen million people died from infectious diseases every year (Organization, 2018). Infections affect individuals more than other diseases and can develop over weeks or months (Grant and Hung, 2013). Infectious diseases spread fast across geographic borders and result in significant mortality, morbidity, and worldwide economic harm (Baker et al., 2022). Since infections are the cause of around 13% of all cases of cancer that are reported, that's why these have a significant role in the global cancer rate (de Martel et al., 2020). Vaccines have been regarded as effective, durable, and safe ways of controlling strategies against infectious diseases, and many studies have previously reported the impact of vaccination on preventing the spread of specific infections at the population level (Drolet et al., 2015; Rodrigues and Plotkin, 2020; Pritchard et al., 2021; Hamson et al., 2023).

A deeper understanding of host-pathogen interactions and host immune responses at the individual level indicates that these interactions are highly complicated, and specific vaccinations might not be practical or safe for every patient (Yu et al., 2022). Researchers explored a one-dose, one-vaccine-fits-all, strategy about twenty years ago (Wei et al., 2021). Nowadays, precision vaccination has become feasible due to the application of system biology techniques and advancements in biotechnology, which have improved the knowledge of diversity in pathogen interactions and host immune responses (Lee

et al., 2023; Lee et al., 2023; Soni et al., 2020). For example, in viral infections, regardless of the vaccine formulation, repeated vaccination administration preexisting levels of both the antibodies and $CD4^+$ T-cells may attenuate the post-vaccination production antibodies and the expansion of vaccine-induced $CD4^+$ T-cell (Jansen et al., 2019). Determining the severity of the disease can be made possible through precision medicine, including genetic testing and post-vaccination serological testing, to identify poor responders and those at risk of increased severity. With such testing, patients can be identified on time, given priority during the vaccination dosages, and eligible for extra prophylaxis after receiving their entire vaccination regimen. Several factors have been identified, such as immunosuppression, old age, chronic diseases like kidney disease, HIV infection, and diabetes mellitus, as well as specific genetic abnormalities as the cause of different immune responses (Körber et al., 2021). More effective vaccinations or adjuvants can be developed through precision vaccinology and given access to more effective precision vaccines (Tsang et al., 2020; Van Tilbeurgh et al., 2021).

Precision medicine is a critical approach adopted from P4 medicine, i.e., predictive, preventive, personalized, and participatory, recognized as an advanced healthcare policy (Collatuzzo and Boffetta, 2022). Precision medicine has been described by the U.S. Food and Drug Administration (FDA) as a novel strategy for tailoring disease prevention and treatment by considering people's genes, lifestyles, and environments (Akhoon, 2021). Precision medicine, commonly called personalized medicine, aims to provide patients with the most efficient and safest medical intervention (Ho et al., 2020). Precision medicine adapted medical interventions to individual characteristics by optimizing effective treatment with fewer side effects, while traditional vaccination focused only on population-wide (Boniolo et al., 2021; G. Xie et al., 2021). Applying precision medicine to vaccination leads to a notion, i.e., precision vaccinology, that potentially addresses the limitations of conventional vaccines, such as combating infectious diseases, cancer, chronic diseases, autoimmune conditions, and allergies. The development of traditional vaccines was aimed at eliciting antibodies, cellular responses, and immune responses and correlating them with protection against infectious diseases (Naithani et al., 2021; J. Xie et al., 2021). These approaches lack a complete simulation of immunity induced by natural infection, so they offer partial effectiveness (Alsaiari et al., 2023). Recent advances in vaccine research bridge this gap by emphasizing natural immune responses induced by infection and understanding the development of more effective vaccines (Pulendran and Davis, 2020).

Precision vaccines utilize analytic approaches and advanced technologies, including bioinformatics, gene expression profiling, and high throughput sequencing to identify potential vaccine candidate antigens and predict individual immune responses. The aim is to discourse the prerequisites of vulnerable populations such as immunocompromised, older people, and infants by optimizing tailored vaccines for their specific immune profiles, making them more responsive and flexible to challenges in pathogenesis (Kennedy et al., 2014; Pezeshki et al., 2019). Previous studies have described the tools for vaccine formation, determining the immunogenicity necessary to produce protection. All licensed vaccines have targeted routes of administration that maintain a controlled and localized antigen presentation to avoid the potential risks associated with a systemic vaccine. The vaccines might have varied effects depending on how they are administrated, reflecting the unique immunity each target tissue has developed. Moreover, by optimizing vaccine formulations, regimens, and dosages for specific populations, the efficacy and safety of vaccines can be improved (Embregts and Forlenza, 2016; Laupèze et al., 2019; Michaelides et al., 2023; Zhang et al., 2015).

Precision vaccinology also improves public health outcomes by minimizing adverse reactions, especially in vulnerable populations (Lee et al., 2023). It has also been extended to target non-infectious diseases such as cancer by analysis of mutanome, i.e., a complete set of all mutations in a tumor (Castle et al., 2012; Kreiter et al., 2015; Sahin et al., 2017), next-generation sequencing aids in understanding specific genetic events that contribute to cancer initiation and proliferation. A detailed cancer mutanome mapping in individual cancers leads to personalized vaccine development (Mortazavi et al., 2008; Sahin and Türeci, 2018). Recent studies have shown precision vaccines generate immune responses against antigens derived from cancer-specific mutations known as neoantigens. These vaccines are designed and tailored to each patient's tumor and genetic makeup (Lang et al., 2022). Recent advances in mRNA cancer vaccines hold immense potential as a personalized cancer therapy and have shown promising results in clinical trials by harnessing patients' immune systems (Huang et al., 2022; Morse et al., 2023). Next-generation sequencing has also made it possible to compare tumors in less time and at less cost. It helps identify cancer targets initially (Meldrum et al., 2011). By tailoring vaccine formulations to promote tolerance and modulate immune responses, personalized vaccines propose potential benefits in halting disease progression, attaining reduction in some cases, and relieving symptoms (Katsikis et al., 2023; Lin et al., 2023; Lybaert et al., 2018; Oosting et al., 2022).

Precision immunization is in its early stages. Technical, demographic, and procedural variables must be considered to interpret vaccine safety and immunogenicity accurately. Precision vaccination requires a detailed evaluation of factors such as gender, age, preexisting antibodies, gut flora, and gene polymorphisms on vaccine efficacy, mainly in unhealthy individuals (Cook, 2008; Lobo et al., 2023; Quiñones-Parra et al., 2014; Saco et al., 2018; Weinberger et al., 2008). Government support is essential to address and regulate the disease burden in targeted populations. Researchers should focus on precision immunization strategies and provide healthcare personnel with crucial scientific knowledge.

Meanwhile, healthcare professionals have to play a critical role in encouraging vaccination for high-risk patients to improve their quality of life and chances of survival. Vaccine research organizations should prioritize developing precision vaccines for patients with subclinical health issues to enhance public health outcomes. Cooperation between the government, healthcare, and research organizations is essential to improve precision vaccinology and immunization to meet the various needs of vulnerable populations.

Need for Precision Vaccines

Personalized and precision vaccinations have become crucial in the last few years against infectious diseases, a primary global health concern affecting the most significant number and rapidly expanding (Heesterbeek et al., 2015; Khoury et al., 2018; Sarwar, 2023). These are equally important to treat non-infectious diseases such as cancer (Kensler et al., 2016; Rossi et al., 2021). Traditional vaccinations aim to protect against a particular infection. However, such a conventional method might not work for every individual due to variations in genetic makeup, environmental conditions, and immune response (Giefing-Kröll et al., 2015; Ogra et al., 2001; Scepanovic et al., 2018). A detailed study of the following infections revealed that these diseases can quickly spread over geographical borders and affect the most significant number of people. The global tuberculosis report estimated that more than 10 million individuals contracted tuberculosis in the last five years. More than 1.5 million deaths from tuberculosis were reported in the previous decade, which is comparable to the number of deaths from respiratory tract cancers (Pandey et al., 2023). Since infections are the cause of around 13% of all cases of cancer that are reported, hence they also have a significant role in the global cancer rate. Human papillomavirus (HPV) is the fourth most common cause of cancer in women and solely accounts for 31% of all malignancies related to infections. An estimated 11.7% of women globally have HPV infection, and many of them re-infect at the same point in their lives (Bruni et al., 2010; de Martel et al., 2020). According to recent research, the virus strain and patient epigenetics will likely work together to cause HPV oncogenicity. Despite the availability of vaccines, their reluctance and challenges with distribution in underdeveloped nations have kept HPV a prevalent infection with recognized testing procedures that have revealed problems, including reliability, which varies with less skilled practitioners (Gallagher et al., 2018; Traversi et al., 2022).

Precision vaccination is now possible due to system biology and biotechnology advancements that have improved our understanding of microbial interactions and host immune responses. It is now feasible to produce cell-based influenza vaccines in large quantities, and knowledge of host immune responses can aid in predicting vaccination effectiveness (Lai et al., 2020; Pezeshki et al., 2019). The significant vaccine response and safety variation during the COVID-19 pandemic recently demonstrated the critical necessity for precision vaccination. Research has shown that 10% of people do not react well to the COVID-19 vaccine. The advancement and extensive application of mRNA vaccines amidst the COVID-19 pandemic further enhanced the precision vaccine development. Determining the severity of the COVID-19 condition can also benefit from precision medicine, which includes thorough background checks on health and genetic testing. Numerous risk factors and predictive biomarkers for the severity of COVID-19 have been identified, including age, gene variations, several comorbidities such as cardiovascular disorders and obesity, non-O blood types and ACE1 polymorphisms (Clark et al., 2020; Figliozzi et al., 2020; Franchini et al., 2021; Khanijahani et al., 2021; Slaoui and Hepburn, 2020; Toyoshima et al., 2020; Traversi et al., 2021; Yamamoto et al., 2020). The need for a precision vaccine against hepatitis B is also being recognized because 5% of those who received two complete dosages of hepatitis B vaccination failed to develop a serologic response and ended up as non-responders to the vaccine (Walayat et al., 2015).

More potent vaccines could be developed due to precision vaccination and omics-based precision vaccinology, which may help identify non-responders who subsequently get access to these more potent vaccinations (Equils et al., 2023). Improved tracking of vaccine safety is also needed in this era of precise vaccinations. The researchers established the following criteria for vaccine safety. The interaction between the host microbial flora, host immune system, and vaccination components such as antigens, adjuvants, DNA/RNA, and preservatives should be studied using data platforms. Vaccine safety monitoring and communication systems are necessary for human-centered procedures collaborating with local communities (Schoch-Spana et al., 2021). Real-time media monitoring procedures can help scientific and regulatory organizations identify new safety concerns, and reliable communication and monitoring systems should be created. Pharmacovigilance systems need to be updated to process data from unusual events and keep up with the speed of global information transformation. To identify infrequent global incidence stemming from vaccinations advised for more limited patient populations (Hagan et al., 2022; Plotkin et al., 2020; Pulendran et al., 2010).

Impact of Host Factors on Vaccine Response

An important component to consider in vaccination techniques is the effect of host factors on vaccine response. The duration and effectiveness of immunity produced by vaccines are significantly influenced by host factors, including genetics, age, gender, gut flora, nutritional status, obesity immunological history, and hormonal milieu (Bosco and Noti, 2021; Dhakal and Klein, 2019). For instance, the host's genetic polymorphisms can influence the vaccine's immunogenicity and efficacy (Dhakal and Klein, 2019; Ellwanger and Chies, 2019). Differences in vaccination response are also influenced by the composition and diversity of the host gut microbiota, with specific microbial profiles linked to either higher or weaker immunological responses. Furthermore, changing the microbiota by probiotic therapies or dietary modifications modulates immunological responses and enhances vaccinations' effectiveness (Falahi and Kenarkoohi, 2022). Optimizing vaccination results and improving protective effects require understanding how these host factors affect vaccine response.

Role of Genetics in Vaccine Efficacy

Numerous research studies have indicated that the immune response to hepatitis B, measles, or influenza vaccine is significantly influenced by the host's genetic background (Posteraro et al., 2014). Genetic information has recently been widely suggested as a potential biomarker of vaccine efficacy and a tool for developing more efficient and tailored

vaccination regimens (Linnik and Egli, 2016). The impact of an individual's genetic condition on the reaction that is either directly or indirectly brought about by an innate or adaptive immune response has been shown to work with various viral vaccinations such as those for influenza, smallpox, rubella, measles, and mumps (Castiblanco and Anaya, 2015). As genetics has grown over the past few decades, the focus on vaccination research has also been developed. Upon the completion and publication of the genome's first draft of a living microbe in the middle of the 1990s, genomic information updated the perspective of this field. Approximately 300 bacteria, including those that can infect people, have now had their whole genomes determined and retrieved (He et al., 2024; Malard et al., 2021).

To further expand genomic information and move toward a personalized and predictive era of vaccinology rather than a one-size-fits-all approach, high throughput sequencing technologies have thus made it possible to adopt new and more sophisticated approaches (Mina and Andersen, 2021; Pasik and Domańska-Blicharz, 2021). These approaches are essential for disentangling vaccine-induced immune responses (Tomazic et al., 2022). Personalized medicine aims to identify, track, and provide patients with an optimized course of care that considers their molecular phenotype and genetic profile. Thus, comparison, assessment, correlation, interaction, and cross-matching of the emerging omic information would help to improve understanding of the infection onset and progression and physiopathological mechanisms of disease while also assisting in the diagnosis, treatment, and prediction at the individual level (Castiblanco et al., 2013). Through experimental and computational methods, the study of host and pathogen genomes has explained the discipline to include functional and mechanistic insights to improve novel medicines, diagnosis, and vaccines (Lu et al., 2021). For interpretation and differentiation of organization and functionality, new and creative technology-driven approaches are beginning to apply a whole set of omics i.e. metabolomics, metagenomics, adversomics, trancriptomics etc. and genomics. These novel methods are going so far as to describe and correlate corresponding layers of genome-wide data in order to investigate and explain mechanisms that consider interactions ranging from genetics to the epigenomics and genomics (Ahmed, 2022; Shafaati et al., 2024; Tan et al., 2020).

Population genetic studies also give us the means to decipher the underlying genetic variables causing the diversity in susceptibility to pathogen infection and predict pathogen-host interaction to determine host response (Dix et al., 2016). The heterogeneity and diversity of the immune response to vaccinations continue to be a solid barrier to vaccine availability for the broader population. This variation stems from the individual's genetic background and is thought to be connected to immune response gene polymorphisms (Kennedy et al., 2020). There is growing curiosity about the genetic impact of polymorphisms linked with the effect of defining adaptive, innate, and humoral responses to vaccines from the perception of an individual and the population (Cadena et al., 2017). Therefore, precision vaccinology uses findings from molecular biology, genetics, system biology, and translational medicine to define patient subpopulations with comparable characteristics and optimize medical interventions based on the patient's response (Soni et al., 2020).

Age-Related Considerations in Precision Vaccinology

Age is a substantial element that may significantly impact the immunological response (Zimmermann and Curtis, 2019). Immunity rises from childhood until adulthood but then declines with aging, showing a saddle-shaped shift with low levels at both ends. It is frequently discovered that young children and older people's humoral and cellular immune responses differ from adults (Valiathan et al., 2016). Neonates often have unsatisfactory antibody responses due to their immune system's immaturity, which manifests as an overall delayed onset, lower peak levels, and shorter duration following immunization (Abdulla et al., 2023). Neonates also have reduced Th1 effector capacity, low amounts of specific complement system components, and restricted memory cell generation abilities. Infants and early children are typically more susceptible to illnesses (Tsafaras et al., 2020).

Increasing vaccination dosages or including adjuvants may be practical solutions to the issues of low antibody persistence and inadequate immune response (Pollard and Bijker, 2021). However, a new generation of vaccines might be required in some circumstances to address the problem. In a randomized study, newborns received a novel conjugate vaccination comprised of the capsular polysaccharide of Hib covalently attached to a protein vector. The vaccine proved safe and highly effective for infants and young children (Perera et al., 2021). It was believed that preterm or low birth weight babies' immune systems are weaker than those of full-term babies and that they are not functioning correctly. Typically, we administer the same vaccination schedule to both full-term and preterm babies. The hepatitis B vaccine is an exception, though, for premature newborns. As soon as feasible after delivery, ideally within 24 hours, the first dose of the hepatitis B vaccine should be given to preterm children weighing less than 2000 g who were born to HBsAg-positive mothers. The three doses of the standard primary series should be administered by the national immunization schedule at the appropriate times, such as when the newborn weighs 2000 g. The birth dosage, however, should not be included in the primary three-dose series (Gagneur et al., 2015).

For older ones, four types of vaccinations are advised named as pneumococcal vaccine, herpes zoster vaccine, trivalent inactivated influenza vaccine (TIV), and a combination vaccine that includes acellular pertussis, reduced diphtheria toxoid, and tetanus toxoid (Cunningham et al., 2021; Weinberger, 2017). The primary issue with vaccination in older people is that, compared to younger people, they are less able to produce a protective immunological response to the immunization and have a shorter length of antibody persistence (Wagner and Weinberger, 2020). The U.S. Food and Drug Administration has licensed high-dose TIV for patients 65 or older (Woo and Moro, 2022).

Furthermore, immunizing the immune response in older people can also be achieved by including adjuvants in vaccinations. It has been discovered that the adjuvanted TIVs MF59 and AS03 significantly boost TIV's immunological response and effectiveness in older people. Additionally, among people 50 to 70 years of age and older, the AS01B adjuvanted zoster vaccination dramatically decreased the chances of postherpetic neuralgia and herpes zoster (Lecrenier et al., 2018; Ruiz-Palacios et al., 2016). According to a meta-analysis by Tricco et al. (2018), the adjuvant recombinant subunit vaccination for herpes zoster was statistically better than the placebo (94%, 79%, to 98%) and live attenuated vaccine (vaccine efficacy 85%, 95%, credible interval 31% to 98%) (Tricco et al., 2018).

Sex Differences in Vaccine Responses

There have been observable gender inequalities in the realm of vaccine biology. Females often experience more adverse symptoms after immunization and produce higher antibody responses than males (Flanagan et al., 2017). The sex-based variations in vaccine response have been suggested to be caused by the interaction of multiple biological systems. Females express stronger and faster innate and adaptive immune responses than males, which may account for the increased incidence of vaccine-related adverse effects in females. (Fischinger et al., 2019). Depending on the sex, genetic and hormonal factors both can influence the immunological response. For example, increased estrogen levels may help females respond more strongly to vaccinations, but increased testosterone levels have been linked to a lessened reaction (Trigunaite et al., 2015). The X chromosome has almost ten times as many genes as the Y chromosome, with many genes coding for immune-related proteins. Genes and proteins responsible for immune response are expressed more often in females because they have two X chromosomes. These genes and proteins may interact with sex hormones to enhance the immunological response (Ciarambino et al., 2021; Fish, 2008).

The first step in designing precision vaccines is to consider the patient's sexual orientation. Males consistently showed better vaccine efficacy than females (Fig. 1) in the studies that reported sex-specific efficacy data for COVID-19 vaccines (Baden et al., 2021; Logunov et al., 2021; Polack et al., 2020). Precision vaccinology needs to be grounded in data from studies that examine how hormonal state and sex impact health throughout a lifetime. When making decisions in precision medicine, clinicians ought to consider the patient's sex to incorporate essential aspects of their unique characteristics (Miller et al., 2015).

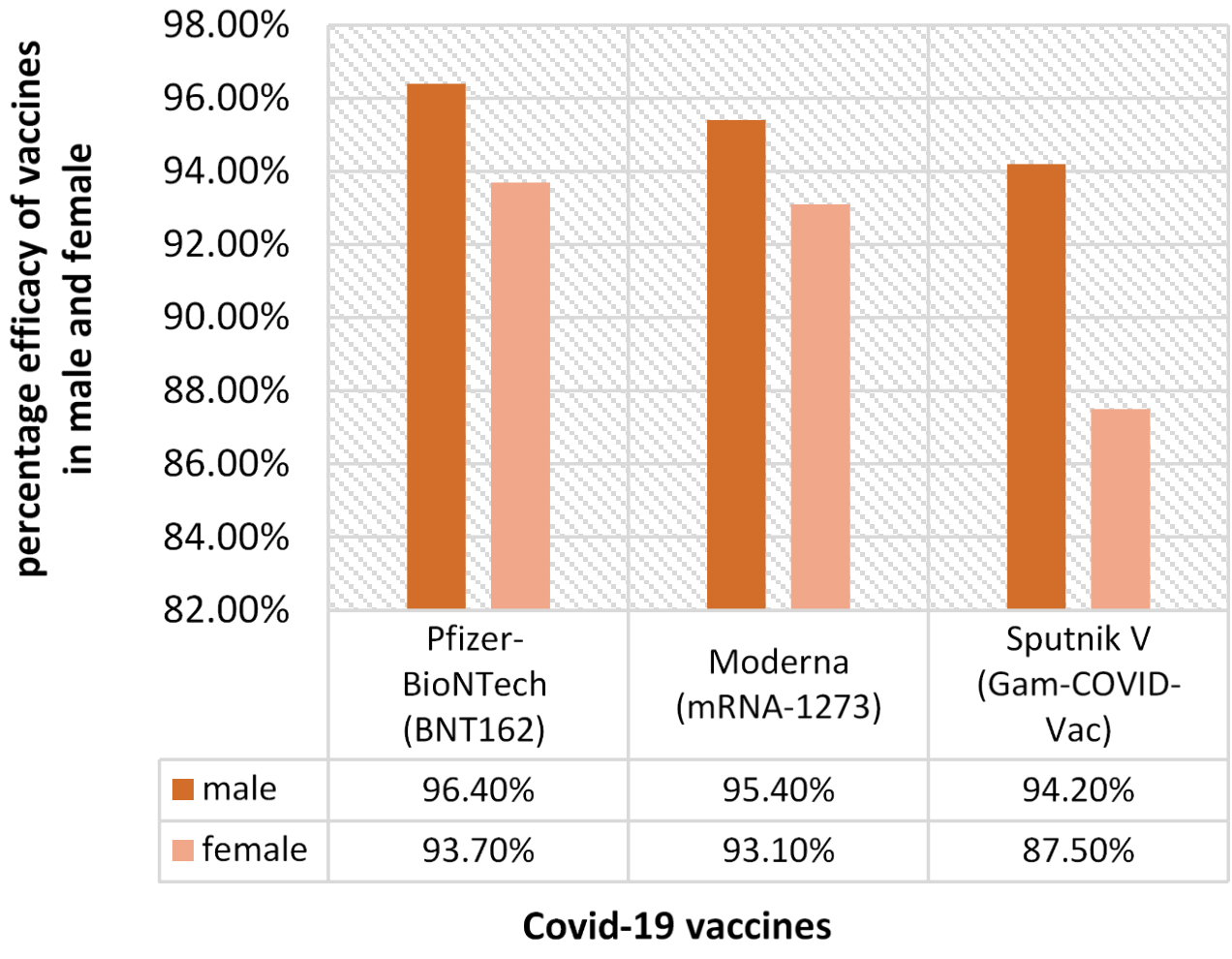

Fig. 1: Analyses of COVID-19 vaccine efficacy in Male and Female

Impact of Host Gut Flora on Vaccine Efficacy

Host gut flora composition varies significantly throughout their lives and in various socioeconomically diverse nations (Syromyatnikov et al., 2022). An infant's innate and adaptive immune systems take two to three years to develop fully. The gut microbiota are crucial to this process because they stimulate the emergence of the immune response, suppressing gut microbiota growth (Laforest-Lapointe and Arrieta, 2017). The Th0 (naïve T cells) differentiate into subsets Th1, Th2, and Th17, which are implicated in autoimmune disorders, humoral and allergic responses, and cell-mediated immune responses, respectively. Immunity generated by vaccinations may be positively or negatively impacted by intestinal microorganisms (Block, 2015). The characterization of gut flora as a vaccination response predictor has been made possible by new high-throughput DNA-based techniques (Nakaya and Pulendran, 2015). Numerous studies on mice have demonstrated gut flora's intricate and powerful involvement in regulating the immune response to mucosal or systemic diseases (Pickard et al., 2017).

The gut flora was crucial for the antibody response and developing virus-specific $CD4^+$ and $CD8^+$ T cells during respiratory influenza virus infection. In developed countries, oral rotavirus vaccination (RVV) prevents severe rotavirus gastroenteritis up to 85%-98%, whereas in developing countries, the rate is approximately 39%-61% (Muhsen and Omar, 2024; Parashar et al., 2013). Pre-vaccination fecal microbiome compositions of RVV responders and non-responders were compared, and the results showed a substantial difference in the total microbiome composition. RVV response was connected with a decrease in species of Bacteroidetes and an increase in *Streptococcus bovis* abundance. A study conducted in Pakistan also identified a correlation between the RVV response and a higher relative abundance of proteobacteria, which includes bacteria related to *Escherichia coli* and *Serratia* and the *Clostridium* cluster XI (Harris et al., 2018; Harris et al., 2017). Precision vaccination strategy becomes more effective if the microbiota associated with the efficacy of each vaccine type is identified (Borriello et al., 2018).

Development of Precision Vaccines

Many techniques and strategies are under consideration for developing precision vaccines with maximum efficacy and the most minor side effects. Here, a few methods are discussed (Fig. 2), which the vaccinologists advised for developing and implementing precision vaccinology. The first and most crucial step is to create a database with information on safety, immunogenicity, and efficacy from numerous vaccination clinical studies. Furthermore, extensive real-world data from vaccination trials must be incorporated since big data can be used to inform or suggest precision vaccination. When a person requires or wants to get vaccinated, relevant data about them may be collected. Later, valuable data from this database may be combined and extracted to create the best possible precision vaccine (Liu et al., 2021). While developing regimens for precision vaccination for healthy persons, i.e., people without a medical condition or other signs, their characteristics like sex, age, genetic polymorphisms, and preexisting antibodies should be considered to assure the safety and efficiency of vaccination (Jia et al., 2020). The ideal precision vaccination could be optimized by varying the timing, doses, and dosage of the various vaccines, the intervals between doses, or the composition of the vaccines with adjuvants (Dowling and Levy, 2022; Facciolà et al., 2022).

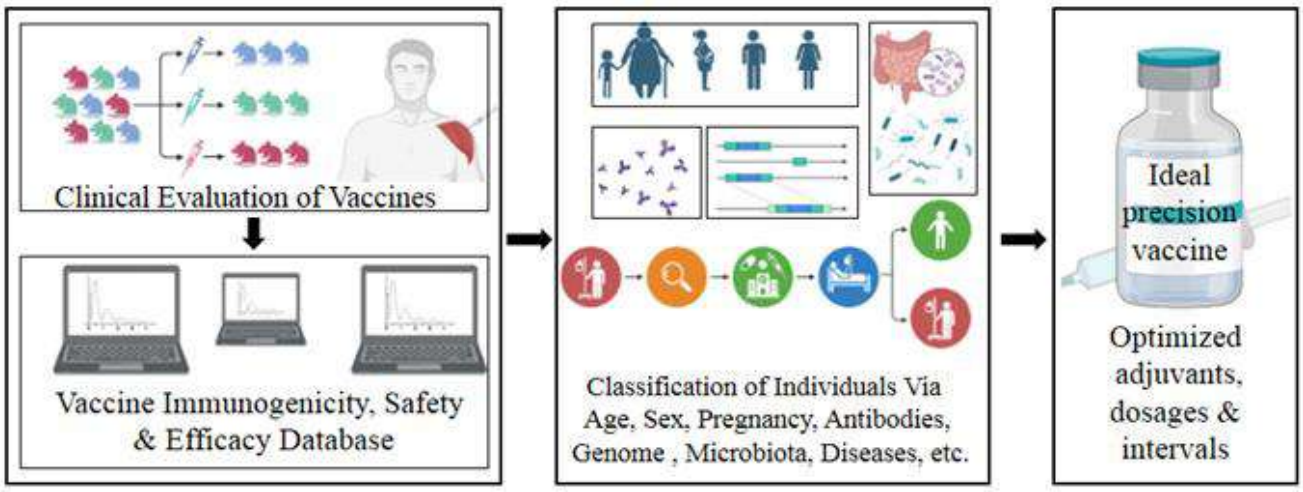

Fig. 2: Development of precision vaccines

Future Perspectives

Precision vaccinology is popular nowadays as a new method of treating and preventing disease that considers the unique genetic makeup, lifestyle, and environment of each (Rahman et al., 2024). It is critical to underline the need to implement precision vaccines for people and shift away from the existing "one size fits all approach" to vaccination. Assessing the vaccination's benefit/ risk profile and the individuals' specific health status will aid in determining if these patients require vaccination, which is a vital component of precision vaccinology. It can improve vaccination effectiveness

while reducing adverse effects in people by modifying dose, schedule, and co-administration (Jia et al., 2020). Traditional vaccination and immunization are relatively simple in healthy people. Still, precision vaccination requires considering the full impact of gender, age, preexisting antibodies, gut flora, and gene polymorphisms on vaccine efficacy (Duraisamy et al., 2020). Critical patients should combine the factors mentioned above with their medical issues. More efforts should be made by vaccine research and development companies to produce next-generation vaccines that can be utilized to treat people with underlying medical problems.

In the future, numerous issues will be addressed in promoting precision vaccinology. To overcome such problems, a database can be created by combining current data from vaccine clinical trials with enormous real-world evidence from vaccination practice (Li et al., 2021). Furthermore, precision vaccinology is a suitable strategy for achieving the desired effects by predicting immune response before vaccination in immunocompromised people, leading to maximal protection and minimal side effects. Though this strategy faces numerous obstacles, the future of precision vaccines looks promising and will eventually become the new trend in human vaccination and immunization strategies.

Conclusion

Precision vaccinology is aimed to apply the concept of precision medicine to vaccines. Precision vaccinology has become increasingly popular as it lowers the possibility of significant adverse reactions, improving safety and public trust in vaccines. Precision vaccinology is a novel method of preventing and treating infectious diseases and cancer, optimized based on an individual's unique lifestyle, environment, and genetic makeup. As the immune systems of early and later lives differ, precision vaccinology can aid in a thorough understanding of early-life immunity, which is essential for the creation of pediatric vaccines as well as for optimizing geriatric precision vaccines. More work should be put into developing precision vaccines for individuals with medical disorders by vaccine research and development organizations. Hence, it is imperative to highlight the necessity of tailoring precision vaccines and departing from the "one size fits all" vaccine approach. Vaccines must be tailored to be more efficacious with minimum side effects. Advancements in system biology determine whether an individual needs to be vaccinated by evaluating the benefits or risk profile of vaccination and their characteristic health status, a critical component of precision vaccinology. Furthermore, precision vaccines can maximize immune response in individuals by modifying vaccination techniques, i.e., schedule, dosage, and co-administration. Hence, precision vaccinology is the new era of vaccinology, and considering factors affecting immune response will fundamentally alter the practice of ongoing vaccination and immunization.

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Chapter 42

SWOT Analysis of Edible Vaccine for Public Health

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ABSTRACT

The development of vaccines in the nineteenth century marked a pivotal point in the development of science. Vaccines are biological preparations that improve immunity to specific diseases. Proteins found in this drug are similar to those found in disease-causing microbes; they are created by weakening or killing bacteria. Immune system stimulation is achieved through vaccines by stimulating the immune system's recognition of antigens. Vaccines of a new form are capable of masking the risks associated with conventional vaccines. This type of vaccine was made using genetically modified plants. Edible vaccines can be produced using plants containing genes encoding bacteria or viruses that cause disease. Plant-based vaccine technologies involve the integration of genes encoding specific disease antigen proteins into the genomes of plants. Despite the many benefits that plant-based vaccines provide to the vaccine industry, these third-generation vaccines still face many challenges. There continues to be a great deal of effort put into developing vaccines against a wide range of human and animal diseases despite all these limitations. This book chapter analyzes edible vaccines using the SWOT (Strengths, Weaknesses, Opportunities, and Threats) analysis framework. It discusses various factors including social, economic, evaluation, environmental aspects, implementation challenges, and potential opportunities for this technology.

KEYWORDS
Edible vaccine, Plant base vaccine, SOWT

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INTRODUCTION

Any biological preparation used to transfer immunity is called a vaccination. An altered version of the antigenic determinant or epitopes of the wild pathogen often makes up the vaccination. A vaccination introduces a minimal quantity of the pathogens so the body can recognize it as well as mount an immune defenses in contradiction of it, but it does not cause the disease in and of itself. It's a type of vaccination that's active (Razna, 2022). Novel formulations and production processes along with various vaccination administration strategies have recently been presented as a way to overcome this constraint. Many investigations on vaccines administered intradermal or at the mucosal interface have shown that the antigen may spread quickly and widely in the body and can trigger humoral and cellular responses in the systemic immune system and protective mucosal immunity, which is mostly mediated by secretory IgA (SIgA) (Brandtzaeg, 2010; Fu et al., 2010) . The edible vaccine concept has gained interest in recent years due to its versatility against various health hazards. Advancements in plant biotechnology allow for the introduction of pathogenic antigens in plant vectors, allowing for the manufacture of antigens in plant parts. This type of vaccine is particularly promising in areas with limited facilities for overall vaccination coverage, as it can be consumed as food. Typically, edible plants are used for vaccine preparation Figure 1 (Karakaş and Tonk, 2022; Parvathy, 2020).Plant-derived vaccines have the potential to strengthen the defenses against infectious illnesses, as evidenced by the first clinical experiment conducted in 1997 (Tacket et al., 1998).

Using current biotechnology techniques, edible vaccines are immunization molecules derived from edible plants that are introduced into the host genome. They constitute an essential pathway for immunity through the mucosal immune system (MIS), acting as the first line of defense against pathogens that target the mucosa. M-cells are specialized intestinal

cells which are able to identify antigens and transfer them to nearby antigen-presenting cells for further processing. These injectable vaccinations are safe to eat and are essential for maintaining immunity(Kang et al., 2018). The immunogen's physical appearance is essential to the kind of immune response that the edible vaccination will elicit (Razna, 2022).An immunological response unique to the vaccination would arise if the vaccine molecule was whole or mostly whole. Oral tolerance will develop if the body breaks down the vaccination molecule into soluble particles. In 2018, Tordesillas and Berin To ensure that the edibe vaccination has the desired effects, selecting the right palnt or palnt portion is imperative (Hopkins, 2023).

Transgenic crops can help lower the expense of producing edible vaccinations. Costs associated with storage and shipping would also go down. These vaccinations are developed using common biotechnology technologies. These vaccinations would be perfect for usage in developing nations. But there could be an issue with these vaccinations' limited shelf life (Athulya and Vethamoni, 2018; Razna, 2022). Edible vaccines are safer than conventional vaccines due to their small antigen portion, lack of contamination, and natural protein packing with plant tissues. They are cost-effective and do not require adjuvants for effective delivery. (Khadwal et al., 2020).They are stable at room temperatures and stimulate mucosal immunity and systemic immunity. They might be produced in large quantities for national immunisation drives. However, differences in the glycosylation sequences in plants and animals raise questions since they may impact the effectiveness of vaccines. The safety of edible vaccines, as they do not require adjuvants and can be used for nationwide immunization campaigns (Razna, 2022). In this book chapter we study the edible vaccine SWOT analysis frame work, social factors, social and economic, evaluation, environmental, implementation, challenges and opportunities of edible vaccine.

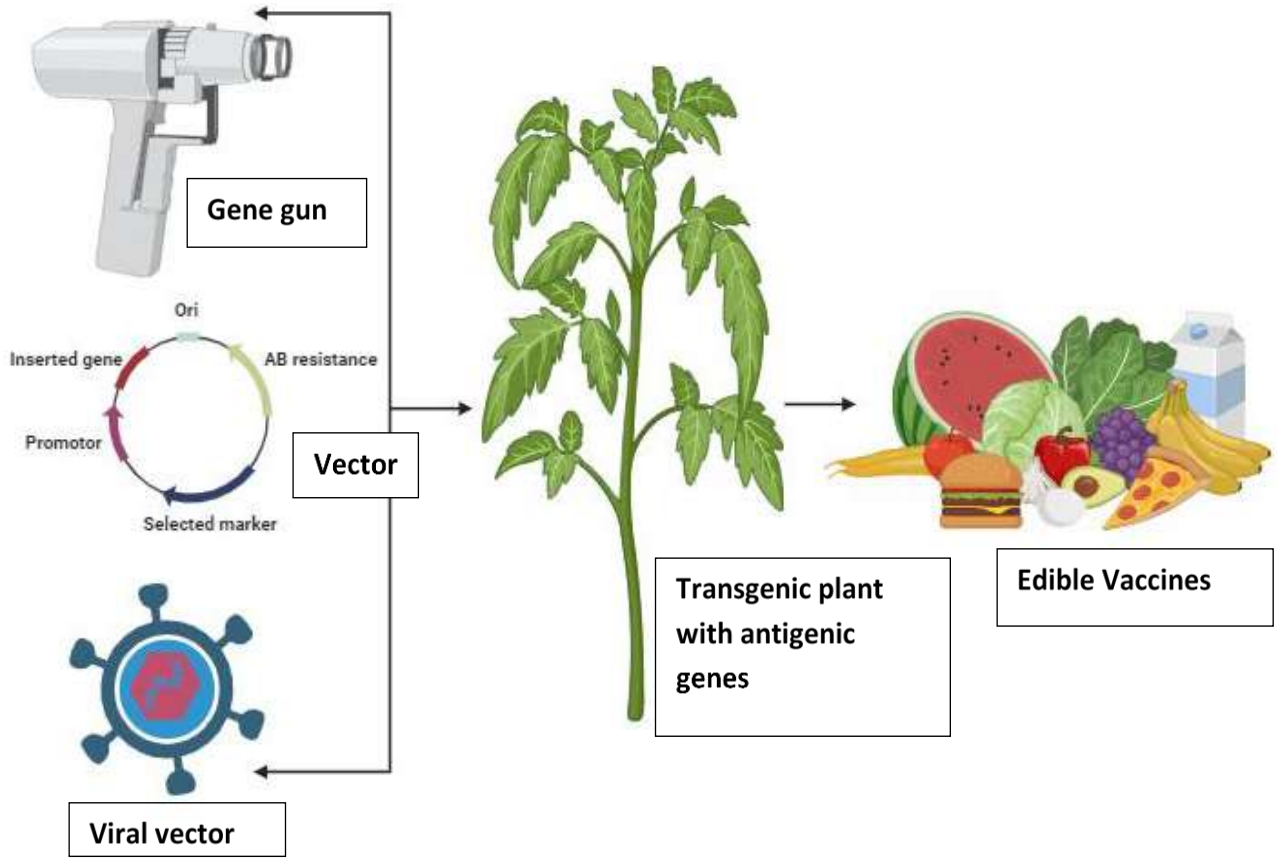

Fig. 1: Production of edible vaccines

Understanding SWOT Analysis Frame Work

An organization's benefits, drawbacks, opportunities, and threats are identified and assessed using the SWOT analysis framework. Researchers in academia and business have given edible vaccinations a lot of attention. Growing in greenhouses, the first plant-derived rabies vaccine provided the benefits of greater containment and high biomass yields. It was created in tomatoes. Bananas and lettuce have also been used to produce plant-based vaccinations.

Strategies

Mucosal vaccine formulation strategies include: Gene fusion technology, which produces non-toxic derivatives of mucosal adjuvants, (iv) genetic material itself, which allows DNA/RNA uptake and its endogenous expression in the host cell; (v) genetically inactivating antigens through deleting an essential gene. (i) Co-expression of an antigen along with a cytokine, which modulates as well as controls the mucosal immune response.

Mucosal delivery strategies include liposomes, biodegradable micro- and nanoparticles, live bacterial/viral vectors, and mucosal adjuvants (Po and Li Wan Po, 1998; Ramshaw and Ramsay, 2000). Prime-boost. A strategy combines many vaccination types and modes of delivery, particularly when more than one antigen or dosage is needed (Ramshaw and Ramsay, 2000). For instance, more MV-neutralizing antibodies may be produced by a single parenteral dose of MV-H DNA (measles virus haemagglutinin) with many oral MV-H boosters than by either vaccination alone (Webster et al., 2002). When compared to conventional vaccinations, the plant-based vaccine now offers a number of significant advantages, such as.

Strength/Other Potential Domains

Cancer Treatment

Sufficient amounts of monoclonal antibodies for use in cancer treatment can be produced by plants. To produce monoclonal antibody (BR-96), which targets doxorubicin for lung, ovarian, colon, and breast cancers, soybeans have undergone genetic engineering. Additionally, non-Hodgkin's lymphoma is being studied (Lal et al., 2007).

Birth Control

The ZB3 protein, which is present in mouse zona pellucida, is intended to be expressed by TMV. The resultant antibodies were able to stop mice's eggs from fertilizing when they were injected (Lal et al., 2007).

Transformation of Chloroplasts

Since the chloroplast genome is inherited from mothers, the protein may not be present in pollen after chloroplast transformation, lowering the possibility that the transgenic would be spread by cross-pollination to nearby crops or weed species. Additionally, it could cause significantly more transgenic protein to accumulate.

Role in Autoimmune Diseases

Oral auto-antigen administration can lead to immunological tolerance, caused by incorrect identification and administration of plants or fruits. To induce tolerance, antigens must be administered at higher doses. Potatoes can inhibit immune assaults and treat autoimmune disorders (Maassen, 1999).

Recombinant Drugs/Proteins

Enzymes and medications including albumin, serum protease, even interferon, which are costly or difficult to make, are being engineered into plants. Tobacco plants, for instance, are manufacturing glucocerebrosidase, which lowers the cost of treating Gaucher's illness. Large-scale recombinant therapeutic proteins within plants may now be produced on an industrial scale. Trichosanthin, angiotensin I, and antiviral proteins are examples of novel chemicals. Hirudin, a transgenic plant-derived biopharmaceutical, is now made for sale (Devi et al., 2019).

The Opportunities/future of Edible Vaccines

The US rejected Zambia's GM maize in food aid due to hunger fears, highlighting the potential impact of resistance against GM foods on edible vaccines. Transgenic contamination, causing over $12 billion in US losses, cannot be avoided (Lal et al., 2007).

The WHO must address concerns about quality control, effectiveness, and environmental impact to ensure the safety of genetically modified foods. Regulation and buffer crops are needed in greenhouse settings. GM crops have not achieved intended reductions in pesticide and herbicide use, and transgenic line instability can cause crop failures.

The inclusion of potent immune/allergens or harmful gene products in terminator crops can lead to side effects like CNS toxicity, sickness-causing cytokines, dementia-causing α-interferon, neurotoxicity, and mood/cognitive abnormalities. Highly toxic herbicides like atrazine, glufosinate ammonium, and glyphosate are needed to combat biotech-resistant pests and herbicide-tolerant super weeds (Lal et al., 2007).

There is limited reliable research on GM food safety, with the CaMV-35S promoter being a common unstable promoter in commercially cultivated crops. This promoter is vulnerable to recombination, horizontal gene transfer, and random insertion mutations, making it a potential cause of growth factor-like effects in young rats (Dresselhaus and Sprunck, 2003).

Geographic engineering may contribute to autoimmune disorders, cancer, drug-resistant illnesses, and viral reactivation. DNA-based vaccinations are dangerous as they can easily be absorbed by cells. Animals and people eating GM products, such as maize, are also at risk (Biselli et al., 2022).

List of major Weakness (Abeysundara et al., 2017)

1. Tolerance can be developed to vaccine molecules.
2. Vaccine dosage will change according on plant characteristics.
3. Dosage of vaccine need to be determined
4. Some plants cannot be eaten raw- cooking may degrade immunogen

5. Plant pathogens have the ability to contaminate vaccines.

Threats of Edible Vaccines

As mention above in chapter edible Vaccines have a lot of benefits on public health but still there are several threats linked with production of edible vaccines such as:

Although there are benefits to ingestible vaccines, there are obstacles to be addressed. Concerns about safety or incomplete information may make some individuals reluctant to eat plants that have undergone genetic modification(Martins et al., 2020). Technical challenges also include preserving vaccine efficacy throughout storage and plant production, as well as differences in the quantity of vaccine generated by plants (Fuenmayor et al., 2017). Additionally possible are allergic responses to the plant material or the manufacturing method. Environmental considerations include the likelihood of increasing pesticide usage and the unintentional transfer of vaccine genes to other plants, which might have unanticipated ecological repercussions. In conclusion, rigorous testing and regulatory approval are required prior to the widespread use of edible vaccinations (Snow et al., 2005).

Economic Evaluation of Edible Vaccine Implementation

The popularity of edible vaccinations and associated technologies has increased due to the world's population growth. As a vaccination, antigenic peptides integrated into a plant's edible portion can be given uncooked. Conventional vaccinations have significantly reduced the onset of illnesses, improving life expectancy; however, edible vaccines can achieve the same result at a lower cost and with better accessibility. A number of factors, including low production costs and the convenience of administration, storage, and transportation, have fueled the demand for the creation of edible vaccines (Aryamvally et al., 2017).

Molecular farming, often known as plant-made medicines, is a potential approach for large-scale synthesis of vaccines in plant cells. In contrast to mammalian cell lines such as Chinese Hamster Ovary (CHO) or bacterial cells, plants may be cultivated more cheaply and widely, which lowers the cost of vaccination antigens and treatments. A creative use of this substitute system is the creation of vaccinations in edible tissues that are meant to be eaten orally in order to transfer protein antigen without other processing.(Shi et al., 2023).

Over the past 30 years, infectious diseases like Zika, Ebola, monkeypox, SARS, measles, polio, cholera, and diphtheria have increased globally due to factors like urban congestion, pollution, and global warming. The need for vaccines has increased, but some countries lack equipment or purchase them at higher costs. Edible vaccinations may be a more cost-effective and stable option (Sahoo et al., 2020)

Operational Consideration (Production and Distribution)

Preparation of Edible Vaccines

Foreign DNA can be introduced into plant genomes through gene-gun bombardment or Agrobacterium tubefaciens, a naturally occurring soil bacterium. The plasmid infects plant cells, integrating into their genome and producing a hollow tumor. Antibiotic resistance genes are used to select cells and plants containing the foreign gene (Schumann, 2001L). The production of transgenic plants is species-dependent and takes 3-9 months. Reducing this time to 6-8 weeks is currently under investigation. Some antigens self-assemble into viral-like particles, which are not infectious (Lal et al, 2007). Genetic engineering involves inserting desired genes into plants to produce encoded proteins for edible vaccines, which are safe for individuals with compromised immune systems.

Stable Genomic Integration

The stable expression technique is the most commonly used approach in edible vaccination clinical trials, allowing for the incorporation of multiple genes for multicomponent vaccine manufacturing. This technique allows the expression of foreign antigens in specific organs and tissues. Other methods include agrobacterium-mediated gene transfer, direct DNA distribution into tissues, and chloroplast engineering, which has gained popularity in recent years due to its advantages over nuclear engineering.

Transient Expression using Viral Vectors

Viral vectors are employed in this technique to transfer genetic information into cells. The choice is made to use a recombinant plant virus, which carries the vaccine gene and causes the plant to manufacture the antigen following systemic infection. Since the virus may proliferate and produce more copies of the gene, transient expression results in higher levels of expression than steady expression. However, because the viral vectors must be injected into individual host plants, transient expression is more difficult to initiate.

Methods for Transformation of Gene into Plants

It has been claimed that the following techniques are applied in the creation of edible vaccines.

Plasmid/Vector Carrier System: Agrobacterium Tumifaciens Method

One method of producing extracellular vegetables (EVs) relies on the kind of microbe to transfer into cells of plants the genetic instructions for an infectious agent or microorganism "antigens," which are proteins that trigger a specific

immune response in the receiver. The soil contains A. tumifaciens, which is used in a process known as transformation to introduce a little amount of DNA into plants. One plant cell can regenerate into the entire plant. It has been documented that when genes are administered orally to animals and effectively expressed in experimental host plants, serum antibodies are produced. Agrobacterium rhizogenes and A. tumefaciens, two vegetable pathogens, have the ability to incorporate their DNA (T-DNA) into the nuclear genome of the infected cell (Yoshida et al, 2011). Following the infection of a vegetable tissue and the introduction of external genes into the suitably modified T-DNA of the Agrobacterium cells, research was conducted on the stable integration of genes into the plant's genome and the resulting creation of a transgenic protein that serves as an edible vaccine.

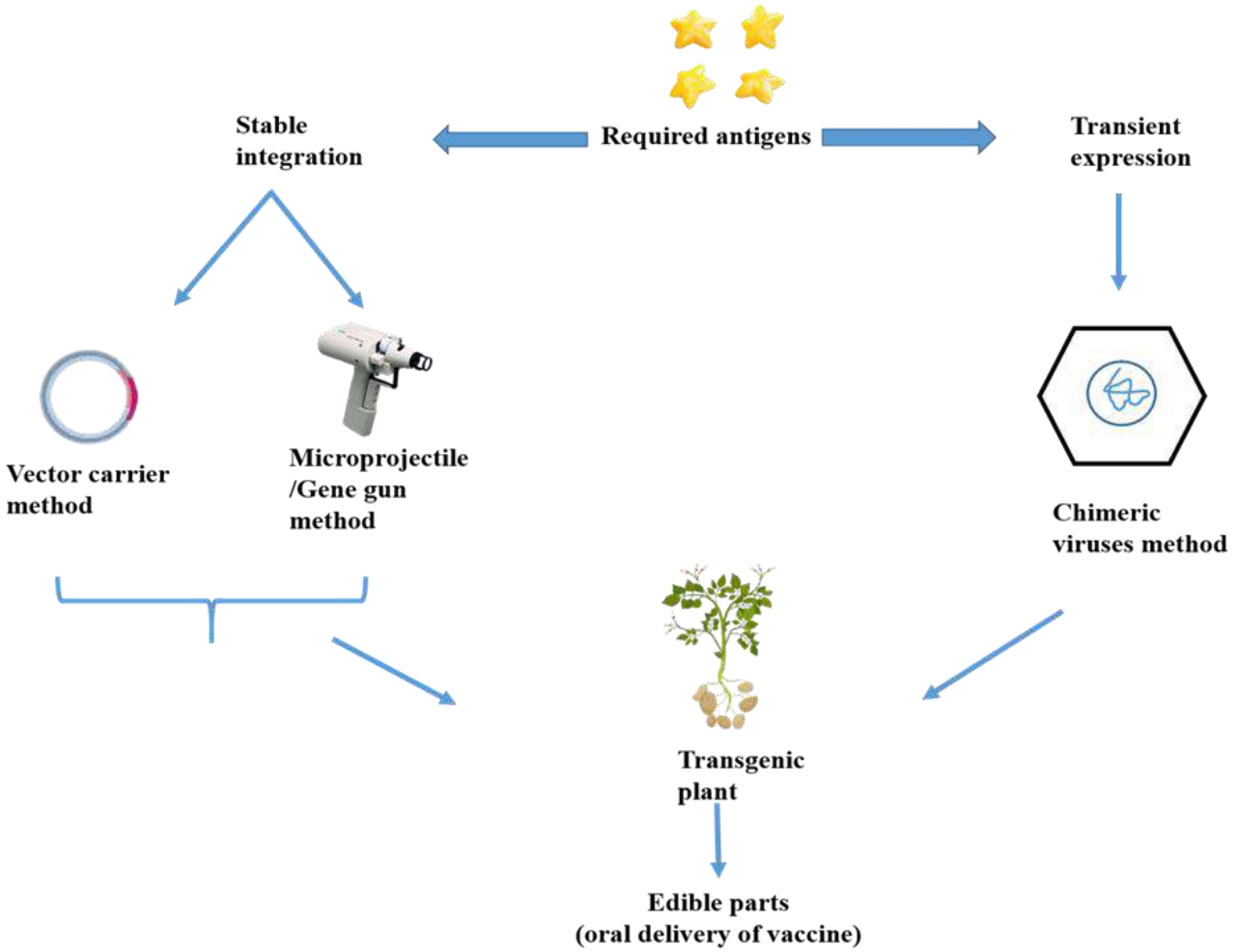

Fig. 2: Methods of production of edible vaccines

Micro Projectile Bombardment/Gene Gun Method

For plants that cannot be changed by A. tumefaciens mediated gene transfer, the micro projectile bombardment method—also referred to as the gene gun or biolistic method—is a means of nuclear transformation (Mubeen et al, 2016). A particle cannon is used to accelerate the bombardment of metal micro particles with specific DNA sequences against plant tissue. Once within the cell, the foreign DNA is released by the micro particles and incorporated into the nuclear genome. A marker gene is used to identify the cells that take up the desired DNA, after which the gene is replicated through culture. This method has advantages such as transferring genes into many cells simultaneously, circumventing difficulties with protoplast use, and being universal in its application, regardless of cell type, size, shape, or cell wall presence. It is also used for transforming organelles like chloroplasts and yeast mitochondria.

Genetically Engineered Plant Virus

Plant viruses are genetically modified to carry the desired genes in order to infect their natural hosts, such as edible plants, where the cloned genes are expressed to varying degrees in different edible sections of the plant. Certain viruses, such the tomato bushy stunt virus, potato virus, cowpea mosaic virus, tobacco mosaic virus, cauliflower mosaic virus (CaMV), and alfalfa mosaic virus, can be modified to create pieces of antigenic proteins on their surfaces. (Hafiz and Eyob,

2015).

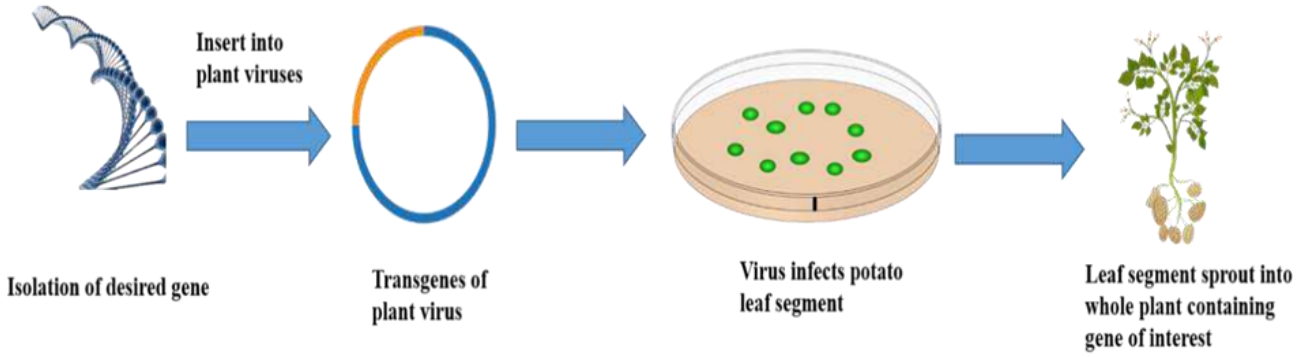

Fig. 3: Genetically engineered plant

Mechanism of Action

Transgenic plants transmit their antigens by a process known as bio-encapsulation, in which the plant's hard outer layer shields the cells from stomach fluids until they break down in the intestines. The M cells covering Peyer's patches or gut-associated lymphoid tissue (GALT) in the intestinal lining absorb the released antigens, which are then transmitted to macrophages and other antigen-presenting cells. Furthermore, local lymphocyte populations create memory cells that swiftly counteract the infectious agent's onslaught as well as serum IgG, IgE, and local IgA responses (Radhakrishnan,2023). There are following factors that affect the efficacy of edible vaccine

- Selection of antigens
- Model system effectiveness.
- The selection of plant species.
- Delivery and dosage frequency
- Controlled and sustained release pattern
- Attitudes and views of the general public about genetic modification

Selection of Antigens

Choosing the appropriate plant expression host and antigen is the first problem. Since not all antigens are compatible with the chosen host plants, this step is crucial in the development of a vaccine that can meet all the requirements. Careful and appropriate selection can be utilized to generate a vaccine that is heat-stable in addition to assisting in determining the vaccine's safety (Sharma and Sood, 2011). In the meanwhile, using proteomics or genomes techniques can identify an antigen candidate of a disease with intriguing traits but lacking sufficient characterization.

Delivery and Dosage Frequency

Choosing the appropriate plant expression host and antigen is the first problem. Since not all antigens are compatible with the chosen host plants, this step is crucial in the development of a vaccine that can meet all the requirements. Careful and appropriate selection can be utilized to generate a vaccine that is heat-stable in addition to assisting in determining the vaccine's safety. In the meanwhile, using proteomics or genomes techniques can identify an antigen candidate of a disease with intriguing traits but lacking sufficient characterization (Rigano and Walmsley, 2002).

Selection of Plant Species

Numerous plant species have been employed in the creation of vaccines to date. Selecting the right species of plant is crucial. In the event that the vaccine is intended for ingestion uncooked, an appetizing, edible plant is required. It is best to pick a plant that the animal already routinely consumes when selecting which one to use as a vaccine.

Challenges' and Opportunities' for Edible Vaccine

The scientific community has focused more emphasis on vaccine development techniques because to the ongoing pandemic, particularly in relation to manufacturing, downstream, and transportation issues. This has made it evident that new, less complicated production and transportation techniques are required, particularly for developing nations.(Garduño-González et al., 2022)

A significant obstacle still remains in achieving the initial goal of an edible vaccine, which is the direct ingestion of transgenic tissue without the need for vaccine protein purification. These include consistent dosing and quality control for a repeatable result. It is acknowledged that direct ingestion is not going to be practical. To address the problems of consistent dose and quality control for repeatable effect, vaccination protein can be quantified in freeze- or air-dried edible transgenic tissues and subjected to little processing. The food processing industries' accessible food processing technologies will help in this regard.(Chowdhury, 2011)

Some of the challenges are as follows

1. Low transgene expression levels
2. Plant-based oral vaccination proof of concept
3. Few PMVs progressed to the Phase I Human Clinical Trial stage.
4. Adapt quickly to address unforeseen pandemic problems
5. Edible portion shelf life and dosage concerns
6. Regulatory approval and public acceptance
7. Enticing significant pharmaceutical firms to PMV for investment

Stabilized nuclear transgenes often express at low levels, but large-scale manufacturing requires >50 mg⁄ kg of antibodies. Chloroplast transformation can provide high expression levels, but most stably implanted nuclear transgenes have insufficient expression levels, hindering efficiency and proof of concept testing. Transplastomic and Magnification transient expression technologies can be used for proof-of-concept and efficacy tests. Plant-derived antigens have been elicited through oral administration to mice and humans. Despite twenty years of development, no plant-based vaccine has progressed past Phase I human clinical trials. Seed tissues have a longer shelf life and are more stable for both humans and animals, making them more promising for vaccine candidates. However, human infections and genetically modified plants will face challenges in regulatory clearance and early public acceptability. Animal illnesses may be the first focus of large-scale manufacturing of plant-derived vaccines.

Environmental Implication and Sustainability of Edible Vaccine

The triple bottom line, which combines environmental quality, social fairness, and economic success, is crucial for a sustainable society. The 17 Sustainable Development Goals aim to end poverty, save the environment, and promote prosperity worldwide. Orally administered edible vaccines show promise in reducing illnesses like diarrhea and hepatitis, especially in underdeveloped nations. The 2030 Agenda for Sustainable Development focuses on the application of edible vaccines as biopharmaceuticals, enabling comprehensive treatment of vaccination status and malnourishment, particularly among children in underdeveloped nations. (Miranda et al., 2020).

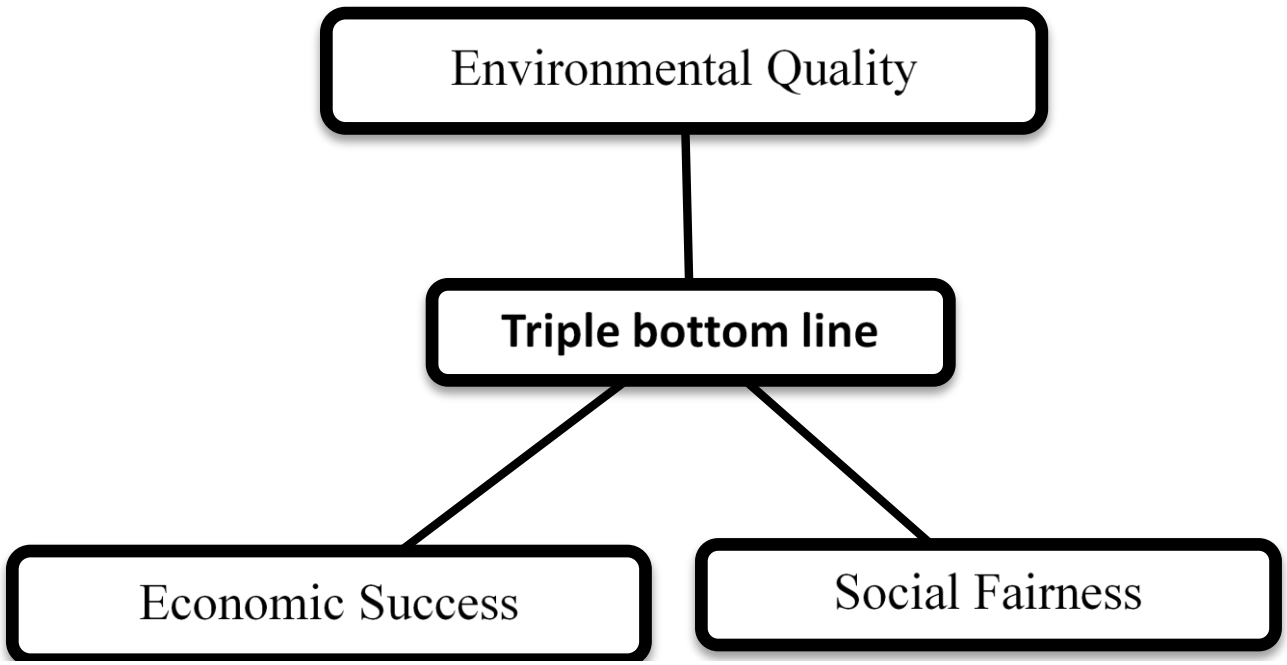

Fig. 4: Triple bottom line: Foundation of sustainable society

Advantages and Disadvantages of Edible Vaccine

Biopharmaceuticals, including edible processed vaccines, can help balance economic growth with environmental and social responsibility. These biotechnology and life sciences can be applied to new technologies, addressing issues like poverty, hunger, and malnutrition in developing nations. The Millennium Development Goals aim to reduce poverty, improve disease management, and improve access to water and sanitation by 2015 (Barzegari et al., 2014). Edible vaccines can also develop tolerance to allergies by inserting a gene or marker sequence into edible plants (Razna, 2022). These edible vaccines are less costly, require no needles, and offer both mucosal and total protection. They are being developed to strengthen defenses against infectious diseases like FMD, cholera, measles, and hepatitis B, as well as treat autoimmune diseases like type I diabetes. The edible vaccine has the potential to immunize generations and prevent starvation (Khan et

al., 2019).

The argument around the relative hazards to human health and the environment is the main focus of public discourse on the acceptability of genetically modified organisms (GMOs) in food production. In order for a vaccine to be helpful in preventing disease, it must be given to those who can respond to it correctly in addition to being effective itself. With that in mind, and based on the public's typically split views on the subject, we can all agree that vaccinations have benefits and drawbacks (Martins et al., 2020).

Table 1: Advantages and disadvantages of edible vaccines (Malabadi, 2008)

Advantages	Disadvantages
Producing vaccinations is far more expensive than growing plants.	The vaccination schedule may change. Different sized bananas has differ amount of vaccine.
Mostly human viruses cannot survive in plants, humans won't be at risk by using it.	Security would become a major concern if the vaccinations were produced in fields or on trees.
There are known purifying processes for plants, commonly utilized in medicines.	People will mistake the edible vaccination for ordinary fruit and eat more of it than is healthy.
Vaccine transportation and storage is both convenient and safe	Environmental factors may affect the transportation in rare cases.
Compared to an animal system, a plant mass production system is simpler.	Plant growth may vary.
Bio encapsulation provides antigen protection.	Harsh digestive environment of the gastrointestinal tract, limited absorption and bioavailability.
Increased shelf life.	Commercial vaccinations have strict expiration dates and refrigeration restrictions, increasing costs of distribution, storage, and management due to ongoing monitoring of pathogens within certain plant parts.
Inducing humoral immunity	May causes side effects

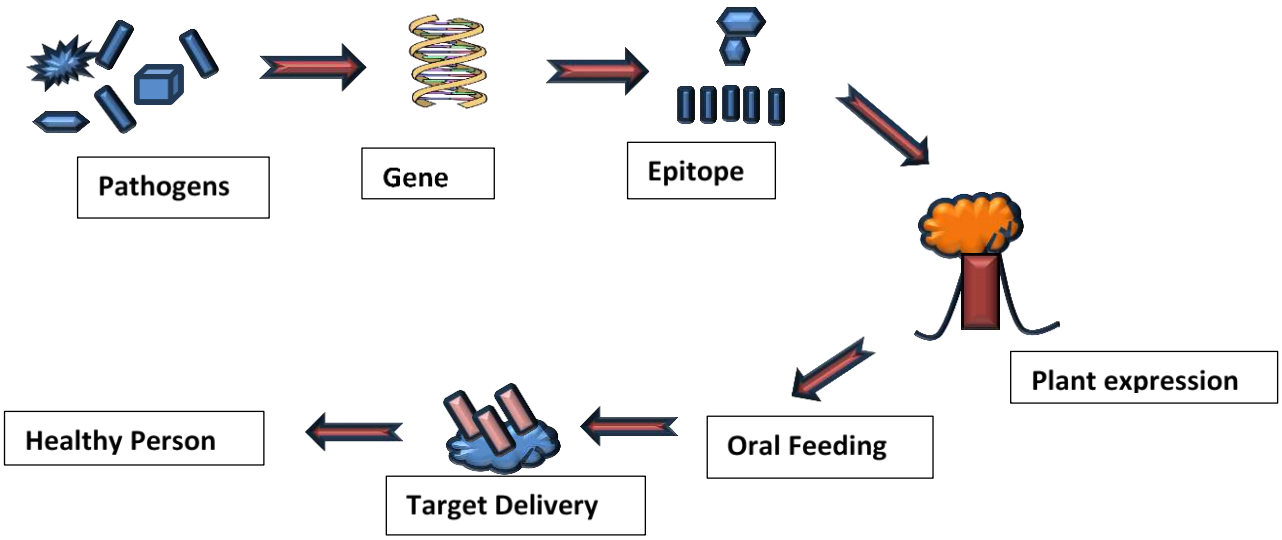

Fig. 2: Plant-based edible Vaccine principle

Examples of Plant-based Edible Vaccine (Mishra et al., 2008)

Sr#	Vaccines	Vector used	Diseases
1	Rabbit haemorrhagic disease virus	Potato	Haemorrhage
2	Hepatitis B virus	Tobacco Potato Lettuce	Hepatitis B
3	Norwalk Virus	Tobacco Potato	Diarrhea Nausea Stomach Cramps
4	Rabies Virus	Tobacco	Rabies
5	Transmissible gastroenteritis	Maize Tobacco	Gastroenteritis
6	HIV virus	Tomato	AIDS

7	Vibro Cholerae	Potato	Cholera

Technological Advancement in Edible Vaccine

The popularity of edible vaccinations and associated technologies has increased due to the world's population growth. As a vaccination, antigenic peptides integrated into a plant's edible portion can be given uncooked (Aryamvally et al., 2017). A brand-new vaccine was unveiled with the ability to conceal the danger associated with traditional vaccinations. Genetically engineered plants were used to create this particular vaccination. The gene-encoding bacterial or viral pathogen can be integrated into plants to produce edible vaccines without compromising their immunogenic qualities. Activating the mucosal and systemic immune responses against an alien pathogen is the primary mode of action of edible vaccines (Kurup and Thomas, 2020).

These edible vaccines are made from plant materials that have been genetically engineered to produce antigen proteins that can stimulate particular immune responses and provide protection against a range of illnesses in humans and animals.

Two different approaches as

- **Nuclear Transformation**
- **Transient Expression**

Gram-negative soil bacteria called A. tumefaciens has the ability to change plant cells by injecting its own T-DNA. The newly introduced bacterial genes cause plant tumors like crown gall and encode for hormones found in plants. The Ti (tumor-inducing) plasmid is used by bacteria to transmit genes. The plasmid was able to be used as a vehicle for heterologous gene transfer into plants tanks to the theory of T-DNA gene transfer.

- In specifics, the recombinant bacterium transfers the introduced heterologous gene into the host plant's nuclear genomic DNA with the aid of the viral gene product in Agrobacterium.
- A. tumefaciens is initially transformed using the recombinant plasmid vector, in which the heterologous gene was added and the hormone gene was deleted.
- Dicotyledons are the primary plant organ used in agrobacterium-mediated transformation. When agrobacterium-mediated transformation is not possible, as with monocotyledons, a transformation process utilizing gene gun technology is used.
- Using a gene cannon, high pressure is applied to plant cells to introduce the heterologous gene. Metal particles, approximately 1 to 3 μm in diameter, are coated with plasmid vectors containing the desired genes.
- This approach has the benefit of enabling the introduction of more than two heterologous genes. (Kim and Yang, 2010)

Conclusion

There are many advantages of edible vaccines over traditional vaccines. They are safe, effective, and cheaper. Plants and algae can be scaled up so easily, just like other edible plants and algae. Edible vaccines are problematic due to the notion that genetically modified crops are harmful, which is prevalent in many developing countries. The technology that makes genetically modified crops safer than ever is constantly evolving and growing. The production of plant-based vaccines also raises some bioethical issues, such as the danger of transgenic plants transmitting allergens to humans and animals. The pathogens involved in some of the plant-based vaccines could reactivate and infect other organisms that consume them because bacteria and viruses are used as the vectors. This interesting biological product shall overcome the challenges it faces due to its advantages and benefits. Accordingly, it is anticipated regulatory approval will be granted eventually to assist in the global fight against disease.

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Chapter 43

Immunization: Role of Vaccines in Preventing Disease Challenges at Dairy Farms

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ABSTRACT

Successful implementation of the vaccination program at dairy farms plays an important role in the prevention of disease outbreaks and health related challenges by making the dairy cattle immune against production and reproduction limiting infectious diseases. However, it confronts many challenges such as selecting and administering vaccine, product cost, drug resistance, hesitation to immunize the herd. Rectification of these problems is only possible through inter-coordinated efforts of government agencies, policy makers, and stakeholders which results into encouraging the vaccine usage, protecting dairy herd health, and ensuring the smooth running of dairy farm operations. Specific strategies should be adopted such as following the Tailored Vaccination Programs (TVP), and Integrated Disease Management (IDM) approach, conduction of awareness seminars and training sessions, provision of the monetary incentives, and promotion of the widespread communication and outreach efforts. Timely vaccination of the dairy herd not only saves the health and productivity of the animals, but also financially supports the farm economics by saving it from the extra burden of expenses in terms of medicine and veterinary bills, and losses such as milk disposal due to antibiotic residues, compromised profit value and time.

KEYWORDS
Immunology, Vaccination, Protection, Dairy herd, Antibodies.

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INTRODUCTION

Understanding the Immunization and Vaccines

Immunization plays an important role in ensuring public health by the prevention of infectious diseases (Morgenstern and Klement, 2020). Vaccines are considered among one of the most effective immunization tools and have been proved to reduce the rate of disease occurrence and death globally (Yupiana et al., 2021). As the health of dairy herd directly affects the production (Gull, 2022) and farm economics (Robi, 2024), the use of vaccines becomes significantly essential in running a successful profitable dairy business (Rainard et al., 2021).

Immunization is the process through which resistance or immunity are produced against a specific disease, either by exposing naturally, or through vaccination (Lianou et al., 2022). Vaccines are biological products which trigger the immune system for pathogen recognition (bacteria, virus, fungi, etc.) (Akram et al., 2023; Tizard, 2021), production of specific immune response against them, without causing the disease (Chen et al., 2021). They consist on specific pathogen-derived antigens stimulating the immune system to produce antibodies and memory cells for future protection (Zhylkaidar et al., 2021).

The Role of Vaccines in Dairy Farming

Dairy farms play a crucial role in the fulfilling the increasing-demand of milk and milk products (Seifu et al., 2023). The incidence of infectious diseases is more in this sector due to many reasons which include close animal proximity, intensive production system, and frequent interactions with humans and the environment (Aslam et al., 2023; Rainard et al., 2022). Several infectious diseases which poses significant health and production and reproduction challenges to dairy animals include mastitis, foot and mouth disease (FMD), bovine respiratory disease (BRD), Pasteurellosis (Mehnaz et al., 2023; Lavon et al., 2023), infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), clostridium species infections, bovine ephemeral fever, leptospirosis, and brucellosis (Eichberger, 2023). By following the proper vaccination of the dairy herd these infectious diseases can be controlled and prevented (Shi et al., 2024). Dairy farms commonly vaccinate their herds against following diseases (Gleser et al., 2023).

Foot and Mouth Disease (FMD)

It the major production-limiting disease of the dairy animals causing severe viremia, oral and foot vesicular lesions, fever, anorexia, and high mortality in calves (Tomazi et al., 2021). Pregnant animals may abort (Ayvazoğlu and Demir, 2022). Some virus strains affect the udder, causing permanent damage to the milk alveoli (Jibo et al., 2023). Recovered cattle fails to regain the lost milk production efficiency and may suffers from hyper panting syndrome due to affected brain area (Tomazi et al., 2021). Vaccines are used target the serotype of FMD virus dominant in Pakistan such as O, A, and Asia-2 (Roshdy et al., 2020). Timely immunizing the herd against FMD not just save the treatment cost, but protects the herd from irreversible losses of production and reproduction (Chen et al., 2022).

Mastitis

It is most common pathological condition affecting the dairy herd globally and causing huge economic losses (Li et al., 2020). It includes inflammation of the mammary glands, reduced milk production, and death in acute and severe cases (Rafique et al., 2024; Zhao et al., 2023). Main causative agents are bacteria such as *Staphylococcus aureus, Streptococcus agalactiae, and Escherichia coli* (Astuti et al., 2024). Vaccinating the herd against these pathogens reduces the rate of morbidity (Nadeem et al., 2024), and increases the production and quality of milk (Raheem et al., 2023; Fernández et al., 2022).

Respiratory Diseases

Diseases of respiratory tract such as Parainfluenza, Pasteurellosis, and Infectious Bovine Rhinotracheitis (IBR) lead to high rates of infection and death in adult cattle and calves (Badr et al., 2023). Infected animals show variable clinical signs such as in mild form coughing, presenting nasal and ocular discharge, and anorexia. Severe form includes high grade fever, pneumonia and death (Baraitareanu and Vidu, 2020). This respiratory distress exerts a significantly negative effect on the herd performance efficiency such as stunted growth decreased production (milk, meat, hair, wool etc.), raised veterinary and medicine costs (Ashraf et al., 2023; Li et al., 2023; Li et al., 2020). Vaccinating the dairy herd against these diseases results into reduction in the severity of illness, chances of outbreaks, and an improved farm production efficiency (Brock et al., 2021).

Reproductive Diseases

Reproductive tracts infections such as Bovine viral diarrhea (BVD), leptospirosis, and brucellosis exert a detrimentally affect the herd fertility (Athambawa et al., 2021), reproduction, and production (Qudratullah et al., 2022). Disease consequences in female animals may include early or late embryonic mortality (Athambawa et al., 2021), abortion, still birth, and congenital disorders or anomalies (Yasin et al., 2023; McCarthy et al., 2021). Whereas, in males it may leads to infertility (Benschop et al., 2021). Losses such as disturbance in the lactation cycle, repeat breeding, reduced production and reproduction capacity (Ábalos et al., 2022), and an increased treatment cost badly impact the farm economics (Refaei et al., 2020). Diseases such as brucellosis and leptospirosis are zoonotic and are threat to the public health (Lowie et al., 2022). Commercially available vaccines target Brucella abortus strain 19 (S19) and RB51, BVD type-1 and Type-2, and 5 strain of Leptospira (Islam, 2020). Vaccinating the dairy herd against reproductive infections significantly helps in protecting it from such loses (Bauer et al., 2022).

Clostridia Species Infection

There are many species of clostridia that infect and cause diseases in dairy cattle, some of which are blackleg, black disease, malignant oedema, tetanus, botulism (Wataradee et al., 2021). The organism produces spores which remain in the soil long lastingly (Mohan and Rajkamal, 2022) and when entering into the host's body leads to toxins production and then death (Gadhavi et al., 2020). Immunizing the herd helps in preventing morbidities, mortalities, and other economic losses caused by clostridial organisms (Schmitt–van de Leemput et al., 2020). The main Closterium (Cl.) species covered by vaccines include *Cl. chauvoei, Cl. septicum, Cl. novyi, Cl. sordellii and Cl. perfringens* Type B, C and D (Dad et al., 2022).

Challenges in Implementing Vaccination Programs at Dairy Farms

While the use of vaccines helps to protect the dairy farm from several infectious diseases, reduced milk production,

reproductive loses, and to raise overall farm profitability (Armson et al., 2020). There are several challenges in implementing the vaccines protocols (Suárez Archilla et al., 2022).

Vaccine Selection and Efficacy

Factors that must be carefully taken into account while selecting the appropriate vaccine for a specific dairy farm include prevalent pathogen strains, biosecurity measures adopted, disease risk, disease epidemiology, and protection spectrum of available vaccines (Baxter-Smith and Simpson, 2020; Brito and Hick, 2024). All available vaccines may not provide optimum protection as several factors such as the vaccines-manufacturing-technique, strains and serotypes difference, cross protection offered, and vaccine administration protocols determine their efficacy level (Khurana et al., 2021).

Storage, Handling and Administration of Vaccine

Vaccine efficacy also depends on factor such as their cold chain aspects of the storage facility, handling of vaccine before and during administration, preparation and administration of the vaccine as directed by the vaccine manufacturer, and maintenance of the vaccine titer by strict following of vaccination schedule (Holt et al., 2021). However, vaccine efficacy and dairy farm immunization programs become compromised due to challenges such as inappropriate vaccines storage (not maintaining 2ºC to 8ºC) (Rainard et al., 2022), failing to prepare vaccine as directed, careless vaccine-handling, failing to administer complete dose, and not following the prescribed schedule (Alamian et al., 2021).

Economic Factor

Affordability of vaccine cost acts as a barrier in the implementation of vaccination program at the dairy farm, particularly when considering small livestock-holders with limited financial resources (Bardenstein et al., 2023). Expenditures related to vaccines purchase, storage, administration, titer testing and maintenance may outweigh the benefits, resulting to incomplete immunization, poor antibody production, and disease outbreaks (Dadar et al., 2021).

Pathogens Mutation

Several pathogens, particularly the viruses, mutate and bypass the protective barriers of antibodies leading to disease outbreaks (Rypuła et al., 2020). Regular field sample collection, testing them for vaccines efficacy, and (Brito and Hick, 2024) strong epidemiological monitoring is required (Arnoux et al., 2021).

Vaccines and Vaccination-failure

There are several factors which lead to no or poor immunity development, even after administration of a carefully stored efficacious vaccines (Moennig and Yarnall, 2021). These include the use of contaminated needles and syringes, vaccination of animals with subclinical diseases, heavy parasitic infestation, compromised immune system and malnutrition (Tuppurainen et al., 2021). For obtaining a better immune response and production of optimum antibodies titers, it is recommended to use the vaccines in completely healthy, dewormed, and well-nourished animals (Milovanović et al., 2020).

Farmer Hesitation

Farmer's hesitation towards immunizing their dairy herd is a result of several reasons such as concerns regarding vaccine safety and side effects, misinformation, cultural believes, lack of awareness, and poor education (Kiplagat et al., 2020). These factors impact the farmers' decision-making process regarding implementation of the vaccination program to their farms (Punyapornwithaya et al., 2022).

Strategies to Overcome Challenges and Improving Immunization

To effectively implement the immunization program at the dairy farm, a multidimensional approach is required (Yupiana et al., 2020).

Farmer Education and Training

It is essential to educate and train dairy farmers, field veterinarians, and farm labor regarding the herd immunization benefits, vaccines administration methods, vaccination schedule, disease prevention measures, biosecurity protocols, and economics benefits of vaccinating the herd (van den Brink et al., 2023). All these topics should be covered through conduction of farmer-meeting, trainings, and seminars (Taddei et al., 2021).

Tailored Vaccination Programs (TVP)

Each dairy farm requires a different Disease-prevention and vaccination program. While designing a TVP, firstly, the specific dairy farm's preventive or immunization requirements are analyzed based on factors such as outbreak risk of a particular disease, area epidemiology, farm's biosecurity management, and financial limitations. Following the analysis, the immunization programs are then modified or customized accordingly, which help to assure the provision of maximum protection against the infections (Sanhueza et al., 2020). TVP helps in providing a guidance for dairy manager about selecting a suitable vaccine brand, designing the herd-immunization-schedule (Madkar et al., 2020). For an effective and

successful TVP development, strong collaboration and coordination are required between the livestock farmers, veterinarian, university researchers, and government institutes that deal with livestock extension, agriculture development, and epidemiology (Patel et al., 2020).

Economic Incentives and Support

Financial circumstances of a farm carry a significantly importance role in the decision-making process regarding the herd immunization (Prasanthi et al., 2022). Lacking financial resources of the farmer often leads to compromising the herd protection, skipping the booster doses, violating the vaccination guidelines, or choosing the inefficacious, more-economical vaccines brands. Dairy farmers can be encouraged to timely vaccinate their herd if institutes such as agriculture extension, livestock extension, policy makers, Non-profit organizations, and pharmaceutical companies provide support such as financial incentives, price discounts, cost-sharing schemes, and subsidies to the dairy farmers. This would reduce the vaccination cost related burden of the farmer (Mohamed et al., 2024; Iscaro et al., 2021).

Integrated Disease Management (IDM)

This includes the use of different techniques, methods, measures, and approaches in coordination to get their synergistic result in minimizing the risk of disease outbreak or disease-spread (Baraitareanu and Vidu, 2020). Moreover, if focuses on implementing the vaccination program, adopting the standard biosecurity measures, analyzing and monitoring the dairy health profile, and using the antibiotics judiciously (Compiani, 2021; Woolums, 2021).

Communication and Outreach

This approach aims to increase the transparency in the communication, and clarity in the processes that take place between the livestock farmers, researchers, policymakers institutes, and the final consumers (Baraitareanu and Vidu, 2020). This will help in the development of farmers trust and make him less hesitant in implementing the immunization program on his dairy herd (Lu et al., 2022). The outreach program mainly aims to transfer the acculturate information to the dairy farmer, and make him well aware regarding the disease protection, immunization schedule, disease outbreak prevention and infection-spread control (Dos Santos et al., 2022). For program execution, several channels can be used including workshops, seminars, educational materials, social media digital campaigns, and in-personal farm visits (Otter and Uzal, 2020).

Conclusion

Vaccination of the dairy herd has become an essential requirement for the success of a dairy farm. Successfully implementing an effective immunization program at the dairy farms does not only help in assuring the disease-outbreaks-prevention, and control of infection-spread, but also supports in achieving the production and reproduction targets by maintaining the lactation length, milk yield, weight gain, breeding, pregnancy, and other production growth parameters.

However, it faces a number of challenges such as vaccines selection and administration, immunization cost, farmers' trust issues, and reluctancy towards vaccination. These issues can only be addressed if the government agencies, policy makers, and stakeholders work in coordination, and make their efforts to encourage the dairy farmers to strictly follow the vaccination program and immunize their herd to ensure the optimum herd-protection. Furthermore, adopting specific strategies such as TVP, IDM approach, farmer meeting and training-sessions conduction, financial incentive provision, and effective communication and widespread outreach-efforts can support in combating the hurdles in successful implementation of the vaccination program. Thus, timely vaccinating the dairy herd not just maintains the health, production, and reproduction of the animals, but also prevents the financial losses.

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Chapter 44

Vaccination and Alternate Control Strategies to Control *Mycobacterium* Species

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ABSTRACT

Tuberculosis is a disease caused by *Mycobacterium species* that results in severe illnesses and poses a danger to worldwide health because of their complicated pathogenicity as well as widespread epidemiology and ability to damage the immune system. Managing numerous bacterial infections which mainly concentrate on immunization as well as nontraditional measures, were discovered in this chapter. For the prevention of infections, different inoculation options are studied along with the detailed cycle of disease as well as epidemiological trends of diverse mycobacterial bacteria. A rare of these procedures consist of the usage of both live as well as dead mycobacteria along with protein-based as well as DNA vaccines that can help to deteriorate damaging strains such as *Mycobacterium bovis*. The occurrence of mycobacterial diseases requires more necessary action, despite the progress in vaccine advancement. A hopeful alternative is phage therapy, which attacks and destroys *Mycobacteria* using bacteriophages. Procedures of immunotherapy are being examined for their capability to improve the host's protected response mostly includes the use of cytokines such as interleukin-2 also Granulocyte-Macrophage as well Colony-Stimulating Factor along with interleukin-24 and interleukin-32. Antibody to control tuberculosis is occasionally observed as an important component of a comprehensive therapeutic method. To control the spread of disease-causing mycobacterial infections, genetic resistance methods in hosts along with environmental and management plans designed to lower the contact and spread. This chapter aims to add valuable contribution to worldwide efforts to reduce the load of these pathogens by integrating these numerous plans in a direction to advance the knowledge of present involvements as well as excite the formation of innovative approaches to fight *Mycobacterium* species in both humans as well as veterinary medicine.

KEYWORDS
Bacteria, Tuberculosis, *Mycobacterium*, Alternatives, Vaccination

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INTRODUCTION

Various collections of bacteria involved in *Mycobacterium* species are liable for important diseases in humans as well as animals, for example, tuberculosis (TB) and Leprosy (Barletta and Steffen, 2022a). Stoppage and control of these diseases are important for public health along with veterinary medicine (Palma et al., 2020). This chapter explores managing mycobacterial infections through vaccination strategies and alternative control measures.

The Mycobacteriaceae family has a genus of bacteria with it called *Mycobacterium*. *Mycobacterium* species have many features like no spore-forming and not a motile bacterium with a bacillus formation (Parija, 2023). Pleomorphism is another feature, which means they can change their formation from round to rod-shaped (Pavlova et al., 2022). They are similar to gram-positive bacteria as well as they need staining of acid-fast to differentiate from other bacterial species (Ullberg and Özenci, 2020). The genus *Mycobacterium* comprises different bacteria that could be disease-causing pathogenic, saprophytic as well as opportunistic, or may be nonpathogenic (Rigouts and Cogneau, 2021).

The fame of *Mycobacterium* species is for causing a disease called tuberculosis, which mostly affects the human breathing system. Yet a variety of other diseases were also caused like affecting different organs and systems (Adamowicz et al., 2023). For example, widespread cutaneal problems in humans are caused by *M. ulcerans*, while cutaneous, hypodermic, and internal infections are due to *M. abscessus* (Tan et al., 2020). Furthermore, species that cause disease in both humans and animals are *M. avium* complex, *M. kansasii* as well as *M. bovis* (Stabel et al., 2021). Various reptiles, fish,

birds, and mammals were also affected by other *Mycobacterium* species.

As predicted one-third of the world's people are diseased by *Mycobacterium tuberculosis* making Tuberculosis (TB) an important worldwide health issue. Presently, there are around 1.5 million fatalities along with 9 million new infections yearly. To protect children from getting severe TB, the only licensed TB vaccine available is the Bacille Calmette Guerin (BCG) vaccine. But in adults, its efficiency against lung TB ranges from 0 to 80% depending on topographical location. Another important factor is that it is not appropriate for persons whose immune systems are compromised. There is an immense need for improving TB vaccines because vaccination is known as the most operative method for preventing and controlling infectious diseases.

After COVID-19, TB is one of the important causes of mortalities from infectious diseases around the cosmos. COVID-19 has a bad impact on TB-related health services that involves late analysis and treatment as well as new vaccinations wrong way up the previous declining trends in TB deaths (Allué-Guardia et al., 2023). The World Health Organization (WHO) developed the End TB Plan in 2015 whose aim is to reduce the deaths cases along with costs related to tuberculosis worldwide. According to strategy, the goal set for 2025 includes a 75 percent decrease in the number of deaths as well as a 50% drop in TB cases in comparison to 2015. A 95% decrease in TB deaths as well as a 90 percent decrease in TB cases will be achieved by 2035. Nevertheless, to meet these striving targets many countries are currently not on track due to their own economic and political reasons.

Effective vaccination along with alternative control methods are important because *Mycobacterium spp*. hurt human as well as animal health (Balseiro et al., 2020). Bacillus Calmette-Guerin (BCG) vaccine is currently available which offers some defense against tuberculosis but is not much curative against other *Mycobacterium* infections. Immunotherapies and new vaccines need more research to give wider as well as more effective defense (Fatima et al., 2020). Various alternative methods include phage therapy along with antimicrobial peptides, a different vaccine in combination with immunomodulators as well as some managemental and genetic modifications that show promise in managing *Mycobacterium* infections (Allué-Guardia et al., 2021). To reduce the spread and brutality of diseases caused by *Mycobacterium spp*. implementation of these strategies is necessary.

An Overview of *Mycobacterium* Species

A varied group of bacterial species that show different levels of disease in humans as well as animals is included in the *Mycobacteriaceae* family which shows different host reservoirs along with growth patterns in agar (Barletta and Steffen, 2022b). Usually, *Mycobacteriaceae* bacteria have different characteristics they are aerobic as well as no spore formation along with Gram-positive in nature, and are unable to be motile having acid-fast bacilli with a curved shape that may show some branches due to the presence of mycolic acid cell wall (Romagnoli et al., 2020). A complex cell wall structure is seen in members of the *Mycobacterium* genus that causes low absorbency. Ziehl-Neelsen acid-fast staining procedure is used to differentiate it from other bacterial species (Dulberger et al., 2020).

There are mainly two groups included in the *Mycobacterium* genus that can be differentiated by noticing their growth rates. *Mycobacterium bovis*, *Mycobacterium tuberculosis* as well as *Mycobacterium leprae* are included in slow-growing *Mycobacteria* and they are answerable for bovine TB (BTB), human TB (TB), and leprosy (Byrne et al., 2020). While opportunistic or non-pathogenic bacteria are included in the fast-growing group that bacteria like *Mycobacterium smegmatis*.

MTBC which means *Mycobacterium tuberculosis* complex is a group of bacteria from the *Mycobacteriaceae* family that includes *M. tuberculosis* as well as *M. africanum* also including *M. bovis*, *M. canettii* along with *M. microti* and some other species *M. pinnipedii*, in addition with *M. caprae* (Kanabalan et al., 2021). Additionally, two new species *M. orygis* and *M. mungi*, are also added to the *Mycobacterium tuberculosis* complex (Islam et al., 2023). Some *Mycobacterium species* cause disease in particular animals which includes *M. bovis* cause disease in bovines (Bespiatykh et al., 2021), M. caprae cause disease in small ruminants including goats and sheep*, M. pinnipedii* usually affects sea creatures like sea lions or seals, *M. microti* infect the voles and *M. orygis* develop an infection in oryxes (Delghandi et al., 2020).

Pathogenesis and Epidemiology

To develop operative control strategies for the prevention of mycobacterial disease the understanding of pathology as well as epidemiology is important (O'Brien et al., 2021). Localized infections or systemic distribution of Mycobacterial infection occur when bacteria enter the host body by inhaling or ingestion that involve the respiratory system or digestive system respectively. The key feature that is involved in pathogenesis is bacteria's capability to live and reproduce within macrophages of Immune cells (Mir et al., 2022). Spread of disease occurs via straight contact, dirty food with water, or aerosolized droplets that enter through the mouth or nose.

The *mycobacterial* species that cause disease in animals especially those that have zoonotic importance and also cause diseases in humans were the primary focuses in the epidemiology of *mycobacteria* (Gebreyes et al., 2020). *M. bovis* is a bacterium that causes disease in cattle but can be spread to humans and develop zoonotic infections. Some other mycobacterial species such as *M. avium* an opportunistic bacterium in both animals and humans often linked with environmental causes (To et al., 2020).

The factors that influenced mycobacterial epidemiology include animal farming practices, environmental circumstances as well as the presence of a reservoir of wildlife species (Pereira et al., 2020). Managing disease in both

animals as well as humans requires an understanding of transmission, control measures, and risk factors is vital. Changes in environmental circumstances along with their effect on the appearance and spread of infection require continuous research to evolve better solutions (Destoumieux-Garzón et al., 2022).

Gross Pathologic Features of MTC Disease

The Latin term "tuberculum" which has meaning of lump or nodule is the main word from which the word "tuberculosis" is derived (Yousaf Kazmi, 2022). The lumps or nodules that form in the disease tuberculosis are called "tubercles". The most important gross lesion in tuberculosis that can be seen by the naked eye is a fixed, yellow to white or maybe grayish tubercle, and size is between pinpoint to some centimeters in width in immunocompromised animals the lacerations may be more diffused (Borham et al., 2022).

In cattle, the culprit bacteria is *M. bovis* which causes Tuberculosis. The nasal and oral routes are mostly seen but by skin contact, inherited and genital routes were also seen in some cases. In the start sub lobular or maybe lobular lacerations are seen that spread to the respiratory lymph nodes (Tiwary et al., 2022). Calcified caseated lesions developed in the lungs and lymph nodes. Caseating bronchopneumonia occurs due merging of nodules in the lungs. Cavitations along with ulcers in the trachea or bronchi are commonly seen in long-lasting cases. In the fulminating course of disease miliary lung lesions are also seen (Stephenson and Byard, 2020). Pleural involvement occurs when the disease spreads to the serosal layer having sessile, pedunculated, or sometimes cauliflower-like erections that lastly calcify and result in forming solid white nodules. Therefore, pearl disease is the name given to pleural tuberculosis (Borham et al., 2022b). The infection spreads to main organs like lymph nodes, skeletal muscles as well as serous membranes together with the peritoneum membrane as well as pericardium layer along with meninges in the nervous system. In advanced respiratory disease and the oral route of infection nodules and ulceration may be existing in the upper portion of the digestive tract and abomasum. Small and large intestines of cattle were also affected by ulcerative lesions (Esteves et al., 2021). There few cases where dermal infections were seen and it restricted to the place of lymph nodes. The uterus or epididymis may be diseased through genital contaminations but these are not common. The newborn fetus is infected through congenital tuberculosis and the disease is spread via umbilical vessels that involve hepatic and abdominal lymph nodes which can turn into general disease. MTC causes inflammation of the uterus that leads to endometritis in the dam and is essential for the transfer of congenital infection (Figueiredo et al., 2021). Although recurrent abortions and infertility result due to extensive uterine disease. The mammary glands shed bacteria in milk due to MTC infection that result in spread and contamination of milk.

There are rare cases in which cattle are affected by ulcerative lymphangitis which is commonly known as "skin tuberculosis." The precise information about which type of organism causes the disease is not known yet, nevertheless, compatibility with *M. bovis* or *M. kansasii* is seen due to low numbers of acid-fast bacilli under the microscope (Konieczny and Pomorska-Mól, 2023). The lesion appears first as subcutaneous nodules attached to the skin. Ulcerative nodules are mostly seen with other nodules that mostly form sideways to the lymphatic system. Lesions are cured after bursting and evacuating the material inside but sometimes nodules unite to form huge masses of connective tissue and pus-filled substance (Naafs et al., 2020). Local lymph nodes were not involved in this and the culturing and identification of animals is not possible. Tuberculin skin tests may or may not show results in infected animals (Srinivasan et al., 2020). Principally *M. bovis* sometimes causes disease in Sheep and goats and leads to MTC disease but normal or natural cases are very rare (Mahomed et al., 2023).

Proliferative enteritis occurs in horses due to organisms of the MAIC causing a disease closely similar to Johne's disease in cattle (O'Connell et al., 2023). Lesions are mostly seen in the intestinal tract but in severe cases, distribution of crudely nodular lesions is seen in the respiratory organs, some parts of the liver and spleen, tissues of the mammary gland as well as vertebrae of the cervical region, and skin is also involved (Urs et al., 2024). Involvement of reproductive system or nervous system lesions is occasional if it occurs.

Vaccination Strategies

Attenuation of Virulent Strains of *Mycobacterium Bovis*

The effectiveness of Bacillus Calmette-Guérin (BCG) was measured experientially. This weekend vaccine requires a single dose most of the time but some may need a booster for optimum protection (Setiabudiawan et al., 2022). All strains of BCG have a 9.5 kilobase (kb) deletion showed during recent genetic analysis and when it is related to infectious strains of *M. bovis* as well as *M. tuberculosis* it includes nine genes. Removing some genes involved in virulence or encrypting enzymes for serious metabolic pathways from infectious strains of *M. bovis* as well as *M. tuberculosis* should advance the BCG vaccine (Seral et al., 2024). In terms of antigenic outline, these mutants may look like infectious strains more closely than that of BCG henceforth showing improved vaccine effectiveness. Methods like jumping gene mutagenesis, and prohibited recombination along allelomorphic exchange have now been developed in molecular biology to deactivate genes in *M. bovis* along with this some screening methods have been recognized to categorize weakened mutants (Sukhija et al., 2023). To dodge any opportunity of return to an infectious strain through alteration an attenuated vaccine would be obligatory to have removal in two different genes to make it used fully in the field. To differentiate between vaccinated animals and those diseased with *M. bovis* would require advanced immunological screening methods. An immunological test makes a separation between vaccinated and non-vaccinated animals could be formed if the novel vaccine strain also

has one or more gene removals, the products of which DTH or another immunological response could be inspected (Aida et al., 2021). A wild-type *M. bovis* strain's esat-6 gene has been removed. In the primary trials, this mutant's vaccination triggered guinea pigs to respond powerfully to PPD-B but not to ESAT-6 protein in a skin test. A forceful reaction to PPD and ESAT-6 was seen in animals inoculated with the wild-type strain of *M. bovis*.

The loss of some metabolic activity in a strain is shown by auxotrophy, or the incapability to grow in a slight medium. Although the method has been functional to several bacterial diseases with victory to create weakened strains with vaccine potentials (Seif et al., 2020). In initial attempts to determine the usefulness of this approach, several attenuated strains of *M. bovis* were developed by chemical mutagenesis of a liquid culture with nitrosoguanidine. Following the screening of strains for auxotrophy, the auxotrophs were tested for virulence in guinea pigs. Two of these auxotrophic *M. bovis* strains, which were shown to be attenuated in guinea pigs, were tested for the protection of cattle against bovine tuberculosis. The calves in this experiment showed solid IFN-Y responses to PPD-A before vaccination, which may have shown that they were exposed to mycobacteria (Cooke et al., 2023). When BCG-vaccinated and control groups were compared, particularly fewer animals established tuberculous lesions following inoculation with any of the two auxotrophic *M. bovis* strains. The calf's previous contact to ambient mycobacteria may have role in BCG's failure to defend the cattle in this trial. The inoculations made from auxotrophic strains had 1-2 x 10° CFU/dose of living microorganisms, which was about 1 log1o extra than the BCG preparation (Conlan et al., 2021). Given that dosages of range 104–106 CFU BCG can produce equal amounts of resistance, it is unsure that this played a part in the enhancement of vaccine effectiveness. The newly-derived weakened *M. bovis* strains' overall efficacy in this setup is inspiring. These two auxotrophic strains required genetic changes that could be exposed despite rigorous hard work (Peres and Brock, 2022).

Many mutants of *M. bovis* have been formed by prohibited recombination to make weakened strains of the bacterium with precise deletions. In guinea pigs, it was confirmed that four mutants that were selected because they could not grow in a slight medium were attenuated. Two of these mutants formed a level of defense similar to that formed by BCG when exposed to infectious M. bovis (Gunasena et al., 2022). A 2bp chromosomal removal existed in one of these mutants, but a 15 kb big DNA removal including twelve genes existed in the future. The efficacy of these two strains as inoculations has freshly been examined in possums. Eight weeks after the strains were dermally vaccinated at an amount of 109 CFU, the possums were tested with virulent *M. bovis* spray. Upon post-mortem eight weeks after the test, the possums vaccinated with BCG or one of the auxotrophic strains showed far fewer lung lacerations and minimized body weight loss in contrast to the non-vaccinated controls (Telford and Goethert, 2020). Only the receivers of the auxotrophic strain inoculation, as opposed to the controls, had a spleen microbial count that was prominently lower. The possum model may help evolve better tuberculosis inoculations because few if any, new vaccinations verified in mouse or guinea-pig replicas prove more defense against tuberculosis than the BCG vaccine. This type of inoculation often produces prolonged (Balseiro et al., 2020b) and long-lasting resistance with fewer doses and has a robust and wide-ranging resistant response but has a risk of return to virulency in some cases as well is not appropriate for animals whose immune system is compromised.

Use of Live Vectors

Genes encoding mycobacterial antigens have been expressed using living vectors, such as the vaccinia virus as well as attenuated *Salmonella* strains. Nevertheless, the effectiveness of these vaccines against *M. bovis* has not been estimated. Meanwhile, most live vectors may be given oral or parenteral route, use of these for wildlife vaccination is remarkable (Osterloh, 2022). Fifty percent of the badgers that administered a dose of a live recombinant injection orally were protected against rabies. Boosting of the immune system by via M. bovis DNA vaccines may be achieved effectively using live viral vectors having mycobacterial genes, later this major boosting method has been confirmed to be fruitful in inducing healthy CMI responses to specific antigens of bacteria.

Killed Mycobacteria

The usage of dead mycobacterial vaccinations is supposed to be harmless than that of live, weakened *M. bovis* vaccines. Administration of this killed vaccine involves a single dose or maybe a sequence of doses which is dependent on the definite vaccine as well as the creature's time of life along with health status (Sefidi-Heris et al., 2020). However, conventional vaccines having killed mycobacteria joined with an oil accessory provoke a Th2-type immune response and offer a slight defense. It has been recommended to use a lifeless *M. vaccac* for tuberculosis immunoprophylaxis. It is supposed that *M. vaccac* applies its defensive assistance by making CMI responses to common mycobacterial antigens as well as by preventing the Koch phenomena of tissue necrotizing elements. In the direction to estimate the efficacy of the dead *M. vaccae* vaccine, calves were administered a sub-cut injection of vaccination having 109 CFU of the (Milián-Suazo et al., 2022) inoculation, surveyed by a trial with *M. bovis*. On comparison of the results the calves immunized with BCG have no protection for tuberculosis. Moreover, vaccinating badgers against tuberculosis with killed *M. vaccae* did not yield any defense. If *M. vaccae* was administered alone intraconjunctivally or through oral route in an initial trial including possums did not show any defense against *M. bovis*. But, defense against the *M. bovis* challenge was produced by the combination of both dead *M. vaccae* and live BCG. Later the spleen microbial count was significantly lesser with this mixture than with BCG (Noguera-Ortega et al., 2020). This study advocates that removing *M. vaccae* could advance the efficacy of the BCG injection as well as the efficacy of the BCG vaccine. This type of vaccine is safe for immunocompromised animals as well as it is unchanging and can be kept for an extended period but it often needs several doses to attain full protection as well as may not deliver robust or long-lasting protection as live vaccines are considered its disadvantages.

Mycobacterial Protein Vaccines

Using defensive protein antigens formed by live mycobacteria is the focus of an alternative approach. Probably, sub-unit immunizations would not make hindrance with investigative trials along with have their efficacy be impacted by animals being sensitive to environmental *mycobacteria* earlier (Amoroso et al., 2021). This vaccine requires many doses for full immunity as well and booster doses are also needed. It is found that proteins present in culture remain made from *M. tuberculosis* powerfully stimulate not only the T lymphocytes in patients having human tuberculosis as well as in experimentally diseased mice and also seen in cattle. The skill of antigens present in culture remains to provide defense has been exposed in several research by using tiny animal models (Ábalos et al., 2022). The highest levels of defense against aerogenic trial with *M. tuberculosis* were obtained by vaccinating mice as well as guinea pigs with culture filtrate proteins (CFP) obtained from tuberculosis. Likewise, it has been exposed that a CFP inoculation produced from *M. bovis* significantly defends mice against virulent *M. bovis*. When *M. tuberculosis* CFP was joined with adjuvants such as dimethyldeoctadecylammonium chloride (DDA) and administered in mice established cellular defense to mycobacterial antigens while the same combination in cattle did not show positive but a slight IFN-Y response was seen (Kaur et al., 2019). Although the administration of a diethyl aminoethyl (DEAE) dextran adjuvant in cattle helped make considerable antigen-specific antibodies as well as IL-2 responses. An *M. bovis-prepared* culture filtrate protein (CFP) vaccine along with lipid A adjuvant that comprised of the recombinant cytokine bovine IL-2 significantly improved antigen-specific antibody responses but minimum stimulation for antigen-specific IFN-y responses in cattle (Wedlock et al., 2000). In tested cattle, this inoculation reduced the overall mean tuberculous lung laceration value and did not reason for any tuberculin skin-test reactivity in the final test. while compared to non-immunized animals, these animals showed an advanced occurrence of extra-thoracic disease distribution.

These data demonstrate how challenging it is to bring strong antigen-specific IFN-Y results in cattle by using low-dose adjuvants. same adjuvants have not yielded the same results when used in cattle or small animal models. None of the sub-unit vaccines currently have formed antigen-specific IFN-y results in cattle up to this point that are equal to those formed by weakened *M. bovis* injections (Lowenthal et al., 1998). To enhance or produce better results for antigen-specific IFN-y responses, preparations with the most superior adjuvants along with the addition of other cytokines such as IL-12 or IL-18 make significant improvement. This type of subunit vaccine is safer than live vaccines because they do not have live pathogens and can be personalized to take in only the most immunogenic parts of the pathogen but may need adjuvants to improve the resistant response and provide shorter time protection as associated with live inoculations (Portielje et al., 2003).

DNA Vaccines

Although the uncomplicated idea behind DNA immunization is fairly straightforward and this kind of knowledge has freshly been established since the early 1990s. This type of injection is administered in a sequence with precise medicating procedures based on the vaccine. An expression of plasmid comprising a part of DNA encrypting a bacterial antigen is how a DNA vaccination is made (Li et al., 2020). The bacterial disease-causing antigen gene is enlarged in an altered bacterium employing this plasmid DNA and the host is vaccinated with the decontaminated plasmid DNA that possibly holds the antigen gene. A living cell is instantly transfected by this plasmid DNA. Inside the nucleus, this antigen-containing gene is decoded into RNA after this the RNA in the cytoplasm is translated into protein (Valizadeh et al., 2022). Eventually, the bacterial protein produced immunization in the host's cells and letting the initiation of strong humoral and cell-mediated responses that are strong and defensive.

In defense of the contradiction of tuberculosis in small animal models used in experiments shows that DNA vaccines have significant capacity. Important features of the TB disease while encrypting a solo protein or maybe epitope these inoculations have the potential to cause strong memory responses such as bringing IFN-y as well as cytotoxic T cell responses and help to provide protection (Duong et al., 2023). Protection is enhanced if Adjuvant molecules such as DNA CpG parts are involved in the vaccine as well and defense is also amplified if the DNA vaccine encodes for numerous proteins otherwise epitopes. DNA inoculations for tuberculosis have been revealed in a mouse model to be able to treat a disease which was a good achievement and news for application in wildlife. The element such as numerous vaccinations seems to be obligatory as a problem. Two of these immunizations produced immune serum globulin G1 (IgG1)-influenced humoral responses along with $CD4^+$ T cell responses while IFN-Y is modest, which is seen in a recent study led in cattle (Abo-Elyazeed et al., 2023). Key advantages of this type of vaccine are that it is unchanging and can be formed quickly and it brings both humoral and cellular protection responses but it is still comparatively new and less broadly used in veterinary medicine as well as regulatory support methods can be long (Montero et al., 2024).

In the field of veterinary medicine, vaccines of all types are vital particularly when it arises to control disease like Tuberculosis caused by Mycobacterium Bovis. The choice of a vaccine is based on a numeral variables like nature of the illness as well as the planned resistant response and the aimed animal type (Vannini et al., 2021).

Alternative Control Strategies

In addition to vaccination, alternative strategies play a crucial role in controlling mycobacterial infections.

Table 1: Vaccination Strategies Against *Mycobacterial* Species

Type of Vaccine	Dose	Mode of Action	References
Inactivated (Killed)	Single dose or series of doses	Stimulates immune response by introducing inactivated pathogens, prompting antibody production without causing disease.	(Tovey and Lallemand, 2010)
Live Attenuated	Usually, a single dose, may require a booster	Mimics natural infection, eliciting a strong immune response with both antibodies and cellular immunity.	(Mok and Chan, 2020)
Subunit	Often requires multiple doses with boosters	Presents key antigens to the immune system, leading to antibody production without risk of disease.	(Foged, 2011)
DNA	Typically, in a series, specific dosing guidelines	Host cells take up DNA, produce antigens, and trigger an immune response.	(Reyes-Sandoval and Ertl, 2001)
mRNA	Usually requires two doses	Cells produce the antigen from mRNA, leading to an immune response similar to live attenuated vaccines.	(Kowalzik et al., 2021)
Toxoid	Initial doses followed by boosters	Introduces inactivated toxins, prompting the body to produce antibodies against the toxin.	(Moylett and Hanson, 2003)

Phage Therapy

Phage therapy's goal is to fight tuberculosis along with other destructive mycobacterial diseases by using bacteriophages as a weapon. Bacteriophages are one of the most important techniques which have natural bacteria-killing capabilities. Bacteriophages are much more selective than antibiotics as well as they can remove mycobacteria without risking the body's helpful microbes (Khusro et al., 2016). when inoculated into the human body these bacteriophages stick to mycobacteria and start transporting their genome to them and after that, the phage increases till it lyses or breaks the bacteria. These newly formed phages will move to other uninfected ones till their individual population also goes destroyed and every disease-causing mycobacterium will have been removed (Ouyang et al., 2023).

Because of its important feature in which phage precisely targets antibiotic-resistant Mycobacterium strains it has numerous benefits. There is a lot of potential seen in phage therapy because of its excellent results in antibiotic-resistant bacteria (Hatfull et al., 2022). Also, bacteriophages can modify itself according to bacteria's resistance and remain to be effective against them. Phage medication is now being studied and the results are inspiring. It can be an active weapon against mycobacterial diseases meanwhile it offers an unfailing, effective, and safe substitute for outdated antibiotics (Melo et al., 2020).

Immunotherapy

Immunotherapy aims to enhance the host's immune response to control mycobacterial infections.

Cytokines

Proteins termed cytokines influence the development, delivery as well as the function of cells and help in directing the activities of the innate as well as adaptive protected systems (Arango Duque and Descoteaux, 2014). Although the recombinant human interferon (rhuIFN-γ) in addition to recombinant human interleukin-2 (rhuIL-2) along with α-tumor necrosis factor (TNF-α), as well as recombinant human granulocyte-macrophage colony-stimulating factor (rhuGM-CSF), are the most important cytokines used in medical applications or clinical experiments but the brief half-life along with high price of cytokine immunotherapy are its disadvantages (Mi et al., 2021a).

Interleukin-2

Th1-type immunization response cytokine IL-2 is elaborate in both immune cell activation as well as control. A study discloses that IL-2 can aim an alteration in the way genes are expressed in MTB-encouraged peripheral blood mononuclear cells (PBMCs) along with this can remove or reduce mucus germs in around 60% of people affected with MDR-TB who are also getting (Daichou et al., 1999). Yet, everyday intradermal inoculation of rhuIL-2 could not show signs or improve bacillar clearance in persons with drug-prone TB, according to information from a double-blind controlled medical trial. So, there is changeability in the scientific results of rhuIL-2 in combination with chemotherapy for MDR-TB or stubborn pulmonary tuberculosis (PTB) (Mealey et al., 2008). Additionally, rhuIL-2 immunoadjuvant treatment was safe for PTB/MDR-TB affected people along with improving the growth and revolution of CD4+ T cells as well as NK cells, thus growing the amount of sputum that is bacteria-negative in TB patients (Mi et al., 2021b). Yet, no visible enhancement was seen in radiographic variations in TB patients. In China, a 24-month multicenter, huge-sample potential scientific investigation is being led to assess the properties of rhuIL-2 adjuvant treatment for MDR-TB (Sheng et al., 2022).

Granulocyte-Macrophage Colony-Stimulating Factor

A monomeric glycoprotein is produced by macrophages including T cells along with mast cells as well as natural killer cells plus endothelial cells and fibroblasts. GM-CSF is a cytokine that has protection and controlling effects. It has been verified that GM-CSF hinders M. *tuberculosis* growth in human WBC especially mononuclear macrophages (Wałajtys-Rode and Dzik, 2017). In the handling of active PTB (APTB), rhuGM-CSF adjuvant immunotherapy verified superior protection as

well as acceptability in affected people which is proved by the outcomes of a phase II experimental trial. Moreover, the mucus microorganisms rapidly turned negative in the eighth week of medication (Y. Zhang et al., 2012a). Moreover, in the MDR-TB mouse model, immunization through IL-2 along with GM-CSF may increase the cure rate of the mice and lesser the microbial burden in the respiratory system as well as spleen and lung lesions, henceforth boosting the efficacy of the initial-line anti-TB medicines. When injected as a solo dose, recombinant GM-CSF adenoviruses (AdGM-CSF) in a mouse model significantly lesser the lung microbial load in contrast to old-style chemotherapy (Francisco-Cruz et al., 2013). This is a new technique found in gene therapy.

Interleukin-24

The IL-10 cytokine family has a new suppressor gene fellow known as IL-24. The silent features like its preserved assembly, chromosomal site, as well as cytokine-like possessions, make it a novel suppressor of IL-24 (Abdalla et al., 2016). According to a study *M. tuberclosis* disease has been exposed to suppress IL-24 appearance in human PBMCs along with minor IL-24 stages in the blood serum of TB patients that may increase TB vulnerability and involve in the development of long-lasting TB (Wilson et al., 2010). Depending upon the initial visit of neutrophils IL-24 can trigger the IL-24 receptor signaling path of $CD8^+$ T cells to make high levels of interferon-γ (IFN-γ) to destroy *M. Tuberclosis*. According to a study IL-24 medication of the mice TB model confirmed an anti-TB effect which demonstrates that IL-24 may be a new immunotherapy treatment (Mi et al., 2021c).

Interleukin-32

An important secretory protein that involved in involved in innate as well as adaptive immune responses is IL-32 which is a cytokine mainly formed by immune cells including T cells along with NK cells and epithelial cells. It initiates the formation of vital inflammatory features in macrophages which include TNF-α also has IL-1β as well as IL-6 MIP-2, and IL-8 whose main function is eliminating *M. Tuberculosis* (Gautam and Pandit, 2021). Consequently, TNF-α can be amplified and cell decease can be stimulated by the increased influence of IL-32 in innate immunity against the disease tuberculosis. According to a recent investigation, heat-killed *M. Tuberclosis* stimulus of human PBMCs can raise the *M. Tuberclosis* clearing capabilities of human monocyte-macrophages by making the formation of a noteworthy quantity of IL-32 (Park et al., 2014). Human IL-32γ formed by type II alveolar consonant epithelial cells from transgenetic mice is significantly lesser than lung *M. tuberculosis*. Additionally, next the down regulation of endogenic IL-32 expression in human THP-1 macrophages by siRNA interfering there was a extensive growth in intracellular *M. Tuberclosis* as well as intracellular inflammatory markers such TNF-α and IL-1β has been seen (Refai et al., 2018). IL-32γ was downregulated whereas IL-32β was amplified in the *M. Tuberclosis* diseased PBMCs of the healthy control group the results indicate that IL-32 helps to stop *M. Tuberclosis* infection. The qualified richness of IL-32 isoforms may have an influence on this result. Afterward, IL-32 is a fresh immunotherapy that displays potential for making protection mechanisms along with stopping *M. Tuberclosis* development (Montoya et al., 2014).

Table 2: Cytokines Used in Immunotherapy for Mycobacterial Infections

Cytokine	Mechanism of Action	Therapeutic Potential	Challenges	References
Interleukin-2 (IL-2)	Enhances T-cell proliferation and activation	Boosts immune response, useful in combination therapy	Toxicity at high doses, cost	(Dhupkar and Gordon, 2017)
Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)	Stimulates macrophage activation and differentiation	Promotes mycobacterial killing, enhances vaccine efficacy	Limited effectiveness as monotherapy	(Y. Zhang et al., 2012b)
Interleukin-24 (IL-24)	Induces apoptosis in infected cells	Targets infected cells, minimizes damage to healthy cells	Delivery to target tissues, stability issues	(Panneerselvam et al., 2013)
Interleukin-32 (IL-32)	Modulates immune response promotes pathogen clearance	Reduces bacterial load, supports long-term immunity	Inflammatory side effects, dosage optimization	(Sasindran and Torrelles, 2011)

Anti-TB Antibodies

It is generally recognized that the cellular immune response acts as a main role in stopping tuberculosis additionally the importance of humoral immunity in tuberculosis is argumentative (Redford et al., 2011). However further research in recent years has confirmed that antibodies also offer a protective influence in contradiction to tuberculosis protection (Rijnink et al., 2021). Immunological damage caused by dangerous molecules can be prevented by the application of M. Tuberculosis antigen-specific antibodies and inflammatory response for example phagosome maturing as well as intracellular bactericidal action may prompted (X. Zhang et al., 2017).

Some hosts lacking in antibodies have a delicate susceptibility to tuberculosis which is shown in a meta-analysis. Antibodies have contradictory defensive effects against many *M. tuberculosis* antigenic determinants (Casadevall and Pirofski, 2006). Human anti-HBHA IgM antibodies may be able to stopover *M. tuberculosis* from penetrating the epithelial cells of TB-affected patients. Although the anti-Ag85A IgG may be able to lessen the danger of active TB along with

decreasing cavities and removing mucus microbes. The minor the level of anti-LAM as well as AM antibodies in TB patients results in the quicker the development of TB along with the advanced incidence of distribution (Abebe and Bjune, 2009).

Moreover, the phagocytosis could be improved as well as CD8+ T cells could be controlled along with tissue injury plus lung swelling and microbial load could be reduced in mice by passively vaccinating with poly or monoclonal antibodies or sometimes blood serum against *M. tuberculosis* antigen (Lawn, 2012). The intranasal otherwise intratracheal dose of anti-Acr IgA antibody otherwise pretreatment with hsIgA was found helpful in declining lung colony counts and also recover granulomatous formation in *M. tuberculosis-infected* mice. On the other hand, the extrapulmonary distribution of *M. tuberculosis* was pointedly lowered by passive transmission of anti-HBHA IgG3 or McAb 4057D2 and IgG2a McAb 3921E4 or anti-LAM IgG (Kim et al., 2011).

Like IgG immunoglobulin Y (IgY), a noteworthy antibody is present in the blood of lungfish as well as reptiles and poultry which may be used as an immune globulin for TB immunotherapy. IgY fits to the class of recognition proteins that the immune system produces in response to external substances same as another Ig (Senger et al., 2015). It has been detected that IgY can intensely increase rat PBMC spread as well as discharge of IL-2 and IFN-γ which represents that the pharmacological achievement of IgY in contradiction of *M. tuberclosis* may be intermediated by regulatory cytokine manufacturing (Tetik, 2024).

Environmental and Management Practices

Operative environmental as well as managemental practices are important for minimizing mycobacterial infections principally in livestock. It is vital to follow to significant environmental in addition managemental values in order to lower mycobacteria (Guardabassi et al., 2018). To stop the entry in addition to the spread of *mycobacteria* will start with severe biosecurity actions like restrictive access and also making sure all equipment is completely washed before usage. The key to preventing environmental infection is the daily routine of cleaning as well as decontamination of animal housing and also supplies. Keeping proper animal care and treatment is also vital although the sick animals must be set aside from healthy ones to stop the disease from scattering (Couto and Cates, 2019). Before reintroducing any novel or giving animals back to the general population it must be quarantined to make certain they are illness-free. Culling may be obligatory to protect the remaining animals in the herd in circumstances where the animals are extremely vulnerable to illness or are sick (Jagielski et al., 2014). Further dropping the risk of spread by managing the animal activities both inside and among accommodations along with the restriction of wildlife reservoirs such as mice or wild birds (Warwick et al., 2023). It is probable to proficiently resist the transmission of mycobacterial species by combination of these methods.

Genetic Resistance

A combination of genetic engineering as well as selective breeding for genetic resistance to mycobacterial infections is a worthwhile long-term method for regulating livestock viruses (Pal and Chakravarty, 2020). Animals that naturally fight mycobacterium diseases in nature are recognized and maintained by selective breeding which in turn advances these assets over following generations to yield a stronger population. Alternatively, genetic engineering introduces or modifies genes that enhance immunity against *mycobacteria* through the use of advanced pioneering biotechnological techniques similar to CRISPR (Choi and Lee, 2016). while collectively these practices lower the necessity for inoculations and antibiotics along with this it also lessens the option of drug-resistant strains of bacteria and viruses. Although it reduces the overall incidence in addition to the spread of mycobacterial contaminations in cattle. All of these activities endorse better herds and improved yield (Pickrodt et al., 2023).

Conclusion

In conclusion, handling of tuberculosis causing *Mycobacterium species* will remains a very hard task in veterinary as well as human medicine. The chapter highlights the vital importance of a comprehensive plan that join the cutting-edge substitute control procedures with important conventional vaccination strategies. The persistence of these illnesses highlights the necessity for new actions, even despite the fact that vaccine developments together with DNA, protein-based as well as live vectors, dead mycobacteria and attenuated infectious strains offer possible pathways for defense. The process of Immunotherapy which uses cytokines to enhance host protection, is a possible alternate to phage treatment that provide powerful means of protection specifically by destroying *mycobacteria*. The weapons in contrast to mycobacterial infections is more strengthened by the characteristics like genetic resistance along with environmental control as well as anti-TB antibodies. A coordinated plan by uniting these various strategies is essential to successfully fight these illnesses. Preserving the efficacy of these treatments, defending the wellbeing of humans as well as animals, although dropping the universal problem of mycobacterial sicknesses will all depend upon the current examination along with enhancement of these methods.

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Chapter 45

Role of Vaccination in Disease Prevention by Immunization

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ABSTRACT

Vaccination is considered one of the most efficient approaches for preventing infectious diseases, which is also regarded as one of the greatest achievements. Worldwide immunization programs have contributed to a decrease in mortality and the desolation of different illnesses. Far-reaching resistance because of immunization is to a great extent accountable for the eradication of smallpox and the control of measles, polio, lockjaw, and numerous other illnesses. The control of measles, polio, lockjaw, and a slew of other diseases, as well as the eradication of smallpox are largely attributable to the widespread resistance that has resulted from vaccination. The immunizations for HPV, chicken pox, and flu have all undergone extensive examination and checks of their viability. Currently, vaccinations cover over 25 avoidable illnesses. Vaccinations are essential in stopping the spread of terrible illnesses because they trigger the body's safe system to create antibodies directed particular pathogens. Immunity to the targeted disease is provided by the immune system, which aids in the total eradication of illness and its dissemination. Smallpox was completely eradicated worldwide in the 20th century because of a vaccine drive spearheaded by the World Health Organization (WHO).

KEYWORDS
Immunization, Vaccine, Stimulation, Eradication, Immune system, Illness

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INTRODUCTION

The process of giving a person a vaccination to ward against disease is known as immunization. Vaccination-induced susceptibility (security) is similar to the resistance that a person would acquire from disease, except instead of contracting the illness, you receive a vaccination. Makes vaccines such an effective kind of treatment for this. While some vaccinations can be taken orally or through the nose, most are given by injection with a needle. A small number of infectious illnesses for which vaccines have been created are measles, mumps, polio, rubella, hepatitis, influenza, human papillomavirus, and pneumococcal disease. Each vaccination is made to improve immunity against a certain infection based on the characteristics of the microbe and the body's immunological response to it (Grohskopf, 2021).

Edward Jenner coined the term Variolaevaccinae to describe cowpox, which is the origin of the terms vaccine and vaccination. When he headed his investigation on the VariolaeVaccincinae, or cowpox, in 1798, he used this word, in which he portray the defensive impact of cowpox against smallpox [Baxby, 1999]. During the latter part of the 1760s, Edward Jenner was finishing his traineeship as a surgeon when he came across the urban marvel that dairy employees would never get the often deadly disease of smallpox because they had previously contracted cowpox, which only has a mild consequence for humans. Jenner (1796) injected an 8-year-old kid with smallpox six weeks after taking cowpox pus from a milkmaid's hand and smashing it into the boy's arm. After that, he noticed that the boy didn't get smallpox (Stern and Markel, 2005). In 1798, Jenner said that children and adults alike may receive Jenner's vaccination without risk, and could be transferred from arm to arm, dropping dependence on insecure provisions from contaminated cows (Baxby, 1999).

The first production of vaccines was developed in the middle of the 20th century. This turn of events made antibodies for tetanus and diphtheria possible. The development of viruses on the chorioallantoic membranes of chick embryos became achievable in the 1930s due to momentous advancements in laboratory methods. This provoked the enhancement of yellow fever and flu immunizations (Barberis, 2016). The improvement of cell culture 15 years after the fact encouraged the development of the polio antibody, and this was the start of the sparkling period of immunizations. Several important vaccines, including measles, mumps, varicella, and rubella, were developed during this time (Saleh, 2021). The late twentieth and mid-21st centuries testified to the encroachment of antibodies against hepatitis B, pneumococcus, HPV, rotavirus, and flu, among others. Immunization proceedings

have confronted difficulties like antibody aversion and production network issues. Late years have seen the development of new immunization methods, including mRNA antibodies (like those for the Coronavirus), which provide additional opportunities to prevent alluring illnesses (Saleh, 2021).

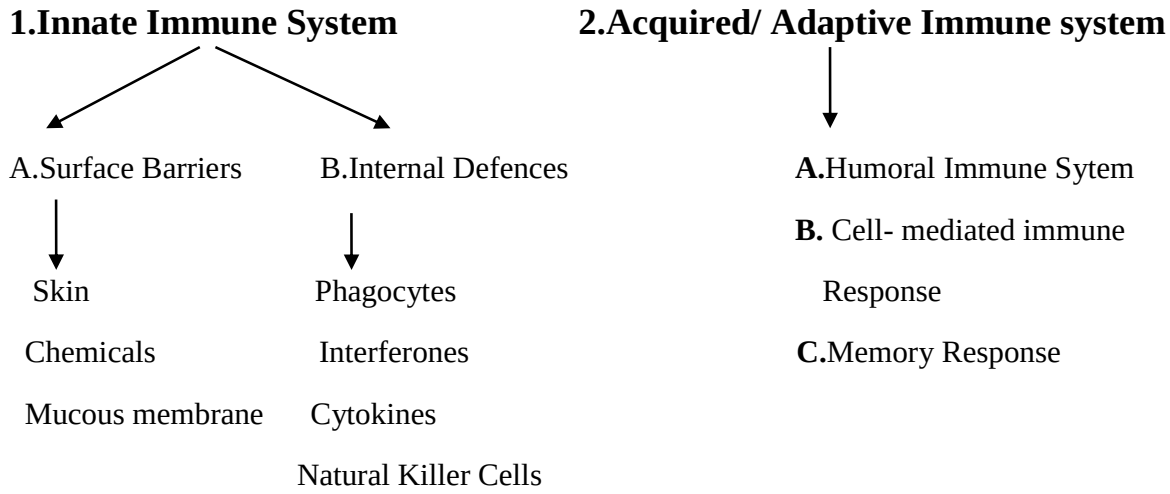
Immunizations as a Barrier

Immunization stops infectious diseases from spreading. Immunization is given to keep people from getting sick. It helps avert the consequences of illness, including the emergence of chronic conditions, cancer, and demise. Antibodies function by boosting the body's defense mechanisms to provide protection against infection. Immunizations can at times create a more grounded, longer-enduring defensive reaction contrasted with invulnerability from a characteristic disease (Pollard et al., 2020). Antibodies make insusceptibility without causing infection. Vaccination is a safer method of developing immunity because disease can result in serious complications. Antibodies function by energizing the body's defense mechanisms to protect against illness and pollution. The invulnerable framework is the collective term for these safeguarding elements. Immunizations replicate and occasionally strengthen the defensive response that the vulnerable framework consistently mounts following infection. The fact that immunization has a significantly lower risk of adverse outcomes is the primary advantage of vaccination over natural infections (Plotkin et al., 2021). Immunization takes advantage of the body's own defenses. To comprehend how inoculation safeguards against the illnesses delivered by microorganisms, (for example, infections and microscopic organisms) (Alberts et al., 2008).

The Body's Defenses

The resistant framework is the body's safeguard system, safeguarding against intruders like microscopic organisms and infections to remain us solid. The essential building units of our body are called cells. Numerous different types of cells, each with a distinct function, are essential to our immune system. Many of these are present in our blood system, especially white blood cells, which are the main component of the immune system in humans. White blood cells are decisively situated all through the body, circulatory system, lymph hubs, spleen, lungs, digestion tracts, and skin. This permits them to manage microorganisms any pace they enter the body (Travers et al., 2007).

Vertebrate Immune System



White Blood Cells can be Divided into two Main Groups

o Watchman cells, which are in charge of innate immunity—your body's most effective defense against microbes. o Your body's capacity to recall pathogens and respond promptly in the event of a re-infection is attributed to lymphocytes that are specialized for particular or "adaptive" immunity.

Different cells of immune system are associated with spscific functions such as macrophages associated with phagocytosis, neutrophils enhance the response of other immune cells, dendritic cells act as antigen representing cells, lymphocytes produce antibodies to destroy antigen representing cells, plasma cells responsible for humoral immunity and mast cells linked with homeoststic functions.

Innate Immunity

The first line of defense against infection is the physical barrier made up of the lining of the intestines, the skin, and the lungs. These tissues and the guard cells that reside within them make up the innate immune system. Some of these cells use vaccination particles or microbes that they swallow to activate lymphocytes (part of explicit invulnerability). Chemicals produced by innate immune cells can amplify the effects of particular immunity and induce inflammation. Anything that the innate immune system considers to be "foreign" receives a uniform response. That reaction probably won't be strong enough to protect against a disease on its own.

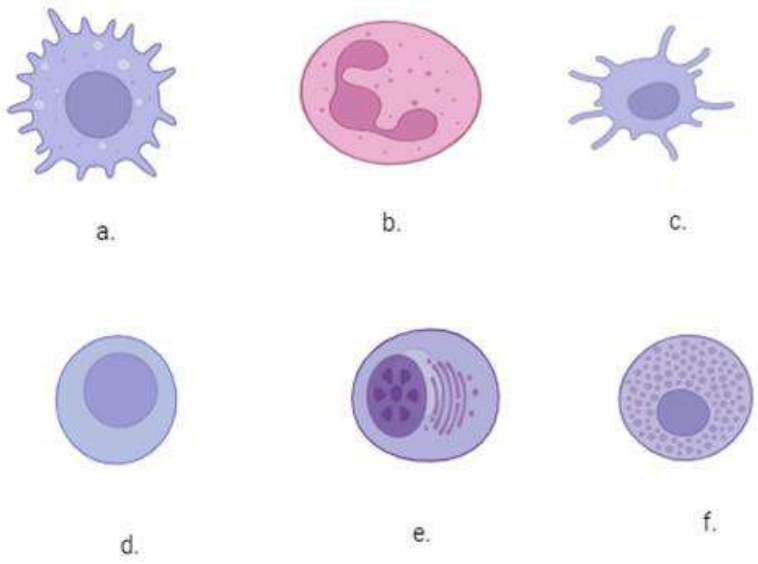


Fig. 1: Different cells of immune system performing specific funtions.

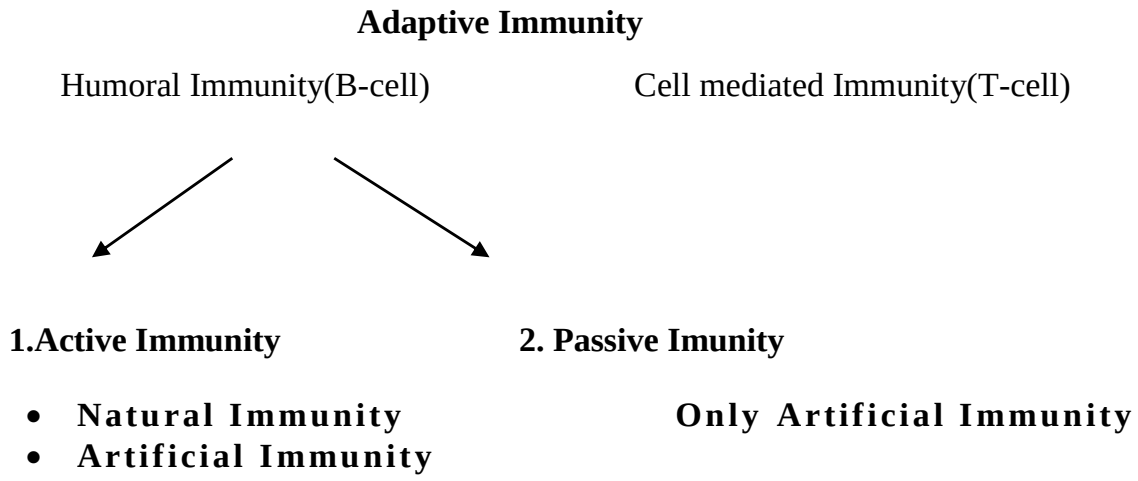
(a) Macrophage **(b)** Neutrophil **(c)** Dendritic cell **(d)** lymphocyte **(e)** Plasma cell **(f)** Mast cell

Adaptive Immunity

Specific lymphocytes multiply after an infection or vaccination as they can identify their target antigens. Following that, some cells develop into effectors can eliminate or prevent infection, while other cells become long-lived memory cells that are ready to react more swiftly and efficiently should the infection reappear.The reason they are 'explicit' is because they are made to particularly target and react to that antigen. B cells and T cells are the two types of lymphocytes (Torrigiani et al., 1996).

T cells release chemicals called cytokines in response to infection, and these molecules result in protective inflammation. T lymphocytes are also capable of eradicating cells that harbor pathogens, such as viruses. B cells generate antibodies; T cells often aid in this process. Complicated proteins called antibodies bind to germs or the poisons they transport in a "lock-and-key" fashion. Antibodies attach themselves to microorganisms and flag them for destruction; when they attach themselves to a toxin, they eliminate the pathogen's ability to do damage (Murphy et al., 2007).

The safe framework's reactions to microorganisms stop the disease, by and large, trailed by the fix of any harm to the body. In any case, serious contaminations can overpower the resistant framework's ability to answer and can prompt extreme sickness or demise. Giving an immunization before openness to contamination creates defensive resistance ahead of time and evades the serious results of the infection. Vaccination is specific to the disease: We need a specific vaccine for each disease because immune responses are very specific. The safe framework independently answers every microorganism it experiences. It can't be 'over-burden' by giving the full scope of right now accessible immunizations or by having numerous antigens in a single antibody. Hundreds of millions of lymphocytes can be produced by a healthy immune system, each focusing on a specific antigen. However, pathogens occasionally overcome the defense system. By teaching the immune system to recognize and recall the traits of a disease, vaccines offer important defense against aggressive infections.These insusceptible reactions are unmistakable, thus we really want to have a different immunization for every sickness. The invincible framework can answer autonomously to every microbe it experiences. Because of this, administering the entire range of vaccines or including several antigens in a single inoculation cannot "over-burden" damage the system (Burnet et al., 1959).



Features	Innate Immune System	Adative Immune System
Cells Involved	Dendritic Cells, Natural killer cells, Mast Cells , Macrophages , Basophils,etc	Killer CD8+ T-Cells, Helper CD4+ T-Cells, B-Cells , Antigen presenting cells
Origin	Ancient	With evolution of bony fishes
Protection	Local	Systemic
Memory	None/ Encoded in genome	Key features
specificity	None Specific	Specific
Primary Function	Control the spread of infection	Development of memory response
Receptors	Germline encoded	Encoded in gene segment
Effector	Cytokines	Antibodies
Response	Rapid	Delayed

Antibodies Work with the Safe Framework's Memorable Capacity Microorganisms

When the immune system detects a pathogen, individual cells proliferate quickly to create antibodies and cytokines to combat the infection. As a result, by producing more T and B cells that are precisely targeted against that infection, the body can combat it more successfully. Most immune cells that are involved in invulnerable reactions only survive for a few days, but a small percentage of lymphocytes survive for months or even years after the illness has been eradicated. These lymphocytes either continue to produce antibodies or maintain a "memory" of the invasive infection (Sallusto et al., 2010). The manner in which the safe framework recollects contaminations is perhaps of its most important resource. This memory implies that the insusceptible framework can mount a much quicker, bigger, and more supported reaction if it experiences a similar microorganism once more. That reaction can better control subsequent infections without producing the unintended and dangerous side effects linked to the illness itself (Ahmed et al., 1996).

Vaccines for Babies work with the Immune System of the Newborn

Before birth, the body's immune system begins to develop. A mother's antibodies shield her newborn from several dangerous illnesses during the early postpartum period, when the immune system is still developing. Typically, this protection lasts about four months (Adkins et al., 2004). The current infant immunization programs aim to strike a balance between the risk of an infection and waiting for the baby's resistant system to act in response to the vaccine.

Ongoing Transporter

People who host microorganisms for months or years after being exposed are known as constant carriers. For example, hepatitis B poses a significant risk to the kid, and exposure to the virus at birth or during the first few years of life can result in the infant being a lifelong persistent carrier of the virus. Hepatitis B vaccines thus start soon after birth. For some diseases, the circumstances are different because the immune system would not mount a defense against them at that age, or because the likelihood of infection during the first few months of life is reduced. For example, the vaccination against Haemophilus influenza type b (Hib) and Streptococcus pneumoniae is delayed until the newborn is between 6 and 8 weeks old, at which point the infant's immune system will be better able to respond. Since maternal antibodies against measles might interfere with vaccination reactions, the MMR (measles, mumps, and rubella) vaccine, which containsmeasles, is not administered until a kid is 12 months old (Mantadakis et al., 2010).

Pre-framed Antibodies give Prompt Security

In healthy individuals, it takes between seven and twenty-one days for an effective immune response to be produced after vaccination. Most vaccines function by stimulating the immune system to generate the antibodies, cytokines, and memory cells required to combat illness. But it takes seven to twenty-one days for this active immune response to fully emerge At times, on account of overpowering and hazardous contaminations, an unwell individual might get pre-framed antibodies as a component of their clinical treatment to forestall or battle the disease. These can be produced in a lab or from healthy blood donors, and they can aid the patient in overcoming the infection much more swiftly. We refer to this as "inactive inoculation." Nevertheless, these antibodies don't stick around in that mindset for very long; it's wiser to develop antibodies by getting vaccinated whenever you can (Walport et al., 2007).

How Immune Vaccines Trigger Responses

Antigens are components of proteins that are present in both infectious microbes and vaccines that are designed to combat them. These antigens stimulate B cells, immune system microbes, and macrophages, among other cells in the resistant framework. When proteins or other antigens are consumed by macrophages and broken down into antigen fragments, an immune response is initiated. A protein known as MHC (major histocompatibility complex) carries some of these fragments to the surface of the cell, where they are shown but stay enclosed in the cleft of the MHC molecule. When T cells identify these exposed antigen fragments, B cells respond by releasing antibodies against the fragments and triggering other immunological reactions. According to Berkower, research suggests that lymphocytes are only able to identify specific antigen segments from proteins that have been

predigested by macrophages; therefore, they are unable to distinguish between an antigen segment from an immunization and one from a contaminating organism (Chisari and Ferrari, 1995).

The different synthetic compounds known as cytokines that are released by activated resistant cells dictate the kind of antibodies that are generated. For example, B cells may release IgE antibodies in response to the cytokine interleukin 4, which may result in allergic responses. Different cytokines induce B cells to release IgG, which is mostly found in blood, or IgA, which is primarily found in bodily fluids, in a preferred manner. Different MHC particles bind to different fragments of the antigen; the MHC atom arrangement and the qualities that appropriate their creation shift broadly starting with one individual and then onto the next. Hence, although the two individuals' safe frameworks might answer similar protein in an immunization, their Lymphocytes might answer various segments of that protein. This variety cultivates contrasts in reactions to antibody antigens. There are no less than nine synthetically unmistakable types of immunoglobulins, and the equilibrium of the different sorts of cytokines that invigorate neutralizer emission decides the last reaction to an immunization. Thus, immunization reactions can vary between people because a similar immunization animates various people to create various measures of the different cytokines. Immunization responses may differ initially, but they may also diverge over time if they lead to a predominantly IgE response. An adversely sensitive response to subsequent inoculations with comparable antigens might be triggered by this reaction (IOM, 1994).

How Various Kinds of Antibodies Invigorate the Invulnerable Framework?

1. Vaccines with Live Attenuation:

Live attenuated immunizations against human viral sicknesses have been among the best confidence mediations in clinical history. These vaccines contain weakened strains of the pathogen that are still capable of replication but do not cause disease. Live attenuated vaccines typically elicit strong and constant immune responses and closely resemble natural infection (PD Minor, 2015).

2. Inactivated Antibodies

Inactivated immunizations have been utilized for north of 100 years to instigate assurance against viral microorganisms. This laid-out approach of immunization creation is generally direct to accomplish and there is an increased well-being profile when contrasted with their live partners. Today, there are six viral microorganisms for which authorized inactivated antibodies are accessible, and a lot more are being developed. Inactivated immunizations contain killed adaptations of the microorganism or explicit microbe parts. These immunizations can't be repeat and accordingly don't cause illness. When compared to live attenuated vaccines, inactivated vaccines typically produce a weaker immune response and may necessitate booster doses (Sanders et al*.,* 2015).

3. Subunit Inoculation

Cleansed antigens or antigenic components of the microorganism are contained in subunit antibodies. These inoculations are frequently safer than live incapacitated or inactivated antibodies since they don't contain whole microbes yet contain a couple of parts, similar to polysaccharides or proteins from microorganisms or contaminations. However,to ensure their aptness, subunit antibodies may require adjutants—substances that enhance the resistant reaction (Wang, 2020).

4. Viral Vector Antibodies

Viral vector-based immunizations utilize an innocuous infection to carry the directions for making antigens from the sickness-causing infection into cells, setting off defensive insusceptibility against it. A resistant reaction is ignited when the host cells create the objective microorganism's antigens. Viral vector vaccinations can drive both humoral and cell-mediated immune responses (Travieso, 2022).

5. Vaccines using Nucleic Acids

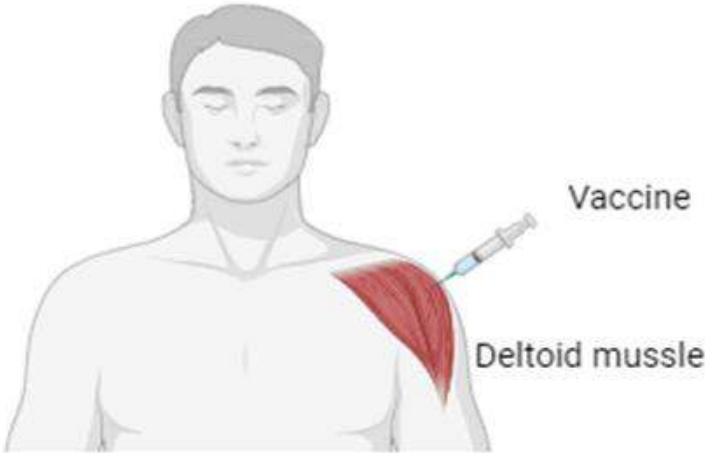
Using genetic material from a disease-causing microbe, nucleic acid vaccines trigger a protective immune response against the specific target. . Depending on the vaccination, the genetic material may consist of either DNA or RNA. Either way, it instructs the immune system on how to produce an antigen—a specific protein from the infection that the body will detect as alien. Once incorporated into the cells, this genetic material is seen by the machinery of the cell that produces proteins and is utilized to create antigens, which subsequently set off an immune response. Once embedded intocells, this hereditary material is perused by the cell's protein-production hardware and used to make antigens, which then, at that point, trigger an insusceptible reaction. Since DNA and RNA technology is still relatively new, vaccines against a variety of illnesses, including COVID-19, the Zika virus, and HIV, have not yet been licensed for use in humans. One of the numerous DNA vaccines approved for use in animals is the one that protects horses from the West Nile virus (Maruggi et al*.,* 2019).

6. Vaccines against Toxins

When they attack the body, some bacteria release toxins—harmful proteins. Similar to how antigens on the surface of bacteria are recognized by the immune system, these toxins are also recognized. These toxins are used to make inactivated versions of some vaccines. Despite not being poisonous, they are referred to as "toxoid" because they resemble toxins.

Pathogen immunization is exceptionally great at forestalling specific poison intervened infections like lockjaw, diphtheria and pertussis (P. Angsantikul, 2018).

Fig. 2: Vaccine administration



Vaccine should be administrated through the appropriate route.

Infections Preventable by Antibodies

Diseases caused by irresistible agents, such as microorganisms and infections, that can be effectively prevented through vaccination are known as antibody-preventable diseases. The incidence, morbidity, and mortality resulting from these diseases have all been significantly reduced worldwide thanks to vaccination programs. The motivation behind this report is to give a far-reaching assessment of different immunization-preventable illnesses, their study of disease transmission, the viability of immunizations, and the impacts of inoculation crusades (WHO, 2012).

Measles

Measles is a highly contagious viral disease characterized by a characteristic rash, hack, and runny nose. Measles complications include pneumonia, encephalitis, and death, particularly in young children and those with compromised immune systems. The measles vaccine which is usually given as part of the MMR vaccination schedule. Due to vaccination, the prevalence of measles has significantly decreased worldwide. However, outbreaks persist in areas with low vaccination coverage, highlighting the significance of maintaining high vaccination rates (Clements et al., 1989).

Polio

The disease, which mostly affects the nervous system and can cause paralysis, is caused by the poliovirus. The introduction of the polio vaccination, particularly the oral polio counter acting agent (OPV) and the inactivated polio inoculation (IPV), has provoked basic abatements in polio cases all over the planet. Overall undertakings zeroed in on polio obliteration have made huge progress, with two or three countries really uncovering endemic transmission. In any case, troubles, such as immunizer hesitance, battle zones, and determined blocks, continue to introduce obstacles to achieving obliteration Grady et al., 2023).

Hepatitis

Hepatitis is an inflammation of the liver that can be brought on by several different viruses, including hepatitis A, B, C, D, and E. Immunizations against hepatitis A and B are widely available and have proven effective in reducing disease burden. Particularly, vaccination against hepatitis B has prevented chronic hepatitis B infection and significantly decreased the incidence of liver cancer. It is essential to increase access to hepatitis vaccines and implement vaccination strategies in high-risk populations to reduce the global burden of viral hepatitis further (Pattyn et al., 2021).

Influenza

Influenza, regularly known as occasional flu, is a respiratory disorder caused by influenza contaminations. While periodic influenza inoculations are open and recommended for explicit masses, including little children, elderly individuals, pregnant women, and individuals with essential illnesses, the feasibility of these antibodies can move depending upon factors like antigenic match and people's invulnerability. Persistent assessment highlighted further creating vaccination suitability and making far and wide influenza antibodies remains needed in influenza contravention and control tries (Bridges et al., 2000).

Other Diseases that can be Prevented with Vaccination

In addition to measles, polio, hepatitis, and flu, a few other infections can be avoided with vaccination. These are some:

1. Tetanus

The bacterial infection known as tetanus, which causes muscle spasms and stiffness, can be avoided by getting the tetanus toxoid vaccine (Randi BA et al., 2019).

2. Diphtheria

The bacterium Corynebacteriumdiphtheriae is responsible for diphtheria. Fever, an irritated throat, and the improvement of a thick, dim film in the throat and nose portray it. Forestalling diphtheria requires inoculation with the diphtheria pathogen containing immunization (Randi BA et al., 2019).

3. Pertussis, also known as Whooping Cough

The bacterium Bordetella pertussis is the reason for the profoundly infectious respiratory illness known as pertussis. The pertussis vaccination, which is usually given as part of the diphtheria, tetanus, and pertussis combination vaccine, is necessary to prevent the illness and its sequelae, especially in newborns and young children (AM Laurie, 2022).

3. Mumps

The salivary organ enlarging, fever, cerebral pain, and muscle throbs are side effects of the viral disease known as mumps. The MMR immune response gives protection against mumps, close by measles and rubella (Davis and Morris, 2023).

Rubella (German measles)

A mild illness with a fever and rash is caused by rubella, a virus. Congenital rubella syndrome, on the other hand, can occur from a rubella infection during pregnancy and can lead to significant birth abnormalities (Leung et al., 2019).

General Advantages of Immunization

Immunization, also known as vaccination, is one of the best general health strategies for preventing infectious diseases and their sequelae. By vaccinating against certain bacteria, people can become immune to potentially harmful diseases. In addition to improving one's health, vaccinations can also reduce the risk of disease, save lives, and accelerate economic growth—all of which impact individuals and networks worldwide (A Nandi et al., 2020). For instance, a successful vaccination campaign led to the global elimination of smallpox in 1980.Vaccination gives security to feeble masses, including children, little children, the old, and individuals with weakened immune systems. Vaccinating these social occasions keeps serious traps and mortality from overwhelming sicknesses, which are ordinarily more outrageous in those with compromised safe capacity ((Dabek et al., 2022).

Immunization is a monetarily wise general prosperity mediation. Diminished medical care costs related to treating immunization-preventable infections, fewer hospitalizations and clinical visits, expanded efficiency because of diminished disease related non-appearance, and reserve funds on long haul inability and restoration costs are among the financial benefits of inoculation (Utami et al; 2022). Vaccination enhances the quality of life and overall health outcomes. Vaccination is important to overall health and has many benefits, from individual insurance to global health security. Inoculation fundamentally affects well-being results and cultural prosperity by forestalling irresistible infections, diminishing grimness and mortality, safeguarding feeble populaces, and advancing monetary thriving (Doherty et al., 2016). Pushes in neutralizer advancement, for instance, mRNA vaccinations, offer promising entryways for accelerating inoculation progression courses of occasions, further creating vaccination feasibility, and working on safe responses against compelling ailments. New immunization stages, antigen plan methodologies, adjuvant definitions, and conveyance frameworks that can evoke strong and enduring resistant reactions across assorted populaces require interest in Research and development (Pardi et al., 2018). In addition, efforts to combat immunization patriotism, advance immunization sharing drives, and support for global antibody acquisition components like COVAX are essential for ensuring antibodies are distributed fairly globally (Gavi et al., 2020).

Antagonistic responses of Immunization

Inoculation is associated with some risk of response, but antagonistic effects are typically extremely rare and extremely mild. Antagonistic vaccination responses are redness and irritation around the immunization site, which are the most widely recognized responses to antibodies. Certain antibodies may cause antagonistic responses that are more severe, such as vomiting, a high fever, convulsions, brain damage, or death. Antibodies have been linked to a variety of adverse medical conditions, including mental imbalance, communication issues, and explosive gut illness. Thimerosal, a mercury-containing chemical added to vaccines, was the subject of several of those instances. Some people supposed that the specific cause of autism—a form of mercury poisoning—was thimerosal, a component of vaccines given to children. Those cases have been trashed. People's perceptions of antibody security were significantly impacted by the deception and dread created by false cases regarding the connection between mental imbalance and vaccinations. Similarly, most people in countries with widespread immunization have never experienced an antibody-preventable illness.

Consequently, rather than the negative effects of diseases that could have been prevented with vaccination, the potential negative effects of the vaccines themselves became the focus of some people's concern. In certain areas of the

planet, inclusion of immunizations diminished because of carelessness regarding sicknesses that can be forestalled with inoculation and concerns regarding the impacts of immunization. Not only were people more likely to get diseases that could have been prevented with vaccines, but vaccination rates fell low enough to break herd immunity, making it easier for diseases to spread. Social orders caused tremendous expenses because of such flare-ups, especially with regards to incapacity, financial strain, and passing. For instance, in the 20th century, whooping cough outbreaks occurred in Japan, England, and Russia, affecting thousands of children and resulting in hundreds of deaths due to a lack of vaccine coverage (Emma K. Brunson, 2024).

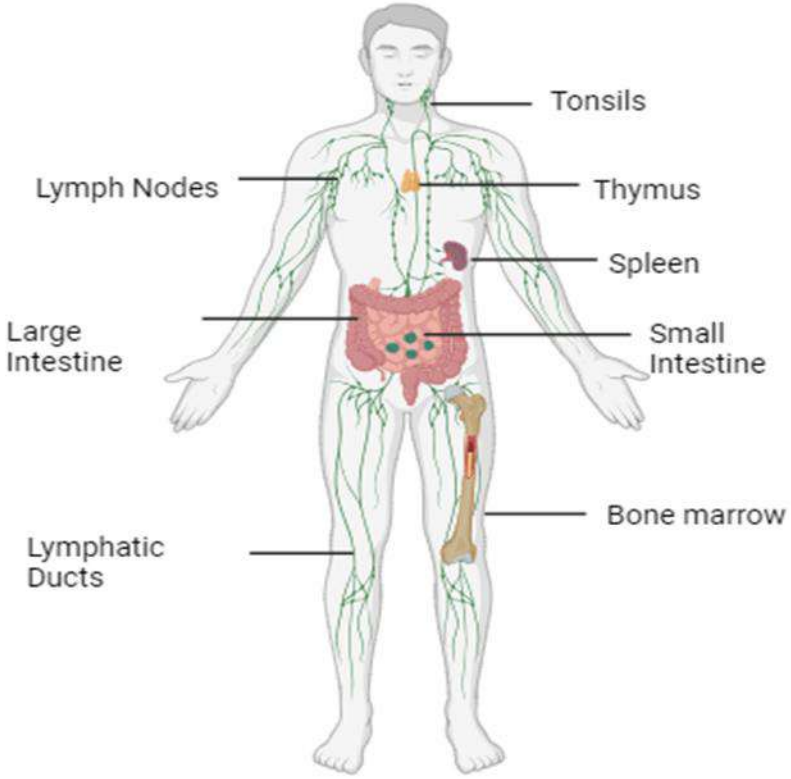


Fig. 3: Human Immune Sytem

Immune system is complex network of organs, cells and proteins that defend body against infection.

Conclusion

The section on immunization is a recognition of human creativity, persistence, and collaboration in the excellent story of general wellbeing. Vaccination emerged as a reassuring sign amidst the dimness, offering a proactive method for managing disease balance by harnessing the power of the safe system. The principal rule of immunization lies in taking action to protections against express living beings, as such presenting resistance and forestalling illness. Inoculations trigger the safe structure to make antibodies and memory cells by familiarizing the body with a harmless kind of microorganism or its parts. As well as killing unequivocal contaminations, inoculation has spread out one more time of insurance medicine and changed the overall prosperity scene. As a financially savvy and simple tokeep up with strategy for shielding populaces from various compelling trained professionals, routine inoculation programs have turned into the groundwork of irresistible counteraction frameworks. The worldwide viability and manageability of inoculation projects can also be upgraded by embracing future headings like propelling antibody improvement, advancing immunization value, helping immunization certainty, and using computerized vaccination arrangements. We can beat these challenges and plan for a superior and more grounded future through helpful undertakings including states, overall affiliations, clinical benefits providers, trained professionals, and organizations.

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Chapter 46

Vaccine Access and Equity; Overcoming Barriers to Immunization

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ABSTRACT

This chapter overviews vaccines development, socioeconomic barriers, vaccine hesitancy and misrepresentation, vaccine distribution, and efforts to overcome gaps in vaccine approach. Vaccination is the major public health success, providing a barrier against infectious diseases. The chapter traces the roots of vaccinology from Jenner's ground-breaking studies to the abolition of smallpox and the current efforts to fight the COVID-19 through vaccine improvement. It highlights the in human life probability and decreasing infant mortality rates. The conversation explores socioeconomic hurdles to vaccination, including revenue inequalities, linguistic and urban-rural disparities. Addressing vaccine hesitancy through public involvements and grassroots initiatives in undeserved inhabitants. Advancements in vaccine delivery, including Ambulances, community outreach, enhance access and handling with Semi-governmental and global collaboration essential for achieving vaccine equity and addressing vaccine distribution. Success stories represent vaccine equity efforts with cultural, racial, and socioeconomic flaws, but ongoing progress require consistent efforts to achieve goals like Immunization Agenda 2030.

KEYWORDS
Vaccine, Covid, Immunization, Cowpox Virus, Pathogens

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INTRODUCTION

A vaccine is defined as a biological product designed to stimulate the immune system to generate antigen-specific immunity against a pathogen, thereby preventing the disease it causes. Typically, vaccines are formulated from attenuated or inactivated versions of the pathogen, or derived components such as proteins and polysaccharides.

Historical Perspective: The Evolution of Vaccines

Just 200 years ago, Jenner's scientific investigations on the prevention of smallpox via cowpox virus inoculation gave rise to the fields of vaccination and immunology. The titans of the biological sciences in the late 19th and the beginning of 20th centuries significantly enlarged this modest foundation (Hilleman, 2003). It makes sense that during the years 1967 to 1977, smallpox became the first—and, to date, the only—communicable illness to be aggressively eliminated. One mystery that has not been answered since smallpox was eradicated is where the smallpox vaccine virus, or vaccinia, came from. Vaccinia, whatever its source, belongs to the Orthopoxvirus species and is genetically different from the viruses that cause cowpox and variola (Larson et al., 2014).

In 1900, children under five made up 34% of all fatalities in the United States, while the average life span at birth was around 47 years. Those who survived these illnesses experienced serious side effects and disabilities, including paralytic poliomyelitis, osteomyelitis variolosa, neurological problems, and visual impairments (Montero et al., 2024).

Presently, in the 21st century, more precisely in 2020, and in light of the worldwide health emergency we find

ourselves in because of the global coronavirus pandemic (COVID-19), research and preventative measures are being implemented as shown in Fig. 1. Their attention has been on the empirical quest for a vaccine to halt the pandemic's vast spread and the following alleviation and control of the disease in order to restore public health (Levine and Lagos, 2016).

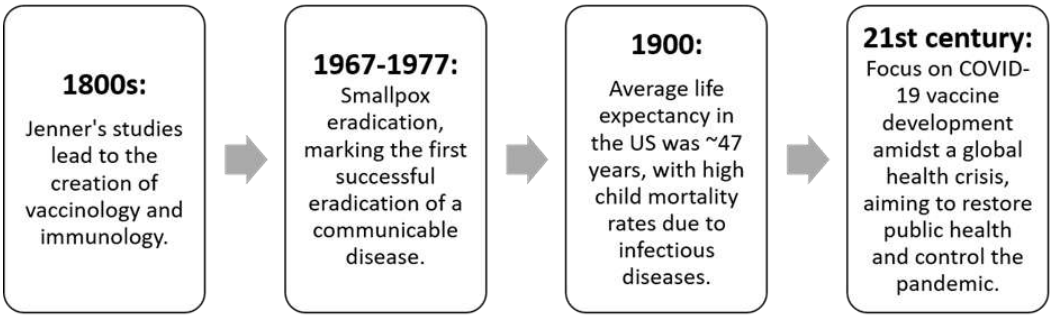

Fig. 1: From smallpox to COVID-19 the evolution of vaccines over two centuries.

Introduction

The groundbreaking findings of the middle of the nineteenth and late 19th centuries as well as the technological advancements of the last 60 years, form the foundation of the science of vaccination. The demand for novel vaccinations in the public health, technological viability, and financial incentives for turning basic research into a commercial are what propel vaccine development (Hilleman, 2000). Many people list vaccination as being one of the greatest contributions to public health. Today's vaccination discussions are getting more complicated as a result of the availability of additional vaccinations and vaccine combinations as well as the widespread, quick, and nonhierarchical growth of global communication channels. People are becoming more skeptical of vaccinations, looking for other vaccination schedules, and occasionally postponing or refusing immunization due to the rapid global exchange of general concerns and occasionally doubt around vaccines (Larson et al., 2014).

Vaccines have saved lives by substantially reducing and eliminating illness worldwide, in conjunction with the introduction of pure water and sanitary conditions. Since its inception in the 1800s, the field of vaccine development has made substantial progress. Numerous novel vaccines, vaccination classes, and strategies for achieving protection have been made possible by our growing understanding of the body's defense system and how invasive diseases activate it (Depelsenaire et al., 2017). Vaccines, together with clean water and hygienic settings, have significantly reduced and eliminated disease globally, saving millions of lives. The area of vaccine development has advanced a long way from its beginnings in the 1800s. Our increasing knowledge of the body's immune system and how invasive illnesses trigger them has led to the development of several new vaccines, immunization regimens, and protective tactics (Hobson-West, 2003).

The Food and Drug Administration oversees the Vaccine Adverse Event Reporting System (VAERS). VAERS is a silent monitoring system that depends on voluntary reporting of disease following vaccination from doctors and other professionals. Such reporting methods have a variety of well-documented shortcomings. These comprise, among other things, inconsistent report quality, skewed reporting, under-reporting, and complexity of establishing the causal relationship between vaccinations and the adverse event. Comprehending the Safety Data Provided by Vaccines (Varricchio et al., 2004).

Data indicates that the number of measles cases in the USA is increasing, and there have been over 30,000 cases in the European region in the recent years (Fig. 2). Approximately 20 million children worldwide are still not receiving enough vaccinations. Global public health is also at risk of newly developing infectious illnesses including Zika, Ebola, and malaria (Mao and Chao, 2020). Throughout history, vaccination has shown most successful strategies in reducing mortality and morbidity from infectious illnesses. Past centuries, vaccination has been most successful in controlling at least ten major illnesses, including rabies, measles, mumps, rubella, typhoid, yellow fever, pertussis, and influenza type B disease (Zepp, 2010).

Understanding Vaccines: A Brief Overview

- Several vaccination formulations include an adjuvant, which boosts the response of the adaptive immune system.
- When a vaccination is given, the immune system recognizes certain pathogen components (antigens) in the vaccine and mounts a particular defense.
- Immunological memory refers to the process by which the vaccination "trains" and primes the body's defenses to react to the pathogen efficiently upon exposure.
- As a result, if a vaccinated person is later exposed to the same infection, their immune system will be ready to mount a strong response that will either stop the disease from spreading or lessen its severity.
- Every vaccination is painstakingly created and put through a rigorous testing process to guarantee that it stimulates a certain immune response that is secure and protected. This highlights the complex interplay and balance between the immune system's dynamics and the vaccine's composition.

Socioeconomic Barriers to Immunization

Income Disparities and Access to Vaccines

The features of residential locations are linked to the differences in immunization service utilization between urban

and rural areas among teenagers with low incomes. Many federal and state initiatives are in place to alleviate the provider shortage, which policymakers and public health professionals widely regard as a significant obstacle to health services access in rural regions (Tsai et al., 2021). Since the start of the vaccination program, differences in income across racial and ethnic groupings have decreased significantly, yet in certain situations, they have grown. (Walsh et al., 2016).

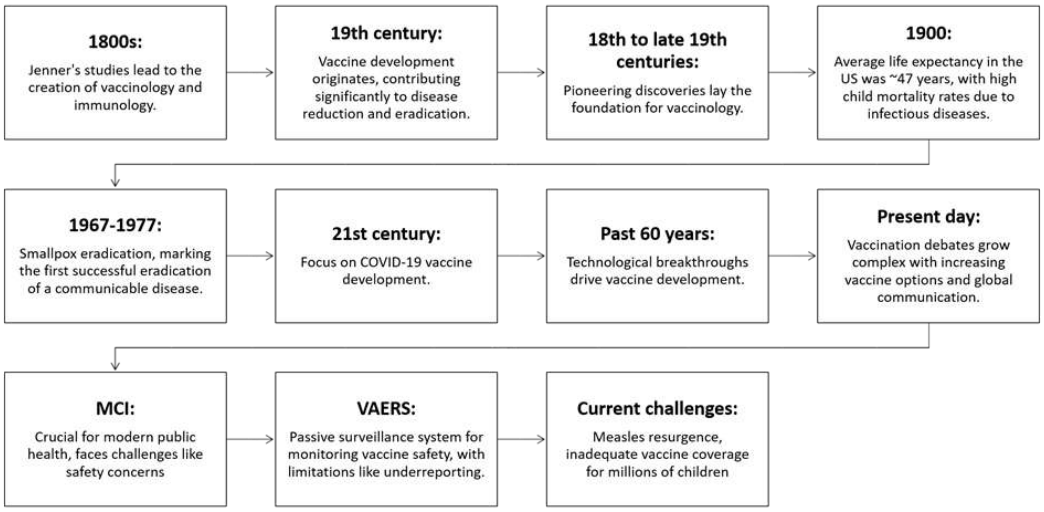

Fig. 2: A historical journey of past achievements and future challenges of vaccination

Language and Cultural Barriers in Vaccine Outreach

Due to challenges in accessing historically under-vaccinated subpopulations, regular vaccination coverage in numerous nations has halted after the Expanded Program on vaccination's early success. The achievement of overall immunization targets is threatened by these subpopulations' inadequate vaccination rates. For instance, despite years of progress, global efforts to eradicate measles and polio have revealed that certain populations have proven particularly challenging to immunize, which has led to avoidable child fatalities and disabilities, ongoing polio-endemic areas, and recurrent measles outbreaks (Ozawa et al., 2019). In order to estimate the number of target groups, share lessons gained on the basis of consistent definitions, and allocate resources accordingly, it is imperative to have a clear definition of "hard-to-reach" people, sometimes referred to as high-risk or marginalized communities, or reaching the final mile (Fig. 3) (Ozawa et al., 2019).

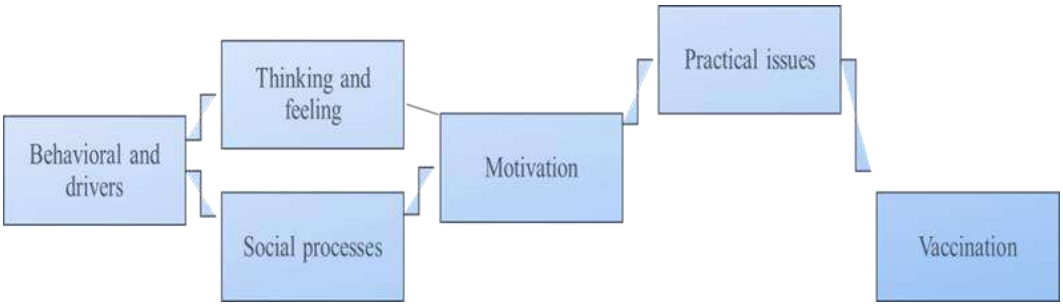

Fig. 3: Reaching the final mile by overcoming the barriers in vaccination coverage

Structural and Systemic Obstacles

Healthcare Infrastructure and Vaccine Delivery Systems

When introducing a new vaccine was an already-existing delivery system alongside another vaccine that was already part of the standard pediatric vaccination schedule, it was most effective (i.e., as a combo vaccine). The introduction of new vaccinations had no effect on the coverage of immunizations that were already part of the standard schedule. (Hyde et al., 2012). The shelf-life of vaccines associated with demand and supply chain (SC) parameters, are also considered in this study to ensure the robustness of the model. To solve the model, two recently developed metaheuristics namely, the multi-objective social engineering optimizer (MOSEO) and multi-objective feasibility enhanced particle swarm optimization (MOFEPSO) methods—are used, and their results are compared. Further, the Technique for Order Preference by Similarity to Ideal Solution (TOPSIS) model has been integrated into the optimization model to determine the best solution from a set of non-dominated solutions (NDSs) that prioritize environmental sustainability. The results are analyzed in the context of the Bangladeshi coronavirus disease (COVID-19) vaccine distribution systems (Chowdhury et al., 2022).

Vaccine Supply Chain Challenges

Ensuring universal access to critical medicines and safe, effective, high-quality, and reasonably priced vaccines, is a primary objective of the third Sustainable Development Goal (SDG) set forth by the United Nations. This goal is essential to attaining universal healthcare coverage, just as efficient supply chains for health products are necessary to guarantee that

people have access to high-quality medications and vaccinations (Olutuase et al., 2022). The COVID-19 pandemic has brought to light the various obstacles that supply networks must overcome to prevent major disruptions. Supply chains for vaccines are not an exception. To clear the path out of this pandemic, it is crucial that obstacles to the COVID-19 vaccine supply chain (VSC) be recognized and given priority (Alam et al., 2021). Millions of individuals receive preventative vaccinations every year, such as the yearly influenza shot, participation in children's immunization programs, or vaccinations against other infectious illnesses (Fig. 4). The goal of preventive vaccination is to stop a disease epidemic by immunizing against it before it manifests. Reactive vaccination, in addition to preventative immunization, can be administered in the case of a bioterrorism strike or during an infectious disease outbreak. Vaccination programs cannot be successful without sound logistics, even if vaccination demands medical intervention. The increasing amount of research on vaccination logistics shows how important it is (Duijzer et al., 2018).

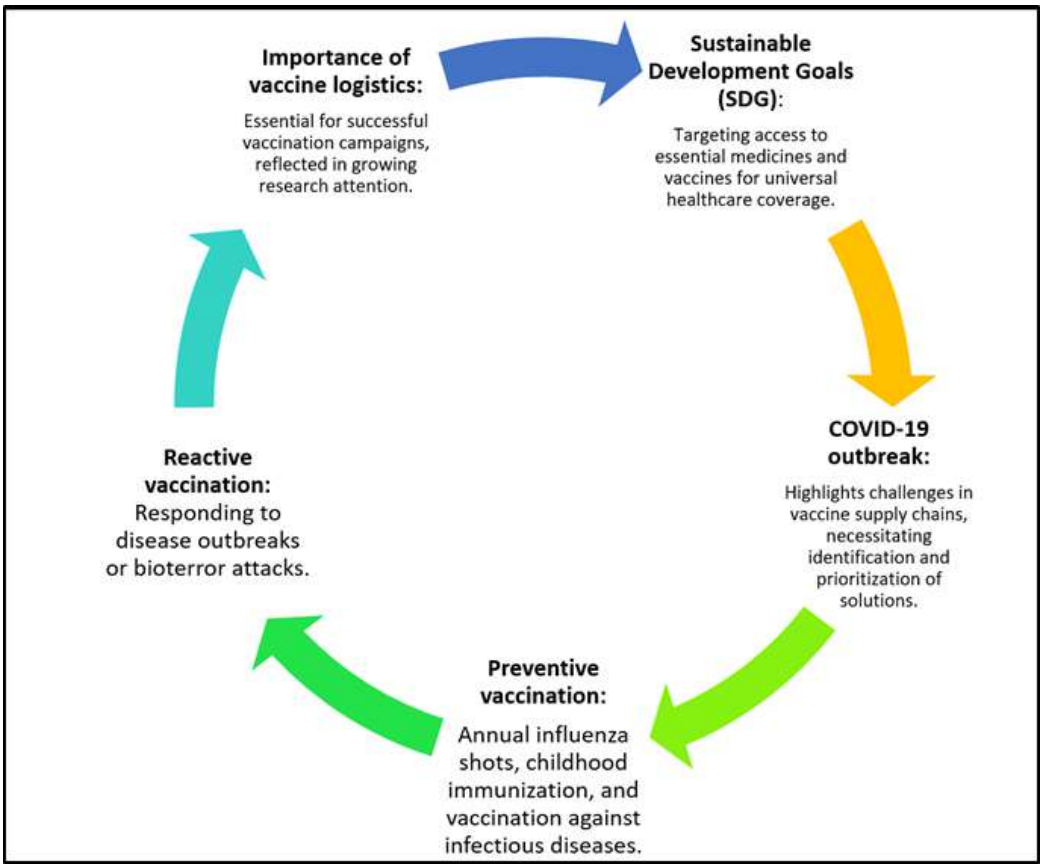

Fig. 4: Vaccine Revolution about Eradicating Diseases and for global health

Regulatory Hurdles and Policy Implications

The first Global Vaccine and Immunization Research Forum (GVIRF) was held in March 2014 and was organized by the World Health Organization, the Bill and Melinda Gates Foundation, and the National Institute of Allergy and Infectious Diseases, a division of the National Institutes of Health. The objectives of the first GVIRF were to monitor the advancement of the scientific and technological agenda of the International Vaccine Action Plan, pinpoint obstacles and possibilities, encourage collaborations in the field of vaccine research, and make it easier for all relevant parties to be involved in the process (Ford et al., 2016). We look at the vaccination policy as a force behind the creation and innovation of vaccines. Similar processes have led experts to recommend the use of approved vaccines in the US, UK, Canada, and Australia (Weintraub et al., 2021). These processes include programmatic feasibility, public demand, expert evaluation of disease the epidemiology, disease burden, and severity; vaccine antigenicity, efficacy, and safety; and increasing cost-effectiveness (Seib et al., 2017). However, neither biomedical researchers nor innovation scholars have given prophylactic immunizations the same level of attention as medications that cure disease. The COVID-19 epidemic serves as a stark reminder of the significant financial and human consequences associated with this carelessness (Xue and Ouellette, 2020).

Addressing Vaccine Hesitancy and Misinformation

Adult vaccine hesitancy is common, as the COVID-19 pandemic has shown, varying widely contexts in response to various circumstances, most notably vaccine safety signals. Interventions based on a "knowledge-deficit" strategy, such as information or instruction that is not adapted to address the values or judgments that support vaccine decision-making, may boost uptake but are unlikely to resolve hesitation, according to recent evaluations (Tuckerman et al., 2022). Vaccine-preventable infections in children may have returned despite global increases in vaccination rates because of the growth of non-medical vaccination exemptions. One of the primary causes of the declining vaccination rates is vaccine hesitancy,

which the WHO lists as one of the top ten worldwide threats to public health. Misinformation about vaccines can be found online in news sources, websites, and social media. Artificial intelligence (AI) facilitates its quick and widespread spread (Garett and Young, 2021).

Understanding Vaccine Hesitancy

20% of people in Eastern Europe and 17% of people in Western Europe, respectively, believe that vaccinations are not necessary for children (Fournet et al., 2018). In North America, a smaller proportion of people—13%—believe that vaccinations are not necessary for children. In South Asia and South America, the majority of people are in favor of vaccinations for children; only 2 and 3%, respectively, disagree that vaccines are not necessary for children (Vulpe, 2020). Hesitancy and confidence over vaccines have been identified by the World Health Organization as two of the most urgent problems facing world health (WHO, 2019). It could be more useful to define vaccine hesitancy as a collection of attitudes and beliefs related to vaccine decision-making, even though some have characterized it as a delay in accepting or refusing vaccinations despite the availability of vaccine services (Clark et al., 2022).

Debunking Myths and Countering Misinformation

The quantity of inaccurate data and misinformation around vaccines has grown in the last decade, highlights for the effective strategies to combat vaccine deception (Whitehead et al., 2023).

Debunking disinformation is a crucial scientific and public policy objective because it can cause people to make incorrect decisions regarding critical issues and is hard to eradicate (Alam et al., 2021). Elderly viewers of a daily news program on Dutch Television were the target audience for recruiting participants. During the National the influenza virus Vaccination Campaign in October 2020, 980 senior individuals participated in the study. We carried out a two-arm randomized masked parallel trial, drawing on recent behavioral science and psychology literature. Participants were assigned to view a movie that dispelled myths about vaccinations, provided facts about vaccines, and discussed social norms (Yousuf et al., 2021).

Promoting Vaccine Confidence through Education and Communication

Despite being the most reliable source of knowledge about vaccines, healthcare professionals (HCPs) have limited access to easily navigable, multidisciplinary vaccine communication educational resources. Virtual reality games (VSGs) are cutting-edge, user-friendly, and efficient teaching tools for the medical field (Doucette et al., 2024). In addition to scientific and economic data, a variety of psychological, sociological, and political factors influence public opinion regarding vaccine acceptability. Policymakers and other leaders must be aware of and consider these issues. The level of public faith in vaccines varies greatly, and fostering that trust requires an awareness of vaccine beliefs and risks, political or religious affiliations, and socioeconomic status (Larson et al., 2011). Over the research and strategies that are currently available for vaccine communication, such as the whole-team approach, developing trust, initiating the discussion early, using a presumed strategy to make vaccine suggestions, motivational interviewing parents who have vaccine-related concerns, and additional strategies for answering parent queries. We also go into organizational tactics such as evidence-based methods for boosting vaccination rates and effectiveness. Organizational strategies is to reassure parents who are hesitant about vaccinations, widely recommended vaccines are secure and efficient, their importance is likely to increase as coronavirus disease 2019 vaccine creation, administration, and assessment unfold (Mbaeyi et al., 2020).

Innovations in Vaccine Delivery

Vaccines are safe and US vaccine industry, which backs vaccination in all its forms, keeps pushing for innovation. The US is currently missing resources to fund creativity in vaccine delivery technologies, despite a few historical recommendations to establish a pay for promoting investments in vaccine safety and recent legislation (the 21st Century Cures legislation, Public Law 114–255) that encourages innovation for new vaccines. (Thompson et al., 2020). Innovations in disease prevention and extinction initiatives are driven by advancements toward quantifiable goals, assessments of novel approaches and techniques, programmatic experiences, and field-tested lessons. (Goodson and Rota, 2022). The creative approaches employed by business and academia to get above the obstacles. They include of additives, mucoadhesive, granular delivery methods, virus-like particles, live-attenuated vaccines, vaccine manufacturing, difficulties faced by regulatory bodies, and the potential market effect of nasal vaccines (Jabbal-Gill, 2010).

Mobile Clinics and Outreach Programs

Mobile medical centers are a vital for providing healthcare to underprivileged populations in the US. Pediatricians have long employed mobile clinics for basic medical services, but there isn't much information available to support them in expanding this approach. Reduced in-person physician visits throughout the COVID-19 pandemic and the ensuing drops in routine kid vaccination rates demonstrated how crucial it is to use a range of care delivery methods to reach patients (Leibowitz et al., 2021). Ensuring universal access to vaccination was one of the Global Vaccine Action Plan's (GVAP) main goals from 2011 to 2020.4 This strategy takes into account populations that are more vulnerable to vaccine-preventable diseases, including the elderly, pregnant women, and people with long-term illnesses that compromise their immune

systems (Giles et al., 2018).

Community Engagement and Grassroots Initiatives

Research from around the world and in India has shown how crucial community involvement is to meeting vaccination targets and advancing health equity at the national level. Community engagement, however, is still a little-used strategy and lacks clarity. Additionally, there is a dearth of research on the benefits of community involvement in obtaining vaccine outcomes (Dutta et al., 2020). Previous investigations have suggested the use of community engagement (CE) approaches as a means of enhancing public trust in health services, including vaccinations. This study assesses the suitability of community engagement (CE) initiatives, including the community health worker model, in mitigating vaccination hesitancy (VH) by utilizing trusted relationships (Fig. 5). The study examines a methodology that addresses health disparities among Ireland's Traveler population as a case study of community involvement strategies in an ethnic minority community (Buggle, 2021).

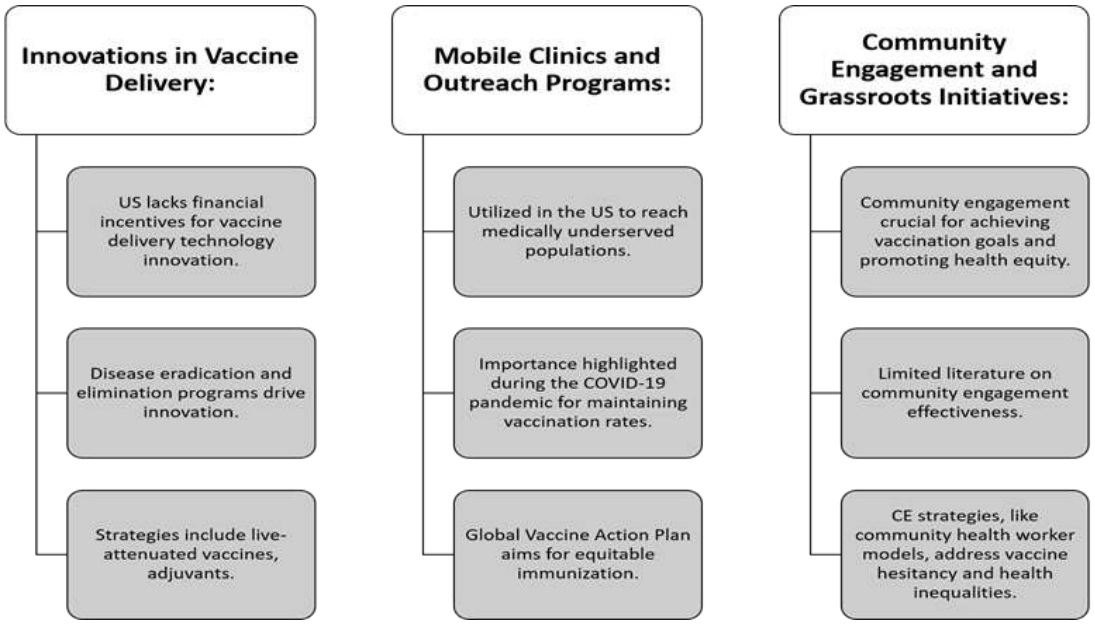

Fig. 5: Empowering vaccination and innovations in vaccine delivery strategies

Leveraging Technology for Vaccine Access

As the COVID-19 pandemic has shown, the mRNA vaccine technology platform may allow for a quick response to several emerging infectious diseases (EIDs). In addition to its potential contribution to future EID responses, mRNA technology could have a significant role in speeding the creation and availability of vaccines for certain neglected tropical diseases (NTDs), which are primarily found in underdeveloped parts of the world (Sparrow et al., 2022). US Healthcare information technology (IT) initiatives can help identify children who need vaccinations quickly or in real time. They can also lay the groundwork for flexible and customized vaccine-oriented parental communication or clinician warnings (Stockwell and Fiks, 2013).

Public-Private Partnerships in Vaccine Distribution

South Africa followed suit, embracing public-private partnerships (PPPs), which are frequently employed as a way to mutually benefit from the resources, know-how, and abilities of the private sector, in order to enhance public sector delivery. One such collaboration that has been examined in this study is the Biovac Institute, which was founded in 2003 to address vaccine supply, production, and research and development (Walwyn and Nkolele, 2018). Partnerships between government and private sectors are considered as presenting an innovative approach with a good probability of generating the desired outcomes when the market fails to provide health benefits to individuals who need them (Reich, 2002).

International Cooperation and Global Vaccine Equity Initiatives

The COVID-19 Vaccines International Access Facility (COVAX) pledged early in the pandemic to provide equal access to vaccination supplies for every nation. Nevertheless, COVAX has struggled and will not even reach fifty percent of its 2021 delivery objective of 2 billion doses because of a lack of funding and contributions (Bajaj et al., 2022). The COVID-19 pandemic's early vaccination supply shortage prevented the world from meeting demand. Numerous affluent nations went home, obtaining vaccine doses for their own people through exclusive bilateral agreements (a practice known as "vaccine nationalism"), while manufacturing nations like India instituted temporary export limitations. 2, 3 the global vaccination disparity that is still noticeable today was sparked by these occurrences. Worldwide vaccine production has increased significantly with an expected 24 billion doses produced

by mid-2022 (Dzau et al., 2022).

Multisectoral Strategies for Sustainable Immunization Programs

Immunization contributes to 14 of the 17 Sustainable Development Goals (SDGs), including eradicating poverty, hunger, and inequality, and has a direct influence on health (SDG 3). Consequently, it is recognized that vaccination is crucial to achieving the SDGs, particularly in low- and middle-income countries (LMICs) (Decouttere et al., 2021). Within the framework of overall health funding, sustainable financing for vaccination refers to the sufficient and regular administration and usage of resources to support the fulfillment of immunization objectives. Four key focus areas are outlined in the Immunization Agenda 2030 (IA2030) agenda that are necessary for sustainable financing: ensuring resources are sufficient and predictable, increasing resource utilization, aligning partnerships, and supporting sustainable transitions from external assistance (Saxenian et al., 2022). The objective of the IA2030 is to demonstrate how universal health systems provide extensive immunization programs and resilience for future. IA2030 reflects a core belief in human capacity to cooperate, respect, and shared knowledge. By working together, we can ensure incredible benefits of modern vaccination (O'Brien et al., 2022).

Overcoming Equity Gaps

As one of the finest health expenditures, vaccinations should continue to be prioritized by society, business, public health, and research. The three breakthroughs that the Vaccine Innovation Prioritization Strategy (VIPS) designated as priorities for 2020 were barcodes on main packaging, heat-stable and controlled-temperature chain (CTC) allowed liquid vaccine formulations, and microarray patches (MAPs) (Bajaj et al., 2022). These innovations were ranked in order of importance according to the vaccination hurdles that they may help remove in situations when resources are limited. They were also ranked according to their technical readiness, commercial viability, potential effects on health, coverage and equity, safety, and the economy (Chopra et al., 2012).

Examples of Effective Vaccine Equity Programs

Racial or ethnic disparities were found in adult vaccination coverage for seven crucial vaccines, such as tetanus/pertussis/diphtheria, influenza, pneumococcal, hepatitis A, hepatitis B, herpes zoster (shingles), human papillomavirus (HPV), and hepatitis B (Rosadas et al., 2023). These estimates of vaccine coverage for 2014 were conducted. For example, 43% of adults over the age of 18 who had the influenza vaccination in 2013–14 was covered, with white people having higher coverage (47%) than black people (37%), Hispanic people (33%) and people who indicated that they were of a different race (39%). In order to avoid shingles, 28% of persons over 60 reported having had a herpes zoster vaccination in 2014. Once more, whites had higher immunization rates (32%) compared with blacks (11%), Hispanics (15%), Asians (17%), and people claiming to be of a different race (16%). (Prins and others, 2017). Disparities in childhood vaccination rates endure despite decades of efforts to rectify inequalities. The routine childhood vaccination coverage has been improved by federal interventions like the Vaccine for Children (VFC) program and the Affordable Care Act, which were implemented in 1994 and 2010, respectively. Improvements have not been consistent for all races and ethnic groups. Compared to white newborns, black and American Indian/Alaska Native neonates had a lower likelihood of receiving all recommended vaccinations in 2017—by 7% and 10%, respectively. In 2016, infants in poverty households were 30% less likely to receive all recommended vaccinations (Brumbaugh et al., 2024).

Conclusion

It is unblemished that gaining general vaccine appearance and justness is a significant primacy when we deliberate the improvement of vaccines and the frequent hindrances that aspect immunization labors worldwide. All revelries concerned in public health must be resolute in their commitment and make regular exertions in order to attain this goal. Barriers to vaccine access are plentiful and complex, fluctuating from earnings difference to philological and folk hurdles, structural impairments, and vaccine hesitency. They are not undefeatable, yet. We can overtake and make definite that everyone through globe has admittance to life-saving vaccinations by functioning unruffled and uplifting corresponding deed.

It is authoritative that decision-makers and fascinated festivities pay courtesy to this demand and provide vaccination equity, the chief urgency. This means making funds in resilient vaccine distribution and healthcare setup, growing natives, and employing expertise to inflate vaccine availability and distribution. Additionally, undertaking vaccination indecision and eradicating incorrect evidence entails rising vaccine self-assurance via tutoring, debate, and open information sharing. Over enabling people with data and facts, we may boost global immunization initiatives and build confidence in vaccines. In order to pledge a collective approach to vaccines and undertake the purposes indicated in series such as the Immunization Agenda 2030, it will be compulsory to uphold to recover overall thorough association going forward. By addressing the breaks in socioeconomic status, refining the circulation of vaccines, and promising vaccination.

Public-private enterprises are indispensable to this activity because they excite inventiveness, reserve utilization, and obliging struggles to escalation vaccine handling and access. Moreover, in order to discourse universal vaccine inconsistencies and assure the rational endowment of vaccines to low-income nations and relegated residents, worldwide commonality and assistance are important. We persist strongly in our keenness to equality and inclusivity as we assign the encounters associated with vaccine delivery and entree. Every creature has to obtain life-saving vaccinations, irrespective of their economical state of affairs, race, or place of residency. Together, we can achieve this aim and a figure out a more

durable, healthy world for upcoming compeers.

Let us, uphold our obligation to certify that everyone is talented to and is pickled appropriately while directing vaccines. We distinguish that chasing health and evenhandedness is not only morally mandatory but also needed to attain defensible growth and build a society with superior justness and righteousness. Let's keep assertive for immunization together to make sure no one remnants behind in the search of an innocuous and flourishing world.

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Chapter 47

Role of Cell Adhesion Molecules in Extravasation of Lymphocytes in Immunized Animals

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ABSTRACT

The clinical applications of lymphocyte exudation into novel drugs and disease management are increasingly becoming apparent. This review highlights the key role of cell adhesion molecules, such as selectins, integrins, immunoglobulin superfamily (IgSF) molecules and mucins, in controlling lymphocyte migration both during immune surveillance and in inflammation. Additionally, the regulatory mechanisms which govern lymphocyte adhesion, transmigration, and activation in diverse tissue microenvironments are discussed. A few words about what can be done to achieve the therapeutic potential of targeting adhesion molecules and chemokine receptors, as well as the possibilities afforded by intravital microscopy, genomics, and computational modeling, wrap up the last part. The dynamics of lymphocyte traffic may offer a new avenue to develop novel therapies against immune-related diseases, by which health care can be promoted in turn.

KEYWORDS
Cell adhesion molecules, Extravasation, Lymphocytes, Immunized Animals, Immune response

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INTRODUCTION

CAMs belong to four main families called selectins, integrins, immunoglobulin superfamily (IgSF) molecules and mucins (Wang and Springer, 1998). Each family has its own specific structural and functional characteristics, which play an irreplaceable role in extra storage. Selectins, to cite another example, help lymphocytes to initially cohere to and roll along the endothelial surface, thus triggering adhesion processes. Integrins, however, bring the definitive linking-up of lymphocyte adhesion and transmigration with the endothelium by acting on cell adhesion molecules such as ICAM-1 (Intercellular Adhesion Molecule 1) and VCAM-1 (Vascular Cell Adhesion Molecule 1) (Ma et al., 2004).

Moreover, experimental evidence has confirmed that CAM plays important roles in processes of cell immunity and inflammation**.** For example, the pro-inflammatory cytokines such as TNF-alpha and IL-1β can up regulate the expression of CAMs on endothelial cells, which promotes lymphocyte extravasation into inflamed tissues (Larochelle et al., 2011). Similarly, following immunization patterns of CAM expression change and lymphocyte trafficking in immunized animals differs.

An understanding of CAMs Cell Adhesion Molecules that can appreciate such subtleties is essential to fully delve into the intricacies of lymphocyte emigration following immunization of animals. By explaining how different CAM family's function and are regulated.

The Role of Selectins in Lymphocyte Extravasation

Selectins represent a key family of cell adhesion molecules involved in initiating the lymphocytes extravasation event cascade. It is composed of three members--the initial tethering and rolling lymphocytes L-Selectin mediate the selectins E-Selectin, P- Selectin and along endothelial surface. Thus adhesion is temporarily increasing their transmigration into tissues (González-Amaro and Sanchez-Madrid, 1999).

The expression of E-selectin is mainly related to result in the recruitment of lymphocytes by inflammatory cytokines such as TNF-alpha and IL-1β. Once stimulated, E-selectin binds to glycoproteins bearing specific carbohydrate ligands on circulating lymphocytes, there by initiating the process of rolling and tethering. This transient interaction allows lymphocytes to scan the endothelial surface in search of chemokine cues, thus guiding their migration towards inflamed tissues (Fig. 1).

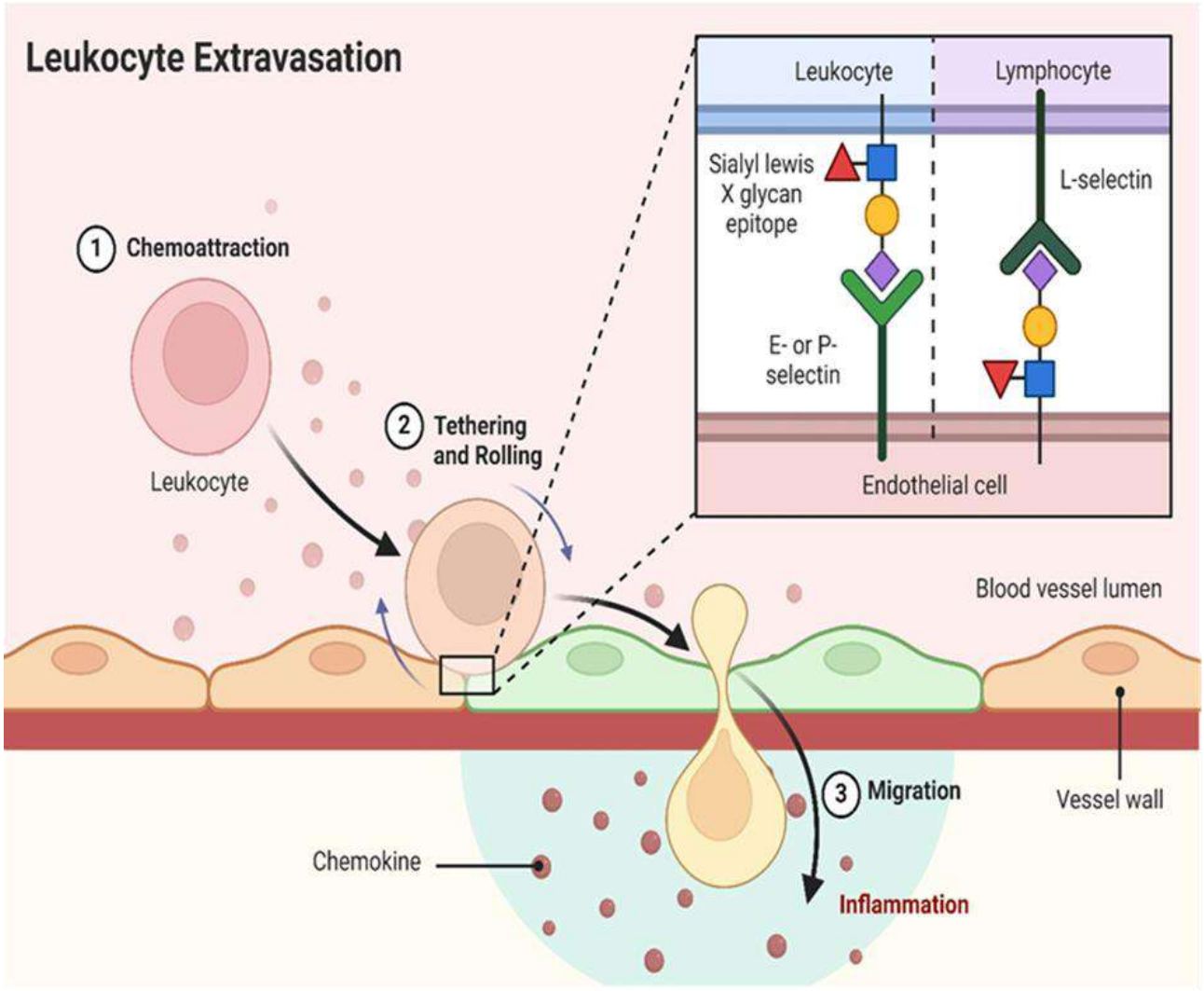

Fig. 1: Role of Selectins in Lymphocyte Extravasation

P-selectin, which is stored both in endothelial cell Weibel-Palade bodies and thrombocyte alpha granules, can be rapidly brought to the cell surface when endothelial cells are triggered P-selectin helps promote platelets to clump and both circulating lymphocytes at sites of vascular injury or inflammation (Bevilacqua, 1993).· When P-selectin interacts with P-selectin glycoprotein ligand-1 (PSGL-1) present on lymphocytes, it carries out the initial sticking and sliding activities of drainage Passage, promoting the subsequent firm adhesion of lymphocytes to endothelial cells (Bevilacqua, 1993).

L-selectin, expressed on most leukocytes including lymphocytes, works as a homing receptor to help guide lymphocytes into lymphoid organs such as lymph nodes (Picker et al., 1993). L-selectin directly binds to peripheral node addressing (PNAd) molecules on high endothelial venules (HEVs) within lymphoid tissues. In doing so it can guide lymphocytes to resting sites within a lymph node or spleen as well (Warnock et al., 1998).

Initial steps of lymphocyte trafficking during extravasation are orchestrated by the dynamic interplay between selectins and their ligands. As lymphocytes are made to stick to and move slowly along the endothelial layer, this not only creates an environment in which their later linkage with integrins or other CAMs can take place to send them transmigrate lymphocytes into inflamed or lymphoid tissues. Rather than repeating itself, it explains the very different roles played by E-selectin, P-selectin, and L-selectin in mediating the extravasation of lymphocytes. Each of these substances is clearly essential to immune surveillance and inflammatory response (Vestweber and Blanks, 1999).

Integrins: Bridging the Gap in Lymphocyte Extravasation

Emerging evidence also suggests that activated integrins, clustering at the sites of focal adhesion to the endothelial cytoskeleton, can transmit outside-in signals which intemperate lymphocyte migration. The human body contains 24 related integrin (α and β), as well as two different types of chains for ligand-binding domains: I and Id. Each of these permutations, or heterodimers, gives lymphocytes unique adhesion properties. Upon activation by chemokines, cytokines or other stimuli, integrins undergo conformational changes that make them bind with higher affinity to endothelial cell adhesion molecules such as ICAM-1 (Intercellular Adhesion Molecule 1) and VCAM-1

(Vascular Cell Adhesion Molecule 1) (Albelda et al., 1994). This activation-dependent binding enables integrins to strongly adhere to endothelial cells, thus stabilizing the bond between lymphocytes and the endothelium (González-Amaro and Sanchez-Madrid, 1999).

All of the integrins involved in lymphocyte extravasation, α4β1 (VLA-4) are not only prominent contributors to lymphocytes ' adhesion and transmigration, but also highly complementary partners. α4β1, found on some lymphocyte's surfaces, helps to form a bond with VCAM-1 excerpted off by activated endothelial cells. This ensures that lymphocytes stay firmly adherent to the surface of the endothelial cells. "At sites of inflammation, where exposure occurs to inflammatory stimuli for prolonged periods, these interactions are particularly important. And it is just here that lymphocytes find themselves on the streets and backgrounds of epithelium all stacked (Camerer et al., 2009) .The interplay between inflamed endothelial cells 'VCAM-1 and lymphocyte-a4b1integrin is particularly important.

Also known as LFA-1 (lymphocyte function-associated antigen 1), αLβ2 integrin interacts with ICAM-1 on the endothelium enabling lymphocytes to become more firmly tied up with endothelial cells through adhesion. In the transmigration of lymphocytes across vascular endothelia, LFA-1 plays an integral role. This process is involved in immune surveillance and inflammatory responses when activated lymphocytes come across tissues (Shimizu et al., 1992). Adhesion mediated by integrins also involves regulation. It is subject to the inside-out and outside-in signaling pathways which are described in more detail below. 'Chemokine receptors' and other cell-surface receptors trigger inside-out signaling -- this increases the affinity of integrins and makes them more active, hence promoting lymphocyte adherence to endothelial cells (Abram and Lowell, 2009). In contrast, transmissions traveling from the outside in (when integrins combine with ligands on the endothelium) trigger intracellular signaling cascades that regulate lymphocyte migration and activation within (Table 1) tissues indemnity (Imhof and Dunon, 1995).

Table 1: Integrins and their role in lymphocyte extravasation (Chen et al., 2007)

Integrins	Cell surface receptors that mediate cell-cell and cell-extracellular matrix interactions.
Function	Mediate adhesion and migration of lymphocytes across endothelial barriers during extravasation.
Ligands	Bind to various extracellular matrix proteins such as ICAMs and VCAMs.
Steps in Extravasation	1. Rolling adhesion 2. Tight adhesion 3. Trans endothelial migration (TEM)
Activation	Integrins undergo conformational changes upon activation, enabling them to bind ligands with high affinity.
Regulation	Integrin activation is regulated by chemokines, cytokines, and other signaling molecules.
Cell Types Involved	Lymphocytes (T cells, B cells) primarily, but also involve other leukocytes.
Pathological Implication	Dysregulation of integrin-mediated processes can lead to immune disorders and inflammatory diseases.
Therapeutic Targets	Integrins are potential targets for drug development in treating autoimmune diseases and cancer metastasis.

Immunoglobulin Superfamily Molecules: Orchestrators of Lymphocyte Trafficking

IgSF members are master players in the extravasation of lymphocytes from blood to tissue. Their biological function mainly lies in mediating the complicated interactions that occur between lymphocytes and endothelial cells (Guan, 2015). Since they boast immunoglobulin-like domains, IgSF molecules can intervene in a variety of ways in immune cell adhesion, migration and signaling, and so they are key molecules to include in the extravasation process.

ICAM-1 interacts with the integrin LFA-1 (αLβ2) on lymphocytes, leading to firm adhesion and transmigration across the endothelial barrier. This interaction is needed to bring lymphocytes into inflammation areas-where ICAM-1 expression picks up dramatically in response to pro-inflammatory cytokines. ICAM-2 One well-known IgSF molecule that is involved in lymphocyte trafficking is ICAM-1 (Intercellular Adhesion Molecule 1) (Vestweber, 2007). It is a transmembrane glycoprotein expressed on activated endothelial cells, intercellular adhesion molecule 2, is also the member of the ICAM family that aids in the leukocyte trafficking via lymphocyte-endothelial interactions stabilization. ICAM-2 is found continuously on the surface of the endothelial cells and binds to LFA 1 on lymphocytes helping their adhesion and thereby can easily migrate into the tissue (Walling and Kim, 2018).

The ICAM family is not the only one lurking in the extravasation but the rest of the IgSF molecules, such as MadCAM-1 and VCAM-1, are in the mix as well during extravasation at various sites in the tissue. VCAM-1, which is found on the endothelium that has been activated, binds to α4β1 (VLA-4) integrin on lymphocytes, and engages to it and stops lymphocytes from migrating to the inflamed tissues. MadCAM1 which is on the endothelial cells in the mucosal tissues facilitate the recruitment of lymphocytes to the mucosal site (Grant et al., 2001). It is a very important element of the immune surveillance and the mucosal immunity.

Moreover, IgSF molecules are also dynamically controlled in response to many diverse stimuli involving inflammation and also vaccination. Endothelial cells produce ICAM-1 and VCAM-1 in response to the pro-inflammatory cytokines like TNF-alpha and IL-1β and so attract more lymphocytes from the blood to the inflamed sites (Khodadust et al., 2022). This same regulation also holds to patterns of IgSF molecule expression in the case of animals are immunized. This regulation point confers greater control of the lymphocyte trafficking and also immune responses for the immunized animals (Butcher, 1986).

Mucins: Navigating Lymphocyte Trafficking in Mucosal Immunity

Given the intricate landscape of Lymphocyte Extravasation, mucins show as central players, especially in a context where mucosal immunology is concerned. The Mucins comprise a family of heavily glycosylated proteins (Cader and Kaser, 2013). They help to orchestrate move lymphocyte traffic within the mucosal tissues, thereby serving as vital agents for immune surveillance and response at mucosal surfaces. A well-known member of the mucin family important to lymphocyte mobility is MAdCAM-1 Expressed by high endothelial venules (HEVs) at locations within mucosal lymphoid tissues such as Peyer's patches. MAdCAM-1 helps recruit into the mucosa lymphocytes, particularly gut-homing ones. MAdCAM-1 binds to the lymphocyte receptor α4β7, thereby activating adhesion and migration of lymphocytes into the mucosa. This interaction is key for maintaining immune homeostasis in the gut and effectively surveying mucosal pathogens (Abraham and Medzhitov, 2011). Besides, on the epithelial cells that line the mucosa surface Mucin plays a crucial role in tracking lymphocytes and modulating immunity (Pelaseyed et al., 2014). The physical barrier formed by mucins prevents pathogens from gaining entry. This is also a basis for emigrating or even invading immune cells. Mucins with treatment of mucin-binding proteins / receptors can interrelate, stop altogether and leave lymphocytes inside mucous tissue. Then this cloaks both the battle cry from neighboring tissue or lymphoid organs nearby where monocytes fight on to defend against new arrivals (diseases). In addition, the immune microenvironment is manipulated locally in a way that lymphocytes "surround" themselves for further comfort there too (Barnes, 2005).

Furthermore, under varied conditions of action Its expression and glycosylation state Mucin can be subject to dynamic regulation. Among these conditions is found various stimulants including microbial pathogens or inflammatory mediators, which cause a change in mucus-producing cells (Bischoff et al., 2014). If pathogens alter the expression of mucins and their glycosylation, for instance, lymphocyte transport and immunity responses in the mucosae may be affected Relating to host-pathogen interactions (Basset et al., 2003) The body's response to irritants inhaled or ingested can lead to heightened mucin production and secretion, additionally impacting the composition and function of the (Figure 2) mucosal immune barrier (Celebi Sozener et al., 2022).

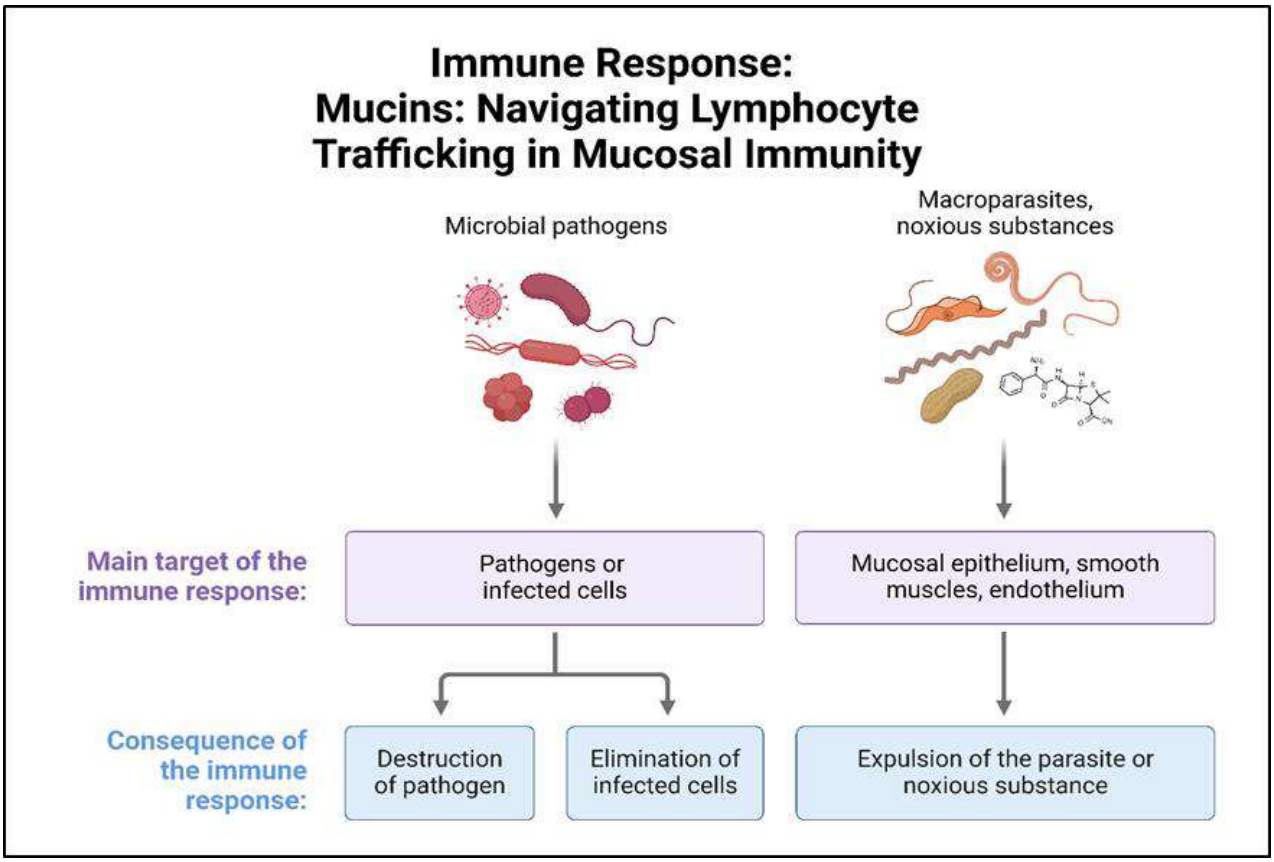

Fig. 2: Lymphocyte Trafficking in Mucosal Immunity extravasation

Chemokines: Guiding Lymphocyte Trafficking with Precision

Chemokines, a family of small signaling proteins, play a critical role in "commanding the episodes" of lymphocytes on immune surveillance and inflammation among other major oil paintings. The interplay between individual chemokines and those specific chemokine receptors that are upregulated on lymphocytes is what dictates whether lymphocytes from specific clusters will move together in the same direction or not (Bromley et al., 2008). These interactions regulate traffic flow at sites of inflammation, infection, tissue damage and other places where lymphocytes accumulate (Moser and Loetscher, 2001).

Chemokines help maintain an appropriate chemotactic gradient for moving lymphocytes. After they are produced by cells through inflammatory mediators or microbial products and the chemokines diffuse to form gradients along which cells will move toward areas of higher chemokine concentration Lymphocytes, each having receptors for chemokines that match the type on surrounding cells, will respond to these gradients of heightened chemokine by moving along them toward more chemokine (Bonecchi et al., 2009).

Inflammatory Mediators: Modulators of Lymphocyte Extravasation

Inflammation is the cornerstone of the immune response, orchestrating a constellation event underway to eliminating pathogens and promoting tissue repair (Patel and Minn, 2018). In the context of lymphocyte extravasation, inflammatory mediators do double duty they not only initiate the recruitment of immune cells to sites with inflammation, but also regulate adhesion properties for endothelial cells so that immune cell enters (Luster et al., 2005). Inflammation is regulated by pro-inflammatory cytokines, which are key mediators of lymphocyte trafficking in health and illness (Turner et al., 2014). Thus, after being activated through the administration of microbial products or other inflammatory factors, cells residing in tissues (e.g. macrophages, dendritic cells and endothelia cell) generate pro-inflammatory cytokines that act in concert to attract lymphocytes local areas of inflammation (Turner et al., 2014).

The upregulation of adhesion molecules then enhances the capacity to which lymphocytes adhere to endothelial cells during extravasation by pro-inflammatory cytokines (Meager, 1999). The expression of endothelial adhesion molecules like ICAM-1, VCAM-1, and E-selectin can be induced by TNF-alpha and IL-1β (Meager, 1999).

For Example, helping with tethering (where the cell first attaches to a sticky protein) as well as rolling firm adhesion between lymphocytes and endothelium (Carman and Martinelli, 2015).

And the responsiveness of endothelial cells to chemotactic signals can change with pro-inflammatory cytokines, so as to guide lymphocyte chemotaxis from only one direction during inflammation (Kolaczkowska and Kubes, 2013). By increasing ectopic production of CCL2, CXCL8, and CXCL10 inflammatory cytokines establish gradients that will draw lymphocytes into inflamed tissues. Within this milieu they are then recruited and activated (Needham et al., 2019).

Consequently, pro-inflammatory cytokines can also cause cells to express more social apparatuses. In this way the course of transfer for lymphocyte cells is changed on account of their distinctive adhesion properties. With molecules like TNF-alpha and IL-1β, e.g., lymphocytes are acted upon. The result is that both integrins and other adhesion molecules grow conspicuously on their surfaces and will easily stick to endothelial cells -- from there they migrate to inflamed organs (Francischetti et al., 2009).

Immunization and Lymphocyte Trafficking: Implications for Vaccine Development

One of the most successful strategies for preventing infectious diseases is vaccination. It harnesses the body's own immune system to provide long-lasting protection against pathogens that cause illness (Look et al., 2010). Immunization--and hence vaccination against infectious diseases--stands out as a most powerful tool in multi-layered public health interventionism. An orchestrated recruitment and activation of lymphocytes is central to the success of vaccination. These cells play a crucial role in bringing about protective immune responses to vaccine antigens (Gasteiger et al., 2016).

Vaccination puts into the immune system the knowledge of how to recognize and deal with certain antigens found in that injected vaccine This recognition initiates a sequence of events which includes recruiting activating lymphocytes that respond to the specific antigens.

Localized Inflammation

Immunization requires the focal production of inflammatory mediators at injection sites, leading to attraction of immune cells such as dendritic cells, macrophages and lymphocytes (Pulendran, 2004). Within the site of antigen deposition Ideal conditions are created for antigen-presenting cells to become active and move where they are desperately needed. These cells then begin to attract the precursor forms of antibody-producing lymphocytes (Cerutti et al., 2013).

Another aspect of vaccination is that it can result in the generation of memory lymphocytes, which are crucial for providing long-term protection against later encounters with that pathogen (Palm and Henry, 2019). Memory lymphocytes will tentatively differentiate and divide rapidly into effector cells upon reactivation when their ability to respond immune any better than normal has been observed (Jameson and Masopust, 2009).

Vaccination can also exert an effect on lymphocytes by changing the expression profile of adhesion molecules and chemokine receptors. By so doing, the former's migratory acritude and distribution in tissues are modified yet again accordingly. Vaccination can alter the trafficking patterns of antigen-specific lymphocytes, rendering them more adept at as ¬homing ' for a fight to injury or infection. Doing so ensures optimum immune surveillance and effector function (Mak and Saunders, 2005).

It is crucial to understand the modus operandi of lymphocyte trafficking after vaccination in order to develop effective vaccines against a raft of infections (Zutshi et al., 2019).

By unraveling both how immunization provokes immune responses and the routes lymphocytes take during and after vaccination, this chapter supplies valuable insights into vaccine design and maximizes its efficacy.

Regulation of Lymphocyte Extravasation: Balancing Immune Surveillance and Tissue Homeostasis

Lymphocyte extravasation is vital for immune surveillance and to safeguard the host organism from attack (Mohme et al., 2017). However, it is tightly controlled to ensure the stability of tissue metabolism and prevent over-reaction of immune cells which would lead to cellular necrosis or excessive inflammation of tissues, which cannot be restored. This fine balance is maintained by the combined action of a number of regulatory mechanisms controlling lymphocyte adhesion, migration and activation within different tissue microenvironments (Swartz and Lund, 2012). One example of such a mechanism is the dynamic regulation of adhesion molecule expression on endothelial cells and lymphocytes in response to microenvironmental signals (Imhof and Aurrand-Lions, 2004). Under baseline conditions, low levels of adhesion molecules such as selectins, integrins, and IgSF molecules allow for occasional interaction between lymphocytes and endothelial cells, permitting immune surveillance without excessive tissue inflammation (Dutta et al., 2016).

This could have a number of consequences. One might expect this to cause the lymphocytes to remain within a particular area of an inflamed tissue and be less able for movement or avoidance.

Regulatory T cells (Tregs) are crucial in maintaining immune tolerance and preventing excessive immune systemic responses (Izcue et al., 2006). Barring the activation of effector T cells (and antigen-presenting cells), help limit tissue inflammation and prevent autoimmunity Ultimately, Tregs can influence the expression of adhesion molecules and chemokine receptors on lymphocytes through three basic mechanisms, thereby affecting the migratory behavior of these cells as well as their location within tissue.

Moreover, lipid mediators and such soluble factors as chemokines, cytokines, cytokines also play a crucial role in the traffic of lymphocytes lymphocyte trafficking or change tissues (Olson and Ley, 2002). Pro-inflammatory cytokines like TNF-alpha and IL-1β can increase lymphocyte entrance and capillary permeability, however anti-protease such as IL-10 transforming growth factor-β (TGF-β) will reverse this inflammatory response and promote repair of tissue damage by detailing our current understanding of the complex control systems that govern lymphocyte penetration provides helpful knowledge on both how immunity interacts with living tissues living systems generally. In what way such control systems actually operate when disease occurs rich new routes can be found for investigating drugs to treat diseases like lupus erythematosus, bronchial asthma and so on (Chiurchiu and Maccarrone, 2011).

Therapeutic Implications and Future Directions

Elucidating the intricate ways of how lymphocytes enter organs will have important implications for the development of new therapeutic strategies for disease caused by immune fighting (Yang et al., 2020). By targeting the molecules and pathways involved in lymphocyte traffic, researchers can shift an immunologic response so as to treat anything from autoimmune diseases and inflammatory disorders to cancer. One approach being followed with enthusiasm is the development of monoclonal antibodies directed against adhesion molecules and chemokine receptors involved in lymphocyte traffic. By interfering with the dialog between lymphocytes and endothelial cells, these antibodies block recruitment of immune cells to favorably disposed tissues with the result being inhibition in inflammation and tissue damage. For example, monoclonal antibodies targeted at integrins like α4β1 (VLA-4) and αLβ2 (LFA-1) have also proven useful for treating inflammatory bowel disease in humans' animals' models (Zundler et al., 2019).

Also, is noteworthy the exploration of small molecules targeting signaling pathways of migration. By such impeding signal chains inside cells downstream from adhesion molecules and chemokine receptor, these inhibitors can modify lymphocyte activation, migration and invasion of tissue (Ward and Marelli-Berg, 2009). Several small molecule inhibitors targeting chemokine receptors and kinases are in clinical development for treatment of inflammatory and autoimmune diseases (Schall and Proudfoot, 2011).

In addition, gene therapy can be used to modify immune responses in a targeted way and alter the migration of lymphocytes to specific tissues (Kershaw et al., 2013). Gene transfer allows the delivery of therapeutic genes encoded regulatory molecules or soluble inhibitors when needed in any type cell or tissue throughout one' It is an in personalized immune modulation which may well have lasting effects and only modest distant impact on the organism as a whole (Albert and Kruglyak, 2015).

The development of advanced imaging techniques and computational modeling methods throws open the opportunity to visualize and quantitatively analyze lymphocyte traffic dynamics in vivo (Slepchenko et al., 2002). Regulators' increasing awareness of when the immune system is operating spatially and temporally in conjunction with other factors could reveal potential new targets for therapy and refine therapeutic strategies for immune disease (Knuesel et al., 2014).

In conclusion, by solving the mysteries of lymphocyte traffic researchers have opened exciting new prospects in designing therapies for diseases of the immune system. Currently with a better understanding about how immune cells traffic through tissue and the factors that determine their destination, researchers can look forward hopeful to one-day remedy diseases of the immune system more effectively and generally improve health care results as well.

Emerging Technologies in Studying Lymphocyte Extravasation

The lymphocyte extravasation study has benefited greatly from the development of innovative technologies that provide new insights into how immune cells move (Bellone and Calcinotto, 2013). For these emerging technologies leverage the progress in imaging, genomics, and computational modeling to untangle the complexities of lymphocyte migration and tissue homing.

One such technology is intravital microscopy, which allows live recording of events within living tissues. Scientists can

use fluorescently labelled cells and high-resolution imaging techniques to observe individual lymphocytes as they interact with endothelial cells, migrate through tissues, or respond to inflammatory cues. Intravital microscopy has the distinctive advantage that it provides an incredible amount of detail about both where and when events occur within the immune response process (Sumen et al., 2004).

Advances in genomics and single-cell analysis have transformed our knowledge of lymphocyte heterogeneity and the fashion. A single lymphocyte can be sequenced from RNA using a technique called Single-cell RNA sequencing (Andor et al., 2019). This method not only tells you the different forms of gene expression within immune cells (i.e., what they are "thinking"), but it also goes so far as to display these diverse pictures that give us glimpses of how an immune system actually functions. Disassembling the transcriptional programs responsible for lymphocyte migration and activation can reveal brand new regulatory pathways that may become therapeutic targets in immune-mediated diseases (Fares et al., 2020).

Additionally, computational modeling offers a powerful tool for simulating and forecasting in silico the dynamics of lymphocyte trafficking (Bailey et al., 2007). By combining experimental data with mathematical models of cell movement and tissue inflammation, researchers are able to simulate complex biological mechanisms and generate hypotheses that can be tested about the ways in which nodes wander from place to place. Computational modeling offers a complementary approach to conventional experiments, helping scientists to make sense of the types of large-scale data that are now flooding in from new assays and leading them to discover emergent properties in how immune cells behave (Council et al., 2009).

Conclusion

Examining the trimolecular model of lymphocyte migration has brought to light the complex interaction of molecular and cellular processes that underpin immune surveillance, inflammation, and tissue homeostasis. From the initial tethering and rolling of lymphocytes along the endothelium to their firm adhesion, transmigration, and tissue homing, every step in extravasation is finely choreographed by a myriad of adhesion molecules, chemokines and cytokines that bind them together. We have looked deep into mechanisms lymphocyte trafficking, peeling off the various roles of cell adhesion molecules and chemokines in regulating immune-cell movement through tissue; likewise, how inflammatory mediators and regulatory pathways control blood. Both basic science and clinical progress have benefited greatly from our growing understanding of the cellular and molecular mechanisms underlying lymphocyte extravasation. We have learned more about this intricate process by studying the ways in which inflammatory mediators, chemokines, cellular adhesion molecules (CAMs), and cutting-edge technologies work together to direct lymphocytes through tissues during immune surveillance, inflammation, and immune responses after immunization.

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Chapter 48

Cancer Prevention through Immunization

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ABSTRACT

With millions of people affected by cancer globally, cancer prevention is a global imperative. The World Health Organization projects that there will be 28.4 million cancer deaths worldwide by 2040, up from approximately 10 million in 2020. Understanding the interactions between genetics and lifestyle decisions including alcohol use, sedentary behavior, and exposure to carcinogens is essential for effective cancer prevention. Changes in lifestyle that include good food, quitting smoking, and regular physical activity can dramatically lower the risk of cancer. Campaigns for public awareness are essential, especially when it comes to preventing diseases linked to tobacco use. They promote immunization and screening programs, encourage healthy lifestyles, and push for limits on smoking. Reducing the effect of cancer requires a holistic approach that integrates lifestyle modifications, early detection, and environmental treatments, despite obstacles like healthcare coordination and cultural differences.

KEYWORDS
Cancer prevention, Lifestyle modifications, public awareness, Cultural difference, Cancer Prevention

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INTRODUCTION

Cancer, a disease name that is enough to even haunt any human of any era. That has been biggest challenge to cope within the world of medicine for ages. Though medicine advancements are beyond the human minds, cancer has been challenging worldwide not just for healthcare workers but also for families, people and affected beings. As it's an old proverb, prevention is better than treatment. Thus, cancer prevention can be a light of hope for the world. By cancer prevention, we mean some vast areas to work on, such as lifestyle changes, public health initiatives and medical advancements. Recently, efforts regarding cancer prevention have been observed widely with a special focus on causes, transmission and life-threatening impact of this disease (Fig. 1).

Cancer Prevention; A Global Perspective

Until we cannot get the deadly consequences of this disease, we cannot reach the seriousness of cancer prevention. Taking preventive measures cannot be understood properly until the global impact of this disease would not be highlighted. Accordingly, World Health Organization (WHO) , up to 2020 almost 10 million deaths have been recorded because of cancer alone and it's declared as the second main reason for death in the world. It's been predicted that the death rate because of cancer has a potent chance of increasing up to 28.4 million by the year 2040. These kinds of alarming predictions are the need for an hour to take some preventive steps against cancer fight (Fig. 2).

Understanding of Cancer and Responsible Environmental Factors

Jumping over preventive measures once need to engulf the key sets of cancer-causing factors. Though many types of cancers like colorectal cancers, breast cancers and ovarian cancers have genetic causing factors but other than genetic factors, environmental factors and mode of life crucially play in cancer causation. Environmental factors include unhealthy lifestyle such as alcohol consumption, inactive routines, fatty foods, and exposure to carcinogens. Other main causing factors can be obesity, chronic inflammations and certain carcinogenic or infectious agents can play a vital role in cancer prevalence. Thus, cancer is a complex interplay between genetics and environmental factors.

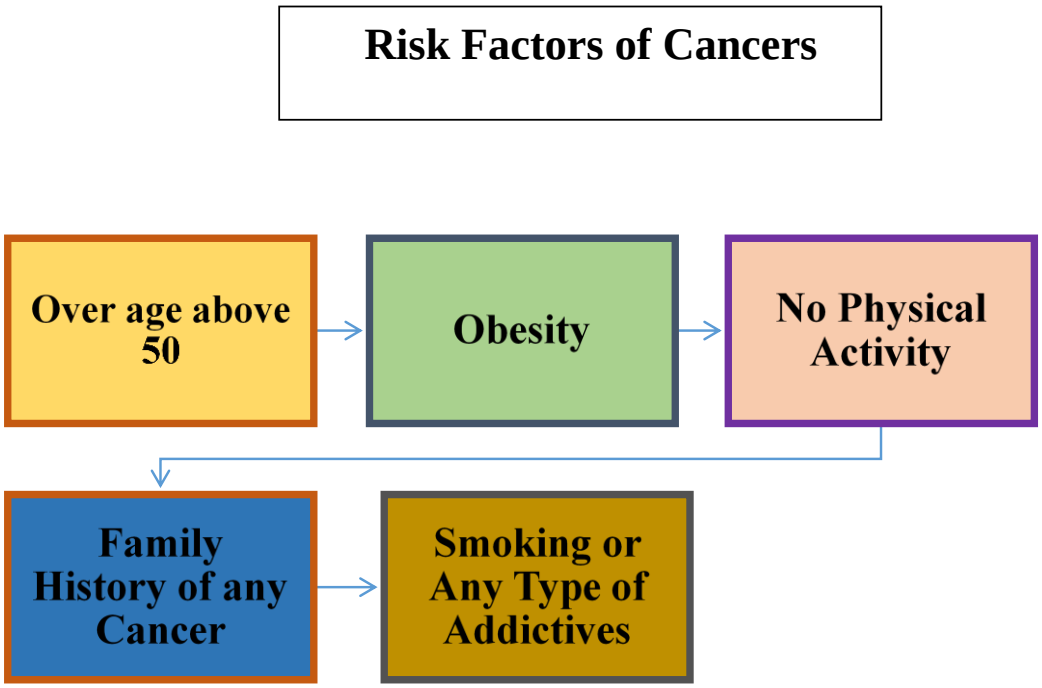

Fig. 1: Risk factors of cancer

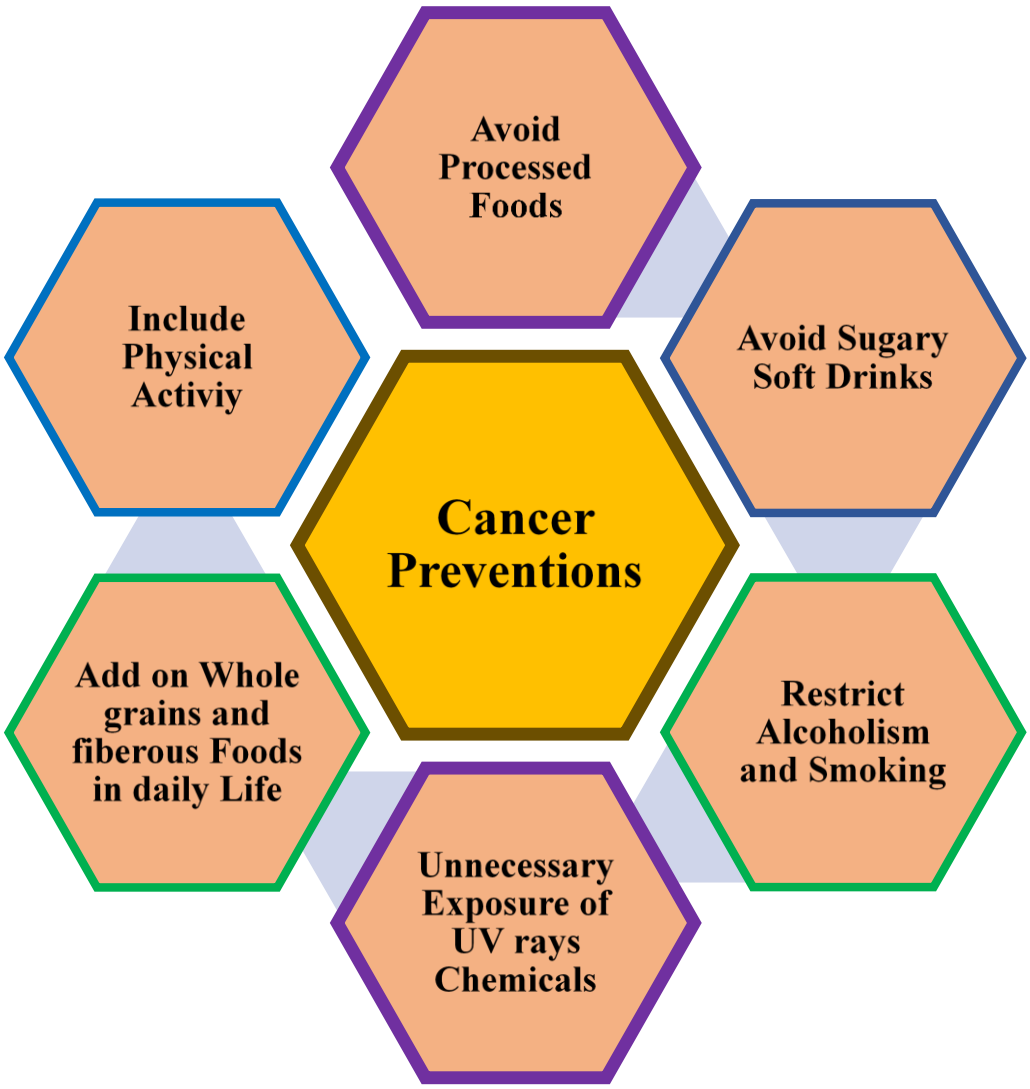

Fig. 2: Strategies to prevent cancer

Minor Lifestyle Modifications but Major Preventive Measures

Including some healthy life-style changes in routine life can make some major changes in health. In some past decades, the human lifestyle has been spoiled a lot which has become a major reason for so many problems. Some healthy

lifestyle choices like physical activities, clean eating and no alcohol consumption and no tobacco usage can lead to a safe and healthy life. With an increasing trend of luxurious life, physical activity has lost its charm in the lives of most individuals. However, it's observed that physical activity is directly proportional to cancer prevention. The more physical activity will be, the more safety from cancer can be seen. Certain types of cancers like colorectal, breast and prostate cancers are types of cancer which can be controlled by adopting some healthy life choices like taking healthy fats and fibrous foods.

Public Awareness

Public awareness has a crucial role in cancer prevention. In some countries, the tobacco usage ratio is drastically high which is responsible for so many tobacco related cancers like lung and throat cancer, so by banning the smoking ads, by restricting smoking and by spreading information about tobacco usage and its worst effects certain cancers can be prevented. By promoting healthy lifestyle publicly like on school levels to higher levels by spreading awareness about healthy lifestyle on childhood levels can be fruitful and a good public health initiative and can be a best strategy to control obesity related cancers. Side by side observing and screening genetic history of people affected by this and administration of cancer vaccines such papillomavirus vaccines, hepatitis C virus vaccines and awareness about chemo preventive strategies can play a visible role in cancer prevention.

Type of Challenges in Cancer Prevention

This means we need to constantly update and improve our strategies for preventing cancer from matching these new discoveries. Designing cancer prevention strategies is way too easy than implementing practically because there can be a number of challenges in the way of cancer prevention. Lack of coordination in health care facilities, inactive preventive services, economic issues, cultural differences and lack of awareness regarding cancer risk factors and importance of preventions, especially in the developing world. Similarly, concepts of cancer biology continue to add different new aspects. We need to know more about cancer diversity and autoimmunity of the body to fight against cancers and how a body inside an environment or conditions can facilitate tumor growth. Because of this diversity sticking to old prevention strategies can't work we need to be updating regarding our preventive measures and strategies. Concluding it as 'Prevention is better than cure' is an oldest but important saying which can be the slogan in cancer prevention campaigns too. Spreading awareness about cancer prevention is crucial because it addresses the root cause before it escalates into a major issue. By promoting and accompanying people in making healthier life choices without tobacco, smoking and with physical activities, we can impart a big role in this battle. In the battle against cancer, all need to work hard and together, including doctors, healthcare workers, governments, institutions and researchers. Today's efforts and teamwork can create a safe future, and these baby steps can build a cancer free world.

How different types of preventive measures can prevent different types of cancers? Cancer is such a deadly disease which is taking millions of lives every year worldwide. Though treatment has its own importance, nobody can deny the value of precautions. Taking precautionary measures can lessen the disease burden on a greater level. Precautions are always better than cure and can hinder cancer cases to a huge extent. By adopting a precaution-based lifestyle and by making lifestyle changes different types of cancers can be prevented (Akbarpour et al., 2021). Such as throat cancer and lung cancer can be prevented by less or no consumption of Tobacco. Because it has been observed that smokers and people who work in cigarette agencies can be more prone to lung and throat cancers (Fig. 3). By spreading tobacco dangers through social awareness campaigns or other strategies like seminars, walks and protests, these types of cancers can be inhibited (Akbarpour et al., 2021).

With the advancements of life following an unhealthy lifestyle such as having junk, no physical activities, just being on phones and social media has pushed human beings towards alarming health conditions. Because of high consumption of unhealthy foods and an inactive lifestyle many cancers like intestinal cancers and liver cancers are spreading. To combat these types of cancers one should include fresh fruits, fresh vegetables, fibrous foods, whole grains instead of refined ones (Bayo et al., 2019). By modifying some food habits and by adopting some healthy lifestyle modifications, certain cancers like colorectal cancers, breast cancers and prostate cancers can be prevented. Similarly, a least included modification that includes physical activity along with an active life routine can be a great step towards cancer prevention.

Because of unawareness regarding the need of wearing sunscreens and taking sun protection is the leading cause of different types of skin and eye cancers. Some dangerous types of skin cancers are being observed among people living in areas of high altitudes because of dangerous UV light exposure. By spreading awareness among people regarding UV rays' dangers and importance of wearing sunscreens and taking sun protection can contribute a lot to skin cancer prevention (Cokkinides et al., 2012).

Vaccinating individuals in their childhood or adulthoods against a certain type of viral cancers like cervical cancers and human papilloma virus cancers can be a huge preventive measure to combat these kinds of cancers. Vaccine against viral related cancers is an effective yet a great step in the world of cancer prevention (D'Souza and Addepalli, 2018).

A person should be conscious of any deformity or issue in any body part. One should get through cancer screening as a routine medical checkup. A beforehand screening can be a protection measure from any dangerous consequences. Antibody screening for breast cancer, cervical cancer, and prostate cancer can be a great preventive measure against cancer protection. Early screening not only can save anyone from cancer causation also in early stages it can be more helpful in cancer treatment (Hung et al., 2022).

Some cancers can occur because of environmental factors. People who used to deal with UV rays sterilization facilities or who deal with carcinogens can be at higher risk so by reducing the exposure or by adopting the safest exposures can help in protection against environment related cancers (Vykhristyuk et al., 2021).

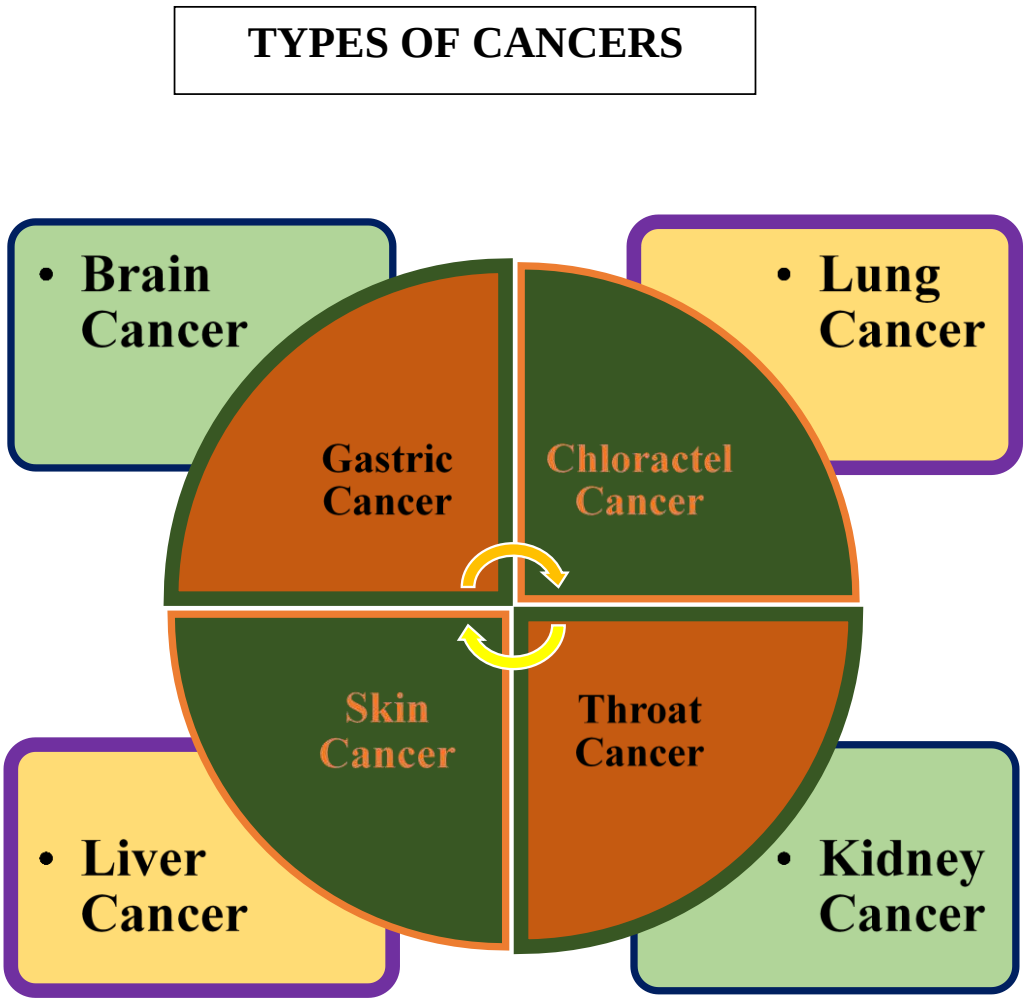

Fig. 3: Types of cancer

In conclusion, effective cancer prevention necessitates a multifaceted approach encompassing lifestyle modifications, vaccination, early detection, and environmental interventions. By adopting these preventive measures on individual, community, and policy levels, we can substantially diminish the burden of cancer and move closer to a world where fewer lives are touched by this devastating disease.

Understanding Immunization for Cancer Prevention

Immunization is essential for preventing cancer because it trains the immune system to recognize and destroy malignant cells. Conventional vaccinations against infectious agents, such as the hepatitis B virus (HBV) and the human papillomavirus (HPV), have had a significant role in lowering the incidence of malignancies linked to viruses, such as liver and cervical cancer, respectively (Finn, 2014). These vaccines prevent viral infections that can result in the development of cancer by preparing the immune system to identify and mount a strong response against certain viral antigens (Finn, 2014).

The creation of therapeutic cancer vaccines, which aim to activate the immune system's ability to identify and eliminate tumor cells, is the result of recent developments in cancer immunotherapy. Tumor-specific antigens or immune-stimulating chemicals that amplify and activate the body's anti-cancer immune response may be included in these vaccinations (Finn, 2017). Furthermore, by enabling the immune system to specifically target and eliminate cancer cells, immune checkpoint inhibitors which decrease inhibitory signals on immune cells have completely transformed the therapy of cancer (Finn, 2017).

Developing successful vaccination tactics to prevent cancer requires an understanding of the intricate interactions between the immune system and cancer. The main goals of research are to discover new tumor antigens, improve vaccine formulations, and clarify the immune evasion strategies utilized by cancer cells (Ward et al., 2017). We may be able to enhance patient outcomes and lower the global cancer burden by utilizing the power of vaccination. Sustained funding for research on cancer immunotherapy is encouraging since it might lead to the creation of novel vaccination strategies that could eventually be used to eliminate cancer (Ward et al., 2017).

The Link between Infection and Cancer

It is commonly known that infections and cancers are related, with some infectious agents being major contributors to the emergence of different types of cancer. Through direct oncogenic effects, immunological suppression, and chronic inflammation, viruses, bacteria, and parasites can all play a role in the development of cancer (L. Zhang et al., 2023).

One of the most prominent viruses linked to cancer is the human papillomavirus (HPV), which is especially linked to malignancies of the cervical, anal, and oropharyngeal regions (Luofei Zhang et al., 2023). After HPV integrates into the host genome, the growth and proliferation of cells are deregulated. In a similar vein, infections with the hepatitis B and C viruses (HBV and HCV) are important risk factors for liver cancer because they can encourage the development of hepatocellular carcinoma (HCC) through persistent inflammation brought on by viral replication (Luofei Zhang et al., 2023).

Infection with *Helicobacter pylori (H. pylori*) has been connected with the development of mucosa-associated lymphoid tissue (MALT) lymphoma and stomach cancer. Chronic gastritis brought on by H. pylori may develop into intestinal metaplasia, atrophic gastritis, and finally gastric cancer (Yusuf et al., 2023).

Furthermore, several parasites' *Schistosoma haematobium* and *Opisthorchis viverrini*, for example, have been linked to liver and bladder cancer, respectively. These parasites contribute to the development and spread of tumors by releasing chemicals that are carcinogenic and causing persistent inflammation (Yusuf et al., 2023).

Comprehending the correlation between infections and malignancies is imperative for executing prophylactic measures, like immunization and antibiotic therapy, to mitigate the incidence of cancer. It has been demonstrated that vaccination against HPV and HBV effectively prevents malignancies linked to viruses. Moreover, early infection identification and management can reduce the risk of cancer (Biondi et al., 2021). Improved cancer prevention techniques and targeted therapeutics depend on an ongoing investigation into the molecular pathways driving infection-associated carcinogenesis.

Introduction

Cancer is a multifaceted condition with several causative agents, including certain viruses and bacteria. Vaccines against these causal agents may use a lucrative target for nullifying cancer (Fan et al., 2023). For example, vaccines against HPV and Hepatitis B were developed to zero human papillomavirus, leading to a significant reduction in cervical and liver cancers, respectively (Jou et al., 2021a). Moreover, immunization against bacterial infection, Helicobacter pylori, has the potential to reduce the risk of infections associated with cancer (Singha et al., 2018). Nevertheless, vaccine efficiency varies from age to age, often attributed to the maturity and immature immune system and presence of comorbidities (Reyes, 2023a).

Vaccines Targeting Viral Causes of Cancer (e.g., HPV, Hepatitis B)

Vaccines have emerged as an effective approach for combating cancer, particularly cancers caused by viral infections. For example, the Human Papillomavirus (HPV) and Hepatitis B Virus (HBV) have been reported to be significant contributors to cervical and liver cancers, respectively (Kechagias et al., 2022).

It has been shown that HPV vaccinations are useful in reducing the risk of HPV infection and the recurrence of diseases related to HPV after local surgical treatment. The British Medical Journal published a systematic review and meta-analysis in 2022 that showed the beneficial effects of HPV vaccination against the recurrence of cervical intraepithelial neoplasia grade 2 or above (CIN2+), a precancerous condition (Lin et al., 2023).

Hepatitis B vaccinations have also been beneficial in limiting the transmission of Hepatitis B and, consequently, in lowering the incidence of liver cancer. It has been reported that these vaccines constitute a significant advancement in public health (Hsieh et al., 2015). Vaccines against HPV and Hepatitis B represent a significant advancement in cancer prevention; they highlight the potential of immunization as a strategy in the global fight against cancer (Hsieh et al., 2015; Kechagias et al., 2022). However, while these vaccinations show promise, their effectiveness can vary across different populations and settings. As a result, ongoing research and surveillance are crucial to monitor their impact and to identify areas for improvement (Escoffery et al., 2010.)

Immunization Against Bacterial Infections and Cancer Risk Reduction

Immunization against bacterial infections has emerged as a significant strategy in reducing cancer risk (Danielsen et al., 2023). One of the main causes of disease in cancer patients and a significant barrier to the effectiveness of cancer treatment is infection. (Rodrigues and Plotkin, 2020). Antimicrobial resistance poses a global threat to exacerbate these challenges and hinder continuing progress in cancer treatment. (Tripathi, 2023). According to recent research, individuals with cancer are more likely to become infected with microorganisms that are resistant to antibiotics. (Zhang et al., 2019). Consequently, there is an urgent need to both develop more effective preventive strategies and comprehend the evolving epidemiology of bacterial and fungal infections in cancer patients (Reyes, 2023b).

One of the greatest scientific discoveries of the twenty-first century has been the development of vaccines. (Jou et al., 2021b). An estimated 6 million deaths from vaccine-preventable diseases have been avoided each year as a result of vaccinations (Kechagias et al., 2022). The population of the world is predicted to approach 10 billion people by 2055 (Finn, 2017), a success that is partly attributable to efficient vaccinations that combat disease and increase life expectancy on every continent (Vraga et al., 2023). In conclusion, immunization against bacterial infections represents a significant advancement in cancer risk reduction (Escoffery et al., 2023).

Role of Immunization in Cancer Prevention across Different Age Groups

Research showed that immunizations against Hepatitis B and the Human Papillomavirus (HPV) significantly reduce the incidence of several cancers (Rimer and Sandler, 2018). However, because of variables including immune system maturity and comorbidities, the efficacy of these vaccinations may vary throughout age groups (Crews et al., 2021).

For example, the HPV vaccine works best when given before virus exposure, so that's why preteens between the ages of 11 and 12 are advised to have it. Catch-up vaccinations, however, are additionally recommended for some age groups who did not receive vaccinations earlier

In the same way, infants are usually administered the Hepatitis B vaccination as part of their regular immunization schedule (Rimer and Sandler, 1971). This vaccine has the potential to prevent (Crews et al., 2021) liver cancer. Adults who have an increased risk of the disease, however, can also receive it.

To sum up, vaccination is an effective method of preventing cancer in people of all ages. To address the issues of vaccine reluctance and to optimize vaccination strategies for various age groups, more study is necessary.

In conclusion, this chapter has explored the significant role of immunization in cancer prevention. We delved into the effectiveness of vaccines targeting viral causes of cancer, such as HPV and Hepatitis B, and their substantial impact on reducing the incidence of specific cancers. We also discussed the potential of immunization against bacterial infections in reducing cancer risk, highlighting the need for further research and development in this area. Moreover, we examined the role of immunization in cancer prevention across different age groups, emphasizing the importance of understanding variations in vaccine effectiveness because of factors like immune system maturity and comorbidities. This understanding is crucial for developing effective vaccination strategies tailored to different age groups. Overall, the potential of vaccines as a preventive measure against cancer is immense. However, it's important to remember that the fight against cancer involves a multi-faceted approach, and while vaccines are a powerful tool, they are just one piece of the puzzle. Continued research, education, and advocacy are essential to fully harness the power of vaccines in our ongoing battle against cancer.

Challenges and Controversies in Cancer Immunization

Challenges in immunization of Cancer

Cancer is an incurable condition in which there are few effective treatment options (Gupta et al., 2022). Despite significant advancements in the last decade or so in the creation of successful cancer vaccines, significant barriers still stand in the way of successful therapeutic outcomes (Bowen et al., 2018). Many obstacles face traditional cancer therapy methods, including limited blood circulation times and poorly soluble anticancer medications (Gupta et al., 2022). Other most concerned problems are:

The Immunological Suppression

Compared to the immune systems in healthy people, cancer patient's function in different conditions and face many problems while trying to generate an immune response. The immune systems are weakened by tumor- and therapy-specific factors.Normally, self-replicating immune cells are the target of radiation and chemotherapy treatments, which aim to eliminate rapidly dividing neoplastic cells. Furthermore, tumors themselves use a range of strategies to weaken and manipulate the immune system. An immunosuppressive tumor microenvironment is fostered by the persistent inflammatory environment linked to tumor progression. All of these circumstances are not only defined by the enervation of T cell and NK cell responses but also by an increase in the suppressive phenotypes of T regulatory cells.

Antigenicity Issues

It is hard to use therapeutic vaccinations to target existing cancers. Antigens that are overexpressed in tumors compared to normal tissue, that the body generally ignores as immune-privileged (such as cancer testis antigens),are typically expressed temporally during development (such as oncofetal antigens), or that result from mutations either stochastic or oncogenic are frequently used to target tumors.

Different levels of immunological tolerance may affect the immune response to self-antigens, and altered antigens may be patient-specific and challenging to target. Variable targets could potentially include tumor antigens. Targeted antigens may be rapidly selected during tumor formation, especially if they are not oncogenic drivers. This could result in antigen escape loss variations, which could lower the effectiveness of antigen-targeted vaccines (Bowen et al., 2018).

Controversies in Cancer Immunization

Adoptive T cell therapy has demonstrated recent clinical success in treating patients with metastatic melanoma, which has increased interest in this type of treatment. It has been claimed that existing peptide vaccines have not worked in the face of this resurgence of interest in adoptive T cell treatment. Additionally, a corollary argument is emerging, suggesting that further research into peptide vaccines or any other active, targeted immunotherapy may not be warranted for cancer.

The ability of adoptive cellular immunotherapy to produce much more circulating CD8 cells with anti-tumor specificity is one of the main justifications for its application in the treatment of cancer. Adoptive therapy is challenging in the current regulatory climate and is also costly and time-consuming. Conversely, vaccines can be used for therapy outside of highly specialized centers more easily.

Lack of understanding of the processes governing the proliferation, activation, and effector function of tumor-

antigen-specific T cells is the flaw in the existing strategies for cancer vaccination therapy. While adoptive treatment is a viable short-term solution to circumvent this process, understanding the immune-biology of the host-tumor connection will be essential to the advancement of tumor immunology and immunotherapy in the long run (Jr and Speiser, 2005).

Public Health Strategies for Promoting Immunization and Prevention of Cancer

Controlling cancer through primary and secondary measures is the first line of defense. In states where cancer mortality is high and the healthcare system is ineffective, more needs to be done. It is vital to investigate the attitudes and cognizance of the intended audience to guarantee the efficacy of cancer prevention campaigns (Karasiewicz et al., 2022).

Three main strategies can be used to mobilize endogenous immune responses to prevent cancer: vaccinations to prevent cancer-associated agent infection; vaccinations to target tumor-associated antigens (TAAs) or tumor-specific antigens; and nonspecific immune modulators that enlist innate immune system components to produce their anticancer effect.

Smoking is one of the causes of lung cancer. The promotion of smoke-free environments and the treatment of tobacco dependence can be implemented with effectiveness. Alcohol sales restrictions can reduce alcohol usage and prevent cancer (Frieden et al., 2008).

Vaccination is a useful strategy for preventing some virus-associated cancers. For example, the hepatitis B vaccination can prevent hepatocellular cancer, and the human papillomavirus vaccine can prevent cervical cancer (Frieden et al., 2008).

Cancer DNA vaccines are DNA plasmids that are circular and contain immunomodulatory or tumor-associated antigens (TAAs) that trigger specific immune responses against the target tumor. Similar to this, mRNA vaccines made in vitro have the potential to encode antigens and, upon internalization, express proteins in order to trigger an immune response. Therapeutic DNA vaccines are thought to be the most effective way to stimulate the immune system in the fight against cancer (Gupta et al., 2022).

Several molecular biomarkers have been discovered for prognostic usage, risk stratification, and decision-making regarding the inclusion of chemotherapy in the treatment regimen because of recent advancements in our understanding of molecular carcinogenesis. A powerful anti-tumor response can be induced by overcoming tumor evasion and self-tolerance through the development and optimization of combination adjuvant therapies (Bowen et al., 2018).

Numerous antigens, including tumor-associated antigens (TAAs) and mutation-derived antigens (neoantigens), which are considered foreign antigens and induce antitumor immune responses in cancer patients, are frequently expressed by tumor cells (Hirayama and Nishimura, 2021).

The latest authorization of therapeutic monoclonal antibodies for the treatment of endometrial cancer, such as dostarlimab, an antibody that blocks the programmed death receptor-1 (Khansari, 2022).

Future Directions in Cancer Immunization Research and Development

Biomarkers: Genomic examination of pre-malignant lesions is now receiving attention in the genomic investigations of various tumors. It is anticipated that assessment of the genetic anomalies will advance a functional understanding of carcinogenesis based on genes and result in the discovery of molecular targets for intervention. Future cancer prevention techniques will leverage a range of novel modalities, to find and verify surrogate biomarkers for use in preventive studies.

Stem Cell

One of the cancer treatment approaches that is thought to be both safe and effective is stem cell therapy. The use of stem cells is still in preliminary clinical trials; one potential application is the regeneration of other damaged tissue. When a tumor is smaller than 3 cm in size and surgery is not an option, ablation is a therapeutic method that eliminates the tumor without removing it. For bigger tumors, ablation is also combined with embolization.

Epigenetic alterations are the mechanism by which normal stem cells or precursor/progenitor cells become cancer stem cells (CSCs). Their involvement in tumor treatment includes the growth, metastasis, and recurrence of cancer, suggesting potential benefits in the management of solid tumors.

RFA is a minimally invasive procedure that uses high-frequency electrical currents (hyperthermic conditions) to eliminate cancer cells. It is guided by images. (Debela et al., 2021). It is anticipated that targeted cancer therapies will outperform other treatment modalities that cause greater harm to both cancerous and healthy cells. Advancement in molecular technologies continues to progress and scientists' focus on targeted cancer therapies will apply more effective cancer medicines that call for collaboration between laboratory scientists and doctors (Khansari, 2022).

Conclusion

In summary, cancer prevention is a vital and complex strategy that necessitates concerted action on a personal, societal, and international scale. Proactive steps to lower cancer's incidence and impact are necessary, as demonstrated by the ongoing fight against this disease, which despite advances in medicine continues to pose a challenge to the medical community. Effective cancer prevention requires an awareness of the serious effects of the disease as well as a comprehension of its multifaceted origins, which include inherited traits as well as environmental variables.

Modifications to one's lifestyle are crucial for cancer prevention. A balanced diet high in fruits, vegetables, and fiber, along with a complete abstinence from tobacco and alcohol, can dramatically reduce the risk of many cancers, including

colorectal, breast, and prostate cancers. These actions not only enhance general health but also provide people the ability to take charge of their own wellbeing. Campaigns for public awareness are equally important. Key tactics in lowering the incidence of cancer include educating populations about the dangers of tobacco use, encouraging healthy lifestyle choices at an early age, and emphasizing the advantages of routine screenings and vaccines. Significant progress can be made in reducing tobacco-related malignancies such as throat and lung cancers by outlawing smoking advertisements, limiting tobacco usage, and educating people about the dangers of tobacco use.

In addition, numerous lives can be saved by early detection achieved by routine screenings and the delivery of cancer vaccines, such as the hepatitis B and human papillomavirus (HPV) vaccines. Increasing access to these preventative interventions should be the main goal of public health campaigns, particularly in high-risk and impoverished communities. It is essential to keep working toward a comprehensive strategy to cancer prevention despite the many obstacles, such as poverty, cultural differences, and poor healthcare coordination. To get past these obstacles, cooperation between several sectors—including governments, healthcare providers, non-profit groups, and communities is crucial. It is also essential to fund research to increase early detection techniques, comprehend the etiology of cancer, and create more potent treatments.

In the end, we can aim for a future where the burden of cancer is greatly decreased by placing a high priority on prevention. We can make significant progress in the fight against cancer by continuing to promote healthy lifestyles, raising public awareness, improving access to preventative healthcare, and developing international cooperation. In addition to having the potential to save millions of lives, this all-encompassing strategy can enhance the quality of life for those afflicted with this terrible illness and their families.

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Chapter 49

Immunoevasion Mechanism of *Brucella abortus* in Dairy Cows

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ABSTRACT

Brucella abortus infection poses a great challenge to dairy farming systems, damaging animal health, crop yields, and public health. This chapter offers an in-depth study of the intricate interplay between *B. abortus* and its host, examining the bacterial virulence factors, mechanisms to evade immune defense mechanisms, diagnostic methods, how it is put under surveillance, what should be done for control, and other subjects. Molecular pathogenesis of Brucella must be humbled by discovery and the use of vaccination, and One Health initiatives might be seriously tried. Interdisciplinary cooperation is the only way forward towards efficient control of this disease. State-of-the-art technologies may be the key to finding out the information on how a particular disease spreads and inventing interventions specifically for every case. While both the economic costs and public health consequences of brucellosis are massive, taking the initiative and acting preventively on control measures and capacity-building will undoubtedly ameliorate the impact and facilitate our abilities to maintain the utmost health of our dairy cow populations.

KEYWORDS
Brucella abortus, Dairy cows, Brucellosis, Immunoevasion

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INTRODUCTION

There are many infuriatingly clever immunoevasion mechanisms utilized by a wide range of pathogens in the intricate world of veterinary science. In this chapter, we will learn about a few of those utilized by *B. abortus* and go through how they help the bacterium escape the immune system of dairy cows. At the same time, as we here are familiar with these mechanisms, we want to determine how to counteract them and pinpoint a *B. abortus* attack on the cow's immune system.

The intricate understanding of pathogen immunoevasion mechanisms is fundamental in veterinary sciences for the development of effective strategies aimed at improving animal health. *B. abortus*, a major pathogen in the bacterial species responsible for causing brucellosis in cattle (Enright, 1990), is a stealthy pathogen in the bovine milieu. In her entirety, we will explore *B. abortus*, in this chapter we will undertake a comprehensive exploration of the molecular mechanisms she employs, to evade this nothing less than formidable defense armamentarium the bovine stacking against her.

Geographical Distribution

B. abortus was once found worldwide in cattle, with rare exceptions such as Iceland. It has been eradicated in several European nations and Canada, Australia, New Zealand, Japan, and Israel from domesticated animals (Corbel, 2020). In the U.S.A. *B. abortus*-free, except in one area noted below. Foreigners and immigrants might sometimes bring an isolated case into a country without *B. abortus* smooth stain infection (Schumaker, 2010).

Brucella abortus - An Overview

B. abortus is a gram-negative bacterium, very much a concern within the veterinary world (Fig. 1) (Seleem et al., 2010). *B. abortus* is a multi-host, highly contagious pathogen that spreads between cattle via three primary transmission routes: direct contact with aborted products or vaginal secretions of infected animals, consumption of unpasteurized milk, and contact with wild animals (e.g., rodents and deer) and possibly ticks may facilitate the re-emergence of *B. abortus* infection in domestic animals (Berhanu and Pal, 2020).

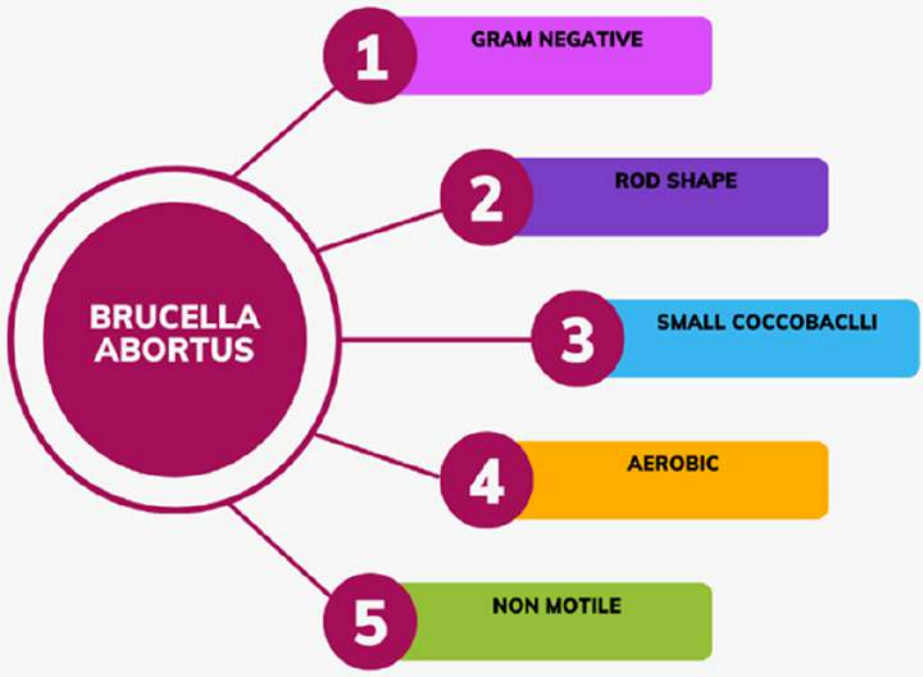


Fig. 1: Morphology of *Brucella abortus*

The course will provide, with the aid of numerous illustrations and diagrams, a solid foundation for the morphology of the bacteria, its life cycle in those animals that can become infected, and the production of brucellosis in the dairy industry.

Epidemiological Characteristics

Brucellosis, being a zoonotic bacterial disease, is no longer regarded as a problem in some countries only to subsequently infect animal populations in every part of the globe (Seleem et al., 2010). B.Acute blazing affections, one of the major viral diseases, is widespread in the Mediterranean and Latin America regions and also in Asia including India. 'Egyptian fever' was designated during the travel of Europeans (Mal d'Adrien) to or from these countries or the import of milk products as the cause. stocked by Europe which drags the illness still but does not do the complete treatment of Brucella since infected cattle are the cause of this disease. In low-income countries, the disease is virtually under-reported and there are no control or too incalculable control measures, which leads to massive health problems, poverty disasters, and livelihood challenges (Watkins, 1995).

Risk Factors

The risk factors could be outdated ways of production, a large ecosystem area, traditional animal husbandry, close contact with the wild species, as well as, actors with no appropriate ignorance. They are also exposed to the working of who can handle animals and live species that is car ringers of these bacteria or with whom they have direct contact or blood contact (Miller et al., 2012). The practice of agriculture is diverse, and hence, it can be a career that can interest a person that is passionate about professions such as veterinary, dairy farming, ranches, kill floor workers, microbiologists, and hunters, to mention but a few (Brassley and Soffe, 2016). The global spread of the epidemic is also believed to be a result of the contact with the African Continent. The workers who live in regions without proper public health protocols and the workers who travel to or produce other raw dairies from the African Continent are also exposed to health risks or diseases. Another category of workers to be infected is those who work in laboratories (Sewell, 1995).

Brucella typically resides in the reproductive organs of sexually mature male and female animals (Fig. 2), and each type of *Brucella* infects particular animal species (González-Espinoza et al., 2021). Infected animals frequently transmit the infection to others. Bacteria excreted by infected animals can endure for several months in damp surroundings. It is commonly transmitted through direct contact with infected animals, as well as through bodily fluids and tissues associated with abortions (Shaapan, 2016).

Bacterium Morphology

B. abortus, a member of the Brucellaceae family, a small coccobacillus-shaped bacterium is revealed through this contrast stain. It is small, measuring 0.5-0.7 mm in width and 0.6-1.5 mm in length as shown here, but causes major health problems in dairy cattle (Tibbetts et al., 2015). Knowledge of its morphology helps us understand its potential pathogenicity.

Life Cycle Dynamics

The life cycle of *B. abortus* is characterized by its remarkable ability to survive and persist in a variety of environmental niches, primarily following transmission in infected animal birth products or contaminated environments (González-

Espinoza et al., 2021). Once introduced into a host, *B. abortus* establishes a chronic persistent infection, primarily in the reproductive organs of female cattle, which presents a significant challenge to eradication if control methods are not rigorously applied (Khurana et al., 2021).

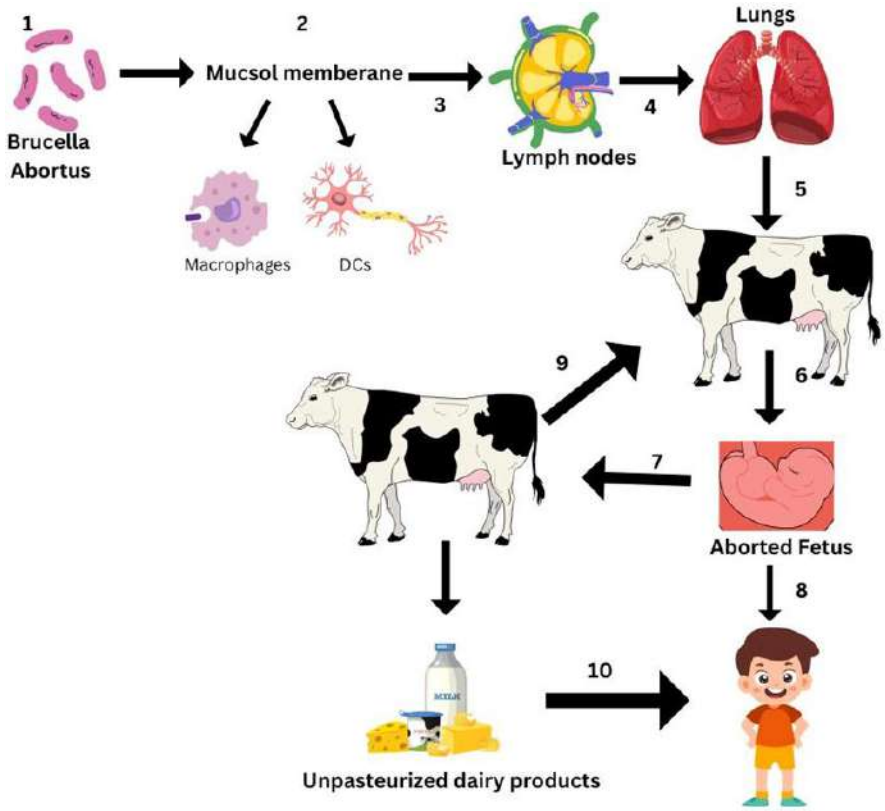


Fig. 2: Life cycle of *Brucella abortus* in cows and humans

***Brucella abortus* Infection in Animals**

Incubation Period

The period from infection to reproductive losses is highly variable because animals may not become infected until any time (e.g., after pregnancy) and abortions generally occur near full term (Givens and Marley, 2008).

Clinical Signs

Again, abortion in addition to stillborns and weak young ancestors would be the fact that in herd pure cattle (Shrode and Lush, 1947). Calves that are born weak often die quickly. Most animals only abort once, and future pregnancies generally progress as normal. Milk yields may be less than usual. There may be no obvious signs of mastitis while the bacterium *B. abortus* is present in the milk (Kibebew, 2017).

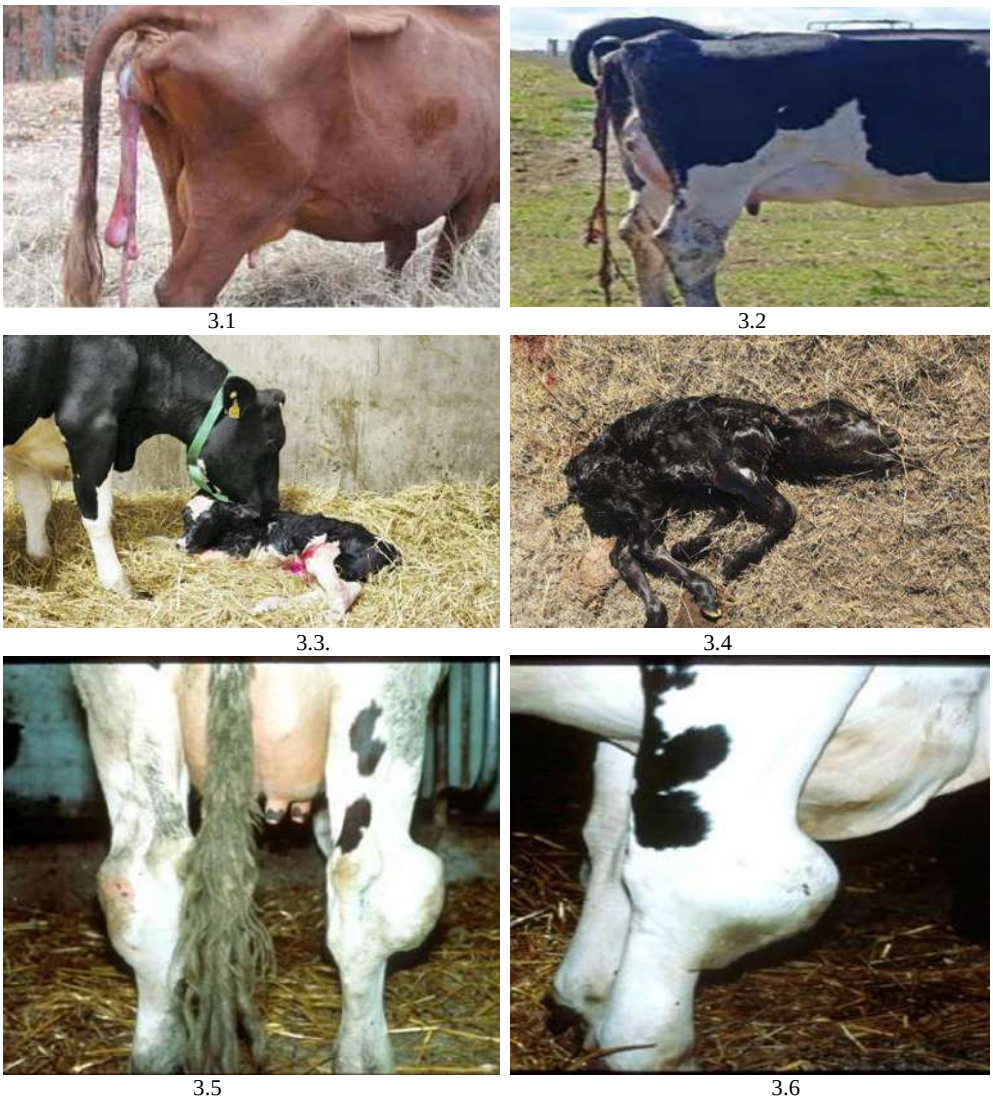
Source of Infection and Route of Transmission

The bovine brucellosis is a serious zoonotic disease, and it could be transmitted from any livestock species such as cattle, sheep, goats, pigs and camels, which may be in contact with blood, placenta, fetus or uterine secretions or might be through the intake of unpasteurized animal-made products like veiled milk and soft cheese (venous disease comes) (Rodríguez-Frías et al., 2021). The spices genus Brucella include at least 3 species among them *B. abortus* (sometimes abbreviated as *B. abortus*) for cattle, *Brucella melitensis* (*B. melitensis*) for goats, (*B. suis*) for pigs for the medical purposes, crucially differing from other species inhabiting farmed or wild animals. The tiny germs, however, include the transfer of disease from various animal organisms to humankind (Lederberg, 2000). The most common clinical symptom of all these infectious diseases is the varying form of fruits, "Glazing-type climate," produced by *B. melitensis* and *B. suis*. Symptoms of other diseases also occur frequently in individuals with this chronic disease (Walke et al., 2004). So these microorganisms are existing as the bearers of this Spanish flu as well as the cause of this coronavirus-originated pandemic of now (Rodríguez-Frías et al., 2021). The living creatures are threefold such that they are the tone for these viruses' epidemics.

Consequences of Brucellosis in Dairy Farming

B. abortus, with its broad socioeconomic implications, goes far beyond the microscopic. Infected animals breed brucellosis, which is among other things a reproductive disease in dairy cows (Mathew, 2017). Infections may result in abortion, unusual retention of fetal membranes, Knee hygroma, and a decreased milk-giving capacity (Fig. 3). When brucellosis attacks, the loss to dairy farmers is great. With reduced milk output, veterinarian bills climb and infected cows have to be taken out of the herd (Griffiths, 2010). The pathogen responsible for those effects is *B. abortus*. It uses its sophisticated

ways of escaping detection from the body's defense system to survive within dairy cattle for generations (Webster, 2020). In this passage, many length of sentences are needed to detail the subtle ways this bacteria succeeds as well as the complicated dialogue between the organism and its hosts'protective forces. As we go on with upcoming discussions, we shall shine ever more light on this reciprocation and how Brucella has lived despite the bovine immune response.



3.1 3.2

3.3. 3.4

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Fig. 3: Consequences of bovine brucellosis; Fig. (3.1 and 3.2): Retention of fetal membranes; Fig. (3.3 and 3.4): Abortion in Cows; Fig. (3.5 and 3.6): Knee hygroma

Life Cycle of *Brucella* in Host Cells

The life cycle of Brucella within host cells. Brucella invade host cells, form Brucella-containing vacuoles (BCVs), and undergo fusion with the lysosome in a controlled manner (von Bargen et al., 2012). About 90% of the Brucella are degraded (~90%), and the remaining 10% survive (Gorvel and Moreno, 2002). BCV traffic to and reach the endoplasmic reticulum (ER), and establish the replicative site after ER replication.

Brucella traffic toward autophagy-like vacuoles (Fig. 4), once in this state, the bacteria can live for years in the host's cells and even leap from cell to cell. Various molecular details of this process are considered potential targets for T4SS: absence of late endosome or lysosome markers, acquisition of ER markers; interaction with secretory pathways, acquisition of autophagosome markers; adaptation to the harsh intracellular environment and the ability to regulate activation in key immunological pathways (von Bargen et al., 2012).

The Intricacies of Host-Pathogen Interactions

While issues such as vaccination, abortion, diagnosis, and host specificity can all be important, we have not delimited ourselves (Godfroid et al., 2014). Now, therefore, we turn this topic into the behind-the-scenes story of countless maneuvers between virus and vertebrate in its shedding and hemagglutination phases. The appreciation of exquisite timing and the awareness of both seducer and seduced is a prerequisite to unraveling these two-faced immune-evasion strategies implemented by *B. abortus* itself. They are used to transform the host environment near and avoid infection into something perfectly adapted to the establishment throughout the life of a chronic asymptomatic carrier state (Rădulescu et al., 2020).

Initial Contact and Recognition

All this begins to go wrong when *B. abortus* comes into contact with its host's immune cells. Providing the bacteria

left its outer membrane intact allows them to attach to host cells (Lund et al., 2005). But all this makes possible in turn--and for many is actually what results--their invasion of the cellular space. After this initial touch, the host immune system begins to recognize how exactly it has been compromised; it implants fixed and dynamic passive and active barriers (Solomon et al., 2010).

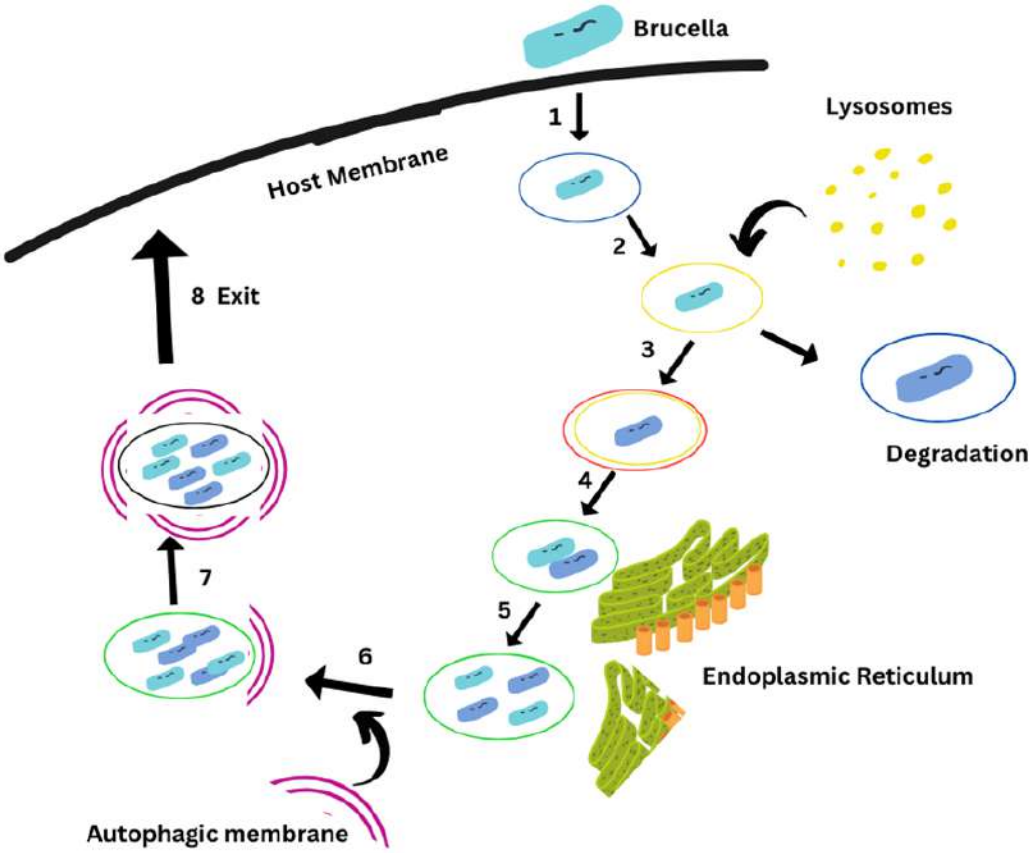

Fig. 4: Life cycle of *Brucella* in host cells

Immune Response Evasion Strategies

However, *B. abortus* has evolved a variety of tactics to evade immunity by the host (Barquero-Calvo et al., 2007). The bacterium can actively inhibit the fusion of the phagosome with the lysosome, and so it remains protected in the host cell, where it can slowly alter the expression of surface molecules and go "unseen" by the host immune system, becoming a stealth presence (Nieto-Sampedro et al., 2011).

Intracellular Survival Mechanisms

Once it enters cells being host to particular viruses, what tactics does the *B. abortus* use so as not to get killed? Just two examples: it manipulates the host cell's signaling pathways to avoid any response by the immune system (Reddick and Alto, 2014); and At the same time, it settles down in phagocytic professionals like macrophages which provide it with a replicative niche. This way they bypass all of your body's normal defenses (Olsen, 2010).

Prolonged Presence Within the Host

By understanding how *B. abortus* suppresses and hijacks the immune system, we hope to begin to understand how it maintains a long-term presence in its host (Pellegrini et al., 2022). The cat-and-mouse format featured here reflects the back-and-forth duel between host defenses and bacterial subversion that define the chronic infection that makes brucellosis such a vexing disease to control in dairy cattle (Špičić et al., 2010).

Strategies of Immune Evasion: A Molecular Viewpoint

In this, we would be discussing the molecular intricacies in *B. abortus* immune evasion tactics, while learning just how exactly it modifies the host's signaling pathways to subvert the immune system defense mechanisms at a molecular level (Fig. 5).

Modulation of Host Cell Signaling Pathways

These results have revealed how it is that *B. abortus* is able to modulate these key pathways in the host cell, and so inhibit their immune activation. This orchestration at the molecular level is key to *B. abortus*' ability to replicate within host cells, and so essential in its promotion of persistent infections (von Bargen et al., 2012).

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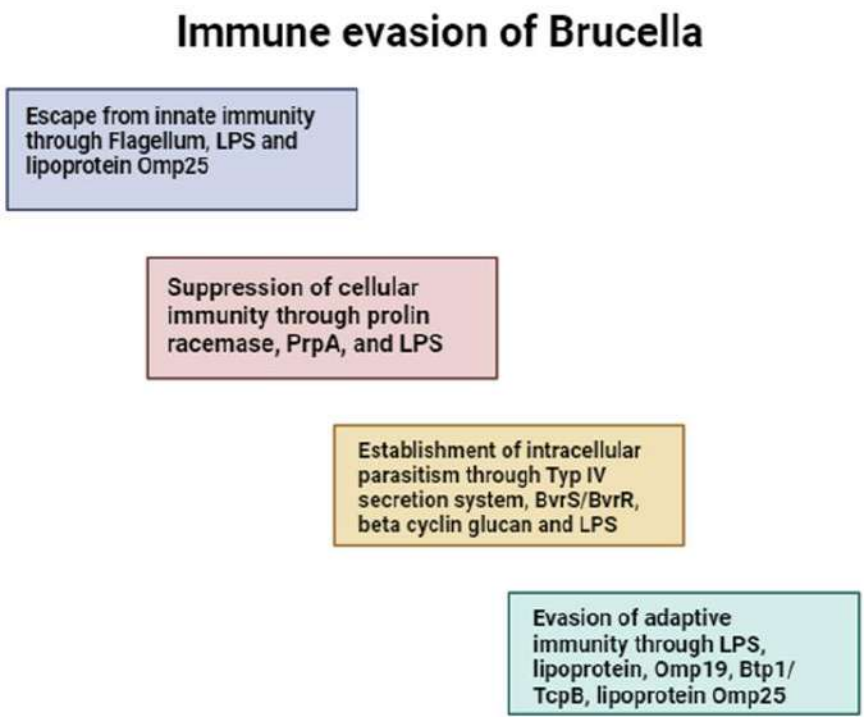

Fig. 5: Immune evasion of Brucella

Virulence Factors and Immune Subversion

Additionally, it also encodes an arsenal of virulence factors that enable it to avoid detection by the host immune system (Jurado-Martín et al., 2021), altering the expression of these factors to avoid heightened recognition and neutralization by the host. Once close to the host, its system of surface antigens, secreted proteins, and more allow the bacterium to persist and proliferate independently within the host (Costerton et al., 1981).

Immunosuppressive Tactics

In addition to this evasion, *B. abortus* deploys a structured series of immunosuppressive tactics. It manipulates and diverts the host immune response, interfering with the production and release of cytokines, which are small proteins that mediate communication between immune cells. This fine-tuning of its immunosuppressive environment allows *B. abortus* to persist (Adetunji et al., 2020).

Manipulation of Host Immune Memory

Moreover, *B. abortus* can pervert the host's immune memory (Steele et al., 2007). To achieve this aim, it manipulates the host's immune response in such a manner that it will not generate a long-term defense and bacteria can keep reinfecting Although a recent story in *Science* suggested that crumbling memory explanations like these might be the making of post-Soviet minds, the longer and longer incubation periods pediatric Brucellosis (Orent, 2004). this co-option of host responses by Mimicry is largely responsible for brucellosis being such an intractable disease (Hybiske and Stephens, 2016).

Impact on Dairy Industry and Economic Implications

This chapter, which is worth pondering over, has taken us on a trip through *B. abortus*'s sophisticated ways of avoiding our immune response--most closely tied to dairy farming where it will surely exact little-noticed as well as potentially mountainous costs (Fig. 6) (Tizard, 2019). The chapter has brought us to the economic aspects of *B. abortus*, and we have come across secondary gain in brucellosis control.

Economic Toll of Brucellosis

This malicious vesiculous disease is significant in several ways to the dairy industry (Segrave, 2008). Large and direct costs include such items as veterinary treatments, diagnostic liquidation of animals and the like, but far greater are the indirect costs resulting from reduced milk production; low rates of reproduction because disease has run its course during mating seasons all year round rather than just one part time in autumn; and a possible loss soon afterwards, when earning power is high but success unlikely without danger (Altizer et al., 2006). These sorts of losses portend hardship for dairy operations, themselves some of the most marginal in any part of agriculture (Dudley, 2002).

Decreased Milk Yields

Milk yields decreased as a result of disease By far the most obvious of symptoms is this, which is bad for all concerned (Sundrum, 2015). There are a number of theories put forward to explain why *B. abortus* causes the cow to drop pounds of milk. Different understandings can provide us with different ideas about how to approach this problem (Alusi, 2014). A logical explanation of why milk yield falls off is a road map for mechanistic control measures to minimize that loss perhaps in many different styles and degrees (Lidfors et al., 2008).

Fig. 6: Summarizing the impact of *Brucella* infection in humans as well in cattle

Culling of Infected Animals

Containing brucellosis means culling (Capparelli et al., 2009). Culling animals that are either infected or suspected of being infected. Culling disrupts the established breeding program and affects the genetic potential of your dairy herd (Berry, 2015). In short, culling is a tough process. The economics of culling reverberate throughout the entire breeding and production cycle.

Strategies for Economic Resilience

Although the road has not been smooth, many elaborate schemes to economically respond to brucellosis have emerged. These interventions, as this chapter has shown range from an even better biosecurity to a focused breeding program to combat the disease (Waage and Mumford, 2008). By increasing the economic resilience of the whole industry, dairy operations are more likely to survive the financial impacts of *B. abortus* infection (Khurana et al., 2021).

Diagnostic Approaches and Challenges

Brucella also known as brucellosis, is a zoonotic rickettsial disease (Seleem et al., 2010). Management measures involving test animals must be taken to prevent it from spreading further or even into human beings. In the battle with *B. abortus* on today's disease management landscape, a critical actor is accuracy and timeliness of diagnosis (Mee et al., 2022).

Serological Tests: Pillars of Diagnosis

Immunological tests are of vital importance. Brucellosis in dairy cattle is overwhelmingly diagnosed by such studies (Ducrotoy et al., 2016). The Rose Bengal Test (RBT), complement fixation test (CFT), and enzyme-linked immunosorbent assay (ELISA) are several tests that identify antibodies that are produced by the host in response to infection with *B. abortus* (Getachew et al., 2016). The pros and cons of those examinations will be looked at here.

Molecular Diagnostics: Advancements and Potential

After development molecular diagnostics makes it possible to diagnose *B. abortus* more quickly and accurately (Kim et al., 2016). Detecting the genetic material of bacteria directly (Sauch as polymerase chain reaction [PCR]) makes the US more sensitive and specific (Hill and Wachsmuth, 1996). Indeed this Suggests golden prospects Its practical use Is, however, also limited by restricted availability and high price.

Challenges in Diagnostic Accuracy

Challenges for diagnosis include the potential for both false positive and negative test results, cross-reactivity with related pathogens, altered sensitivity of tests in different phases of infection, and the infection's general impact on test sensitivity, there is a continued need for both refinement of existing tools and additional technological advances to improve accuracy (Rollo et al., 2016).

Surveillance Strategies and Early Detection:

While individual diagnostics are essential, effective disease control relies on robust surveillance strategies (Schiller et al., 2010). Indeed, the prevention of disease within and across herds depends on the early identification of infections produced by *B. abortus* (Sreevatsan et al., 2000).

Therapeutic Strategies and Future Prospects

As we navigate the complexities of *B. abortus* disease intervention and its reverberations throughout dairy cattle, this chapter has focused on ongoing and forthcoming therapeutic strategies (Singler, 2023). It is the marriage of our current toolbox and future implementations that will propel our field forward and temper the impact of this relentless pathogen (Case, 2017).

Antibiotic Treatments: Current Practices

Antibiotics are one of the main treatment methods for *B. abortus* (Hall and Manion, 1970). We presented a synopsis of antibiotics that have been widely used, such as doxycycline and streptomycin, and a detailed account of their mechanisms. Although they worked well in reducing bacterial numbers, their drawbacks--long treatment time and susceptibility to relapse--were pointed out

Challenges of Antibiotic Therapy

Antibiotics clear a path for bacterial clearance, but their incomplete reach demands thorough attention (Stachelek et al., 2021). *B. abortus* can settle into sanctuary sites to form a reservoir, where antibiotics cannot reach. Delivery of therapeutics may be used along with antibiotic therapy, or in place of it.

Vaccine Development: Progress and Hurdles

Live attenuated vaccines, such as the strain RB51, have shown efficacy in reducing clinical manifestations (de Oliveira et al., 2022). However, challenges related to safety, potential interference with diagnostics, and public perception necessitate ongoing research for safer and more effective vaccines.

Vaccination of Female Cattle against Brucellosis

Strain-19 vaccine

The "Strain-19 vaccine" was the sole brucellosis vaccination utilized in US cattle brucellosis management operations up until recently (Organization, 1970). Strain-19 helps an animal's immune system fight off a brucellosis disease challenge, develop antibodies to the disease's organisms, and eradicate the vaccine's organisms (Elrashedy et al., 2022). Immediately upon birth, calves vaccinated against Strain-19 will pick up anti-brucellosis antibodies from the mother through the colostrum (first milk). For 4-6 months, the calves' blood system will typically contain these developed antibodies, which can neutralize or eliminate live vaccination organisms if given to the calf during this period (Saif and Smith, 1985). Therefore, no heifer calf may be immunized before the age of four months. Because certain calves mature early and acquire a persistent infection from the live vaccination organism (Chase et al., 2008).

RB-51

The novel brucellosis vaccine was first approved by the USDA in 1996, at which point cow vaccination campaigns against the illness started (Moreno et al., 2022). Similar to Strain-19, "RB-51," the new vaccination, is a live vaccine made from the *B. abortus* bacteria that causes brucellosis in cattle. However, the RB-51 vaccination does not induce antibodies that are found by the common serological tests for brucellosis, unlike strain-19 (Ibarra et al., 2023).

As a result, the issue of certain cattle who had brucellosis vaccinations testing positive has been resolved. Similar to Strain-19, only employees of state and federal brucellosis programs and veterinarians with USDA accreditation are authorized to give the new RB-51 vaccination. Heifers must be vaccinated between the ages of 4 and 10 months, and all vaccinated cattle must have an appropriate permanent identity (Nandi et al., 2009). Applying an official USDA brucellosis ear tag and getting an official ear tattoo constitutes permanent identification (Barnum, 2007).

RB51 *Brucella abortus* Detected in Milk from Vaccinated Adult Cattle

Contaminated coagulating milk of mothers fed with an entire dose of attenuated RB51 vaccine during the nursing period that induced the Brucella bacteria in newborn cows (Fig. 7).

(Dougherty et al., 2013). The S19 RB51 viral vector was found in foremilk samples of the calves that had been the vaccinated with S19 vaccine previously (Kalaivanan et al., 2013). The vaccination was segregated in the dairy cattle which has never been vaccinated for 30-60 days after the calving by dipping a long needle into the suspension of *B. abortus* RB51 with the volume of 1.3 x 1010 CFUs. Day 7 (de los Funes-San Luis, 2007): all animals' milk samples were taken every day as well as on and up to day 43, each sampling was taken twice a week until the last ones were collected week until the last ones were collected taken every day as well as on and up to day 43, each sampling was taken twice a week until the last ones were collected.

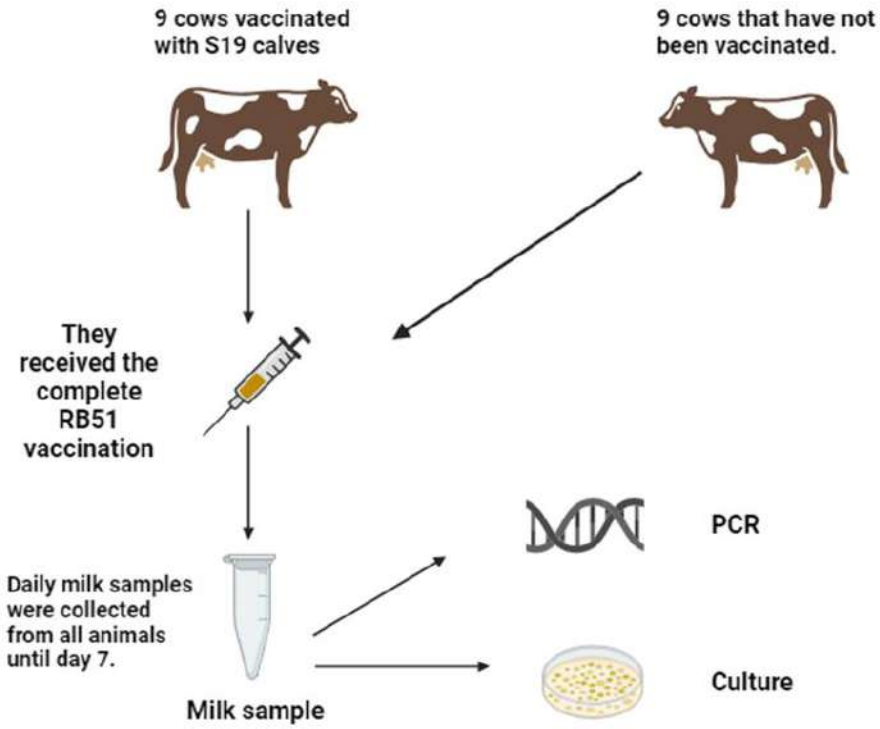

Fig. 7: RB51 *Brucella abortus* detected in milk from vaccinated adult cattle

Testing of the potential shedding of B. abortuis in cow milk was done by bacterial culture and analysis of PCR of milk samples (O'Leary et al., 2006). Field sequencing and subsequent analysis by the specimen series collected did not discover any *B. abortus* strains that matched the reference copy strains. And, the output from laboratory was extremely unfortunate as could have been anticipated. With a multitude of testable variants at my disposal, the lonely PCR gave me a miniscule positive result. On day 1, the response of the sheep being inoculated was less than one week older than cow inoculated before. In a short summary, there are no major health problems causing probable outbreaks associated to the milk or dairy products of the cows given two-step vaccination involving the RB51 vaccinations either before or after the S19 (*B. abortus* Rev 1 for Cattle) procedure (Khurana et al., 2021).

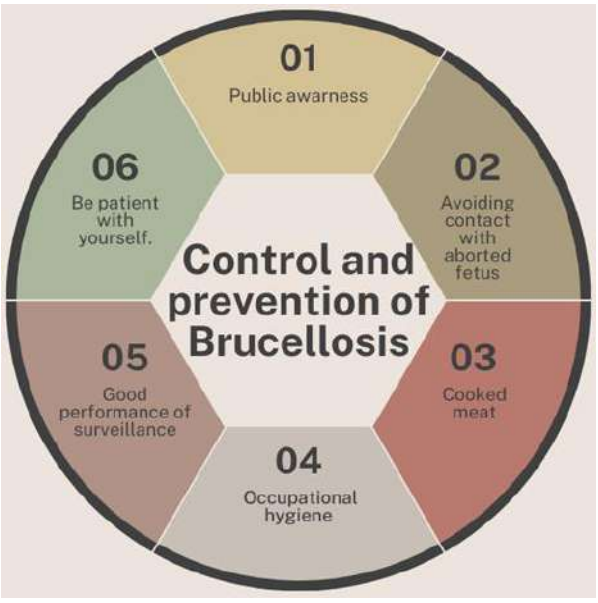

Fig. 8: Control and Prevention of Brucellosis.

Control and Prevention

It's not as critical to separate affected individuals, even though brucellosis infection control and prevention depend on avoiding pathogen exposure to individuals, as the illness cannot be spread among people. As shown in (Fig. 8), when animal eradication (by vaccination and/or the removal of infected animals) is not practical, human infection prevention is predicated on increasing awareness of workplace cleanliness, food safety precautions, laboratory safety, and occupational hygiene (McCormick-Ell and Connell, 2019). The only effective way to control and prevent brucellosis in humans and animals is through multidisciplinary efforts, as neither a veterinarian nor a physician could perform all necessary manipulation techniques alone (Elbehiry et al., 2023). This approach is becoming more and more popular worldwide in recent years.

Future Collaborations and Research Directions

The chapter closes with suggestions for potential collaborations and fields where further research is needed to make use of these new technologies for brucellosis control. International cooperation, interdisciplinary research projects, and new developments in technology point towards a multi-faceted future in the neverending fight against *B. abortus* (Mackenzie et al., 2013).

Conclusion

A *B. abortus*, on the other hand, exhibits a unique capacity to compete with dairy cows' immune system, enabling its ability to develop a chronic infection that is long-lasting, especially in the host population. A lot of process-oriented immunoevasion mechanisms are developed by the Brucella, e.g. formation of the different intracellular vacuoles (BCVs) and controlling their fusion with the lysosome. This ensured the Brucella to stealthily survive and reproduce within the host cells without destroying and hampering the host's innate immune system. The most striking is that their ability to be “in case” the host makes it possible for them to stay in the bog as well as carry the infection to other animalsScientists can approach Brucella management from different angles by focusing on the target pathways associated with gut survival and proliferation, like their interaction with host cell signaling pathways, and the molecular mechanisms that help prevent immune reactions. In the last, the analysis of Brucella's evasion by the immune system paves the way towards improved health in animals, food safety, and reduced economic losses for dairy farmers, which are all key factors for improving the quality of life.

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Chapter 50

Role of Complement Proteins in Phagocytosis of Exogenous Antigens

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ABSTRACT

Proteins that are found in the body's fluids and on cell surfaces are known as complements. Complement effectors, which target specific cells in the immune response, are produced when soluble complement components are activated, setting off a proteolytic cascade. By acting as messengers between complement effectors and target cells, receptors and regulators attached to the cell membrane help keep the complement system from overreacting to pathogen surfaces and injured or stimulated host cells. Protecting the organism against infections and aiding in tissue healing is facilitated by a highly effective system that involves mediators, immune cells, and complement proteins. All sorts of illnesses can start when this "defense machinery" stops working properly. The intricate involvement of complement in inflammatory disorders is well-documented. Deficits in complement, whether inherited or acquired, can greatly promote the development of autoimmunity, while complement activation can greatly increase tissue damage caused by inflammation.

KEYWORDS
complement system, Membrane attack complex, Opsonization, Adaptive immunity, Exogenous antigens

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INTRODUCTION

A century ago, complement was discovered as a temperature-sensitive element in plasma that enhances opsonization by antibodies, thereby aiding in the antibody-mediated killing of bacteria (Markiewski and Lambris, 2007).

The initial study named this component "complement" to indicate its function as a supplementary factor to humoral immunity. As a result, the word implies our long-held notion that the complement system acts as a secondary entity in a subtle manner. This naming approach is based on the historical concept that complimentary components work on the "periphery" of the defensive response, supplementing the functions of other immune system components. The inadequacies of the "complementary" paradigm is evident when recent findings highlight the crucial roles of complement in the innate immune response and its ability to connect innate and adaptive immunity (Carroll, 2004). Today, the complement system is seen as a sophisticated system that orchestrates and connects various reactions in immunological and inflammatory processes, rather than just acting as a bacteria-killing agent (Mastellos and Lambris, 2006).

The complement system consists of regulators, receptors attached to the cell membrane, and other plasma proteins that interact with various cells and immune system mediators (Mastellos et al., 2003). Depending on the pathophysiologic environment, interactions can happen at various stages of the immune response. The many types of complement activation and the intricate network of interactions among its components make it an ideal system for coordinating innate immunity and the adaptive immunological response that follows. This study delves into how complement proteins control and coordinate an early inflammatory response that kills pathogens and injured host cells, which is obviously beneficial for the host. In addition, we discuss how complement plays a part in the pathogenesis of several diseases. Interactions between complement and other immunological components or cells that aid in fighting germs or promoting tissue repair may paradoxically lead to diverse illnesses if the regulatory systems of the immune system are impaired or exposed to unknown stimuli. Overproduction of complement in some pathophysiological conditions can cause harm to the host, whereas pathophysiological circumstances in which complement is completely absent or inadequate might lead to disease development in other cases. Interacting with different cells, the immune system mediates the complement system, receptors connected to the cell membrane, and other plasma proteins (Nathan, 2002).

Complement System

Protection against infections is a critical function of the complementary system, which is the most important part of the immune system(human) (Mathern and Heeger, 2015). This substance is made up of around 30 soluble proteins that are mostly generated by the liver, with a modest contribution from leukocytes. These proteins are found in an inactive (Cochrane et al., 1965) in the plasma, alongside with the membrane bounded regulators and receptors which correspond to different cells and immune system mediator (Mastellos et al., 2003). The complement system is activated with a series of enzymes along with non-enzymatic processes which can result in coating on pathogen- or virus-infected cells with opsonins such as C3b and C4b. Following that, a cluster of proteins gathers and forms a MAC (membrane attack complex), which destroys these cells. Furthermore, activating a complementary system causes manufacturing of Anaphylatoxins which are powerful chemicals that cause inflammation. The complement system helps to eliminate immune complexes and injured cells, as well as aid in the phagocytosis of particles by neutrophils and monocytes (Cho, 2015).

The Complement System Pathways and Activation

Complement activation can occur through three separate routes.

1. Classical Route
2. Lectin Route
3. Alternative Route

Classical Route

Conventional complementary system route is brought to "Antibody Dependent" reason is that it involves the mixing of IgM **/** IgG of Antibodies in the stimulating mechanism. C1q Protein which is segment of the C1 complex consisting of 6 C1q molecules, 2 C1r molecules, and 2 C1s molecules, attaches to Fc region of complement fixing antibodies, often IgG1 and IgM, sticks to pathogenic surfaces more or less cells infected by pathogens. It happens to activate C1r and C1s protease within the C1 complex (Wallis et al., 2010). C1s splits C4 and C2 protease, producing massive fragments C2a, C4b and smaller fragments C2b, C4a. Huge parts combine to form the C2aC4b complex on the surface of pathogen leads to cleavage of C3 in Anaphylatoxin C3a and opsonin C3b. Formation of convertase C3 occurs where 3 complement routes meet and subsequently follow similar procedure to produce MAC (Mathern and Heeger, 2015).

Lectin Route

Lectin complementary route is more alike to a classical route, except it works without immunoglobulins. Instead of detecting antigen antibody complexes, it makes use of germ-line encoded pattern Recognition Receptors (PRRs) such Mannose Binding lectin (MBL) and Ficolins (Janeway et al., 2001). The molecular patterns that viruses and other pathogens utilize to attach to carbohydrates now called Pathogen Associated Molecular Patterns (PAMPs) (Medzhitov and Janeway Jr, 2000). This sets in motion MBL-associated serine proteases (MASPs), such as MASP-2 and MASP-1, which break down C2 and C4 components of the complement system, ultimately producing C3 convertase (Medzhitov and Janeway Jr, 2002).

Alternative Route

In comparison to classical and lectin processes, the alternative complement system route contains three somewhat overlapping steps. Viruses play a key role in the alternate route as initiators (Gupta and Tripathy, 2020). This route produces C3H2O via a steady process that begins in the plasma with the synthesis of C3 convertase. Surfaces devoid of Complement Regulatory proteins trigger the activation of C3. Surfaces that take in C3 cause changes in conformation. Factor D (FD) can divide factor B (FB) into two portions (FBb and FBa) when C3H2O interacts with FB to induce a conformational shift (Janeway et al., 2001). Like C2aC4b enzymes in classical and lectin routes, this enzyme converts C3 into C3a and C3b. When C3b is ready, it attaches to the cell's surface and combines with fb to become C3bBb, the primary convertase in the different route (Walport, 2001). By preventing factor H (FH) and factor I (FI) from rendering the C3bBb complex inactive, properdin increases the complex's stability. In order to activate the branch of the different complementary system route, surface-bound C3 convertases can activate C3 from any source (Harboe and Mollnes, 2008). As a result, C3b builds up in the body and convertases with an extra C3b molecule (C4b2b3b or C3bBb3b) are gradually produced, modifying their target to C5. In the alternate route, the C5 convertase forms Anaphylatoxin C5a and the C5b piece via enzymatic activity, splitting C5. Complex is introduced in cell membranes when C5b attaches to C6 and C7. The formation of the MAC complex C5b6789 is caused by interactions with C8, which in turn induce the attach of several C9 units (Müller-Eberhard, 1985).

Activation of Complement System

Coating the targeted surface with opsonin's C3bi, C3b, and C4b directly destroying target surfaces via MAC; stimulating and activating the immune system by producing potent proinflammatory anaphylatoxins and these three pathways are formed when the complementary system is activated, allowing system to carry out its defensive functions (Walport, 2001). The complement system destroys harmful cells by assembling special complexes called membrane attack complexes (MAC) ((Dunkelberger and Song, 2010). Anaphylatoxins with wider immune regulatory activities can be formed by certain cleavage fragments and complement activation products. All three pathways can produce the cleavage products

C3a and C5a, which have strong effects on immune regulation. All that is produced is C4a by the classical and lectin routes. The presence of C3a and C5a can entice various immune cells to the site of inflammation and infection, including eosinophils, macrophages, mast cells, fibroblasts and monocytes (Figure 1). Neutrophils are drawn towards C5a (Morgan, 1998) while C4a increases endothelial cell permeability, promotes stress fiber production, and carries out various tasks (Wang et al., 2017). Chemotaxis is aided by C3a and C5a, which also control vasodilation and enhanced vascular permeability (Williams, 1983), furthermore to the cytokine production, which includes IL-1β, IL-8/CXCL-8, CCL5, IL-6, and tumor necrosis factor-α (TNF-α) (Monsinjon et al., 2003).

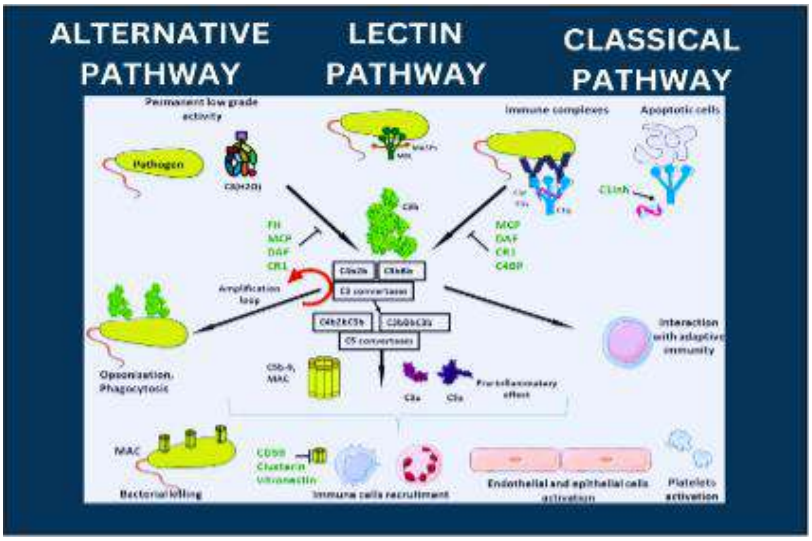

Fig. 1: Illustrate how complement system become activated

Complement as a First Line of Defense against Pathogens

Opsonization and Phagocytosis

The main role of complement in eradicating infections is an indirect one, achieved by depositing complement fragments on the surface of pathogen targets. Pathogens are recognized, consumed, and destroyed by phagocytic cells, which include neutrophils, monocytes, and macrophages, in a process known as opsonization (Figure 2). C3 fragments and IgG antibodies both serve as traditional opsonin's. The process of complement opsonization involves activating the alternative route on pathogen surfaces directly, enabling phagocytes to eliminate them prior to the adaptive immune response and antibody production (Figure 2). According to the article "Complement System Part I: Molecular Mechanisms of Activation and Regulation," phagocytes possess distinct receptors for C3 fragments (Merle, 2015).

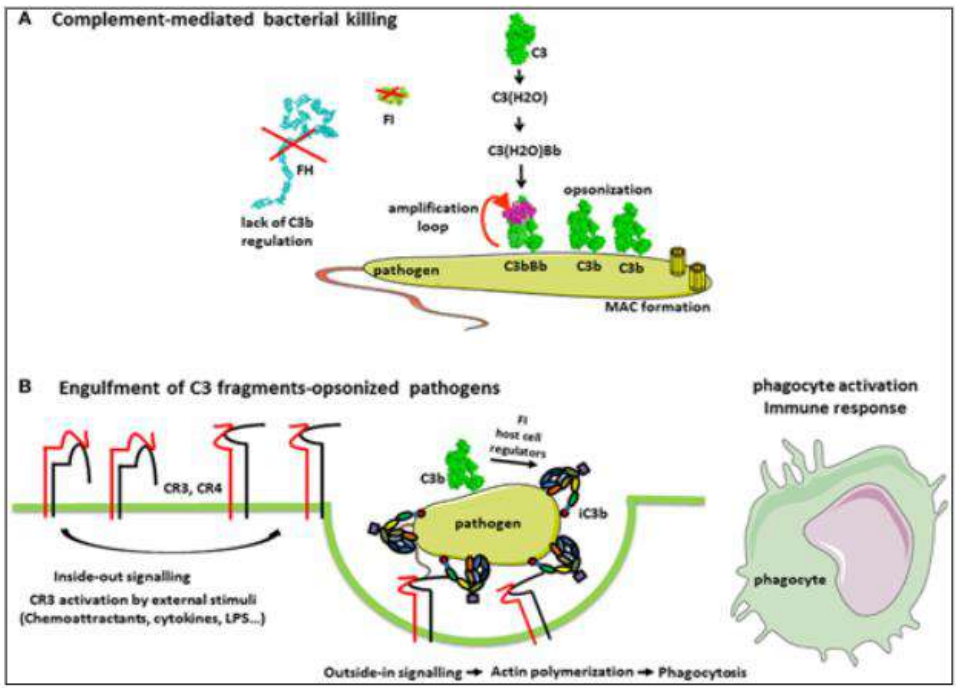

Fig. 2: Illustrate role of complement system against bacteria and pathogens

Fig. 2: (A) Complement-dependent bacterial eradication. C3b is accumulated on the surfaces of pathogens as a result of the continuous activation of the alternative pathway (AP). Due to the absence of complement regulatory molecules in most

pathogens, C3b remains active and combines with FB and FD to create a C3 convertase known as C3bBb. This enzyme complex hydrolyzes more C3 molecules, leading to the opsonization of pathogens by the formation of C3b. Additionally, the cascade progresses towards the synthesis of a C5 convertase and the creation of MAC, which aids in the elimination of bacteria.

(B) Phagocytosis of pathogens that are coated with C3b and iC3b, facilitated by complement receptors. The CR3-mediated phagocytosis process requires extracellular stimulatory signals such as chemoattractants (including chemokines, bacterial formylpeptides, and C5a for neutrophils), cytokines (such as TNF-α), and bacterial products (such as lipopolysaccharide). The integrin CR3 is activated by external stimuli, namely through a Rap-1-mediated signaling pathway, resulting in a conformational shift that increases its affinity for iC3b. The association of CR3 with iC3b stabilizes its high-affinity conformation, leading to the activation of a RhoA-mediated signaling pathway. This signaling pathway then promotes actin polymerization, which results in the engulfment of iC3b-coated targets. The intracellular migration of the phagosome throughout its maturation to the phagolysosome involves intricate actin motions.

The complement component molecule (CCP) domain found on the molecule CR1 controls the activity of C3 convertases. It can be found on several cells in the body, including those that carry oxygen, those that fight off infections, and the specialized cells in the kidney called glomerular podocytes. Particularly C3b and C4b molecules bind to this receptor. The removal of soluble immune complexes is a crucial function of the complement receptor 1 (CR1) on red blood cells. Macrophages in the liver and spleen remove them when they are transported there. Initiation of phagocytosis is not only dependent on C3b-coated target binding to phagocyte CR1. The phagocytosis of targets coated with both IgG and C3b via FcγR is enhanced by the interaction between C3b and CR1. When C3b is opsonized alone, target phagocytosis is improved by immunological mediators like fibronectin or LPS (Griffin et al 1990). However, in this case, the role of CR1 is predicted to be slightly impacted. Elastase, a key protease generated by active phagocytes, can degrade CR1 and convert C3b to iC3b. The extremely effective phagocytic receptor, CR3, identifies iC3b-coated targets (Tosi et al., 1990)**.**

Two separate receptors, CR3 and CR4, attach to a particular segment of C3 called iC3b. These receptors initiate phagocytosis by locating iC3b-coated targets (Uotila et al., 2013). Members of the integrin family CR3 and CR4 interact with intercellular molecule-1 (ICAM-1) as a mechanism for cell attachment. A wide variety of cells contain ICAM-1, including endothelial cells. Integrins can't do their jobs properly without the magnesium ions found in their alpha and beta chains. The integrin subfamily that is particular to leukocytes consists of CR3, MAC-1, αMβ2, or CD11bCD18, in addition to CR4, p150,95, αxβ2, or CD11cCD18. Other members of this subfamily include LFA-1 and αDβ2. These integrins share the β2 chain, which is generally known as CD18. In contrast to iC3b and ICAM-1, the CR3 receptor has its own lectin site. Bacterial sugar ligands may be detectable at this lectin location. Acceleration of the phagocytic response occurs with activation of the lectin and iC3b-binding sites on CR3 (Ross and Větvička, 1993).

When monocytes differentiate into macrophages, the expression of CR3 increases. A little amount of CR3 is present on the surface of resting neutrophils. A substantial rise in CR3 levels is observed upon stimulation of these cells. The receptors are released from their huge reservoirs inside the cell, which is why this happens. The expression of CR4 is low on monocytes and neutrophils, but it increases as monocytes develop into dendritic cells or macrophages (Dupuy and Caron, 2008).

When engulfing targets coated with C3 or IgG, the cellular mechanisms are different. The involvement of CR3 in phagocytosis slows it down, which allows pathogens to enter phagocytes more slowly via an actin polymerization process that relies on the small GTPase Rho (Dupuy and Caron, 2008). Fcγ receptors are unique among receptors in their ability to internalize IgG-coated pathogens through the use of membrane extensions triggered by small GTPases Rac and Cdc42. The phagocytic response that CR3 initiates against targets coated with iC3b is enhanced when other receptors, particularly toll-like receptors (TLRs), which recognize patterns of pathogens, are involved.

Phagocytosis is primarily induced by CR3 and CR4 in conjunction with phagocytic cell activating stimuli such as pro-inflammatory cytokines (Aderem and Underhill, 1999). In response to the stimuli, integrins undergo a conformational change into an active state, which increases their affinity for iC3b and ICAM-1 (Dupuy and Caron, 2008). Because the alternative pathway is continuously and slightly activated, this might function as a control mechanism to avoid undesirable reactions in host cells with low iC3b molecule amounts. In addition to CR3 and CR4, CRIg is expressed by some resident macrophages, like liver Kupffer cells. iC3b is an immunoglobulin family member and an immune system component; CRIg is a receptor for this protein. The process of absorbing microorganisms covered in iC3b can be enhanced by CRIg (Gorgani et al., 2008).

The elimination of infections is the primary goal of phagocytosis. Toxic reactive oxygen molecules produced by a NADPH oxidase complex on the phagosomal membrane and microbicidal components such as lysozyme and proteases from phagocyte granules combine to create the phagolysosome, which eliminates internalized bacteria. Critical for resolving inflammation and infections, CR3-mediated phagocytosis causes phagocytic cells to apoptose (Mayadas and Cullere, 2005).

A Sudden and Intense Reaction to Inflammation

The complement system is responsible for the effective regulation of several stages of an inflammatory response. The pathophysiology of inflammation is multi-mediated and involves many different kinds of cells and tissues. Any event that damages cells can trigger its activation. As a result of an infection, inflammation frequently develops. On the other hand,

physical or chemical harm is the exclusive way to trigger this reaction (Nathan, 2002). While minimizing harm to host tissues and restoring damage done by the original component, the primary objective of this strategy is to remove the causal element.

How long an inflammatory reaction lasts depends on how well the original substance is neutralized and cleared. The duration of acute inflammation typically ranges from a few minutes to a few days, and it is a temporary condition. Fluid and plasma protein release and white blood cell migration from blood arteries to surrounding tissues are the key features. Inflammation is controlled by cellular and vascular responses through chemical mediators produced by plasma or cells. Swelling, redness, pain, heat, and loss of function are the classic clinical signs of inflammation, which were initially listed by Aulus Cornelius Celsus and further clarified by Rudolph Virchow. If the first reaction doesn't get to the bottom of things or fix the damage at the site of inflammation, the body may enter a chronic phase where it dies and repairs tissues all the time. Triggers for chronic inflammation might be triggers for moderate, subclinical reactions.

When exposed to harmful stimuli, any tissue might develop an inflammatory reaction. The reaction of connective tissue that is vascularized is the characteristic feature of this process. Blood vessel blood flow variations and changes in tiny blood vessel size are early indicators of inflammation. Following a brief period of restriction, which only lasts a few seconds, the injured area's tiny arteries dilate, forming new networks of blood vessels. Vasodilation describes this process. Arterioles enlarge and new capillaries develop, allowing for a greater influx of blood to this region. Fluid leaking into an extravascular region occurs as a result of endothelial alterations that increase microvasculature permeability. Reducing the volume of fluid in the blood arteries makes the blood thicker, which slows its flow rate. The process of leukocyte margination begins when there are changes in blood flow. Eventually, leukocytes transmigrate into the interstitial tissue after adhering firmly (adhesion) and rolling (temporarily) to the endothelium. Acute inflammation has accomplished its primary objective, which is to transport plasma mediators and white blood cells to the site of injury (Markiewski and Lambris, 2007).

The Role of The Complement System in Adaptive Immunity is to Regulate B-Cell Activity and Facilitate Humoral Immunity

Exemplary and well-defined components of the innate host defense mechanism include the aforementioned complement system functions—opsonization, lysis, and generation of the inflammatory response through soluble mediators. More and more, people are starting to see that complement's function in host defense goes beyond innate immune responses alone. As early as the 1970s, debate regarding the potential role of the complement system in adaptive immune responses began with the observation that B cells demonstrated C3 binding. Subsequent research demonstrated that C3 deficiency impaired antibody production and provided conclusive evidence that, in some contexts, an optimal complement system was necessary for good adaptive responses (Pepys, 1972). Animal studies showing complement protein deficits further demonstrated the classical pathway's critical involvement in the efficient capture and retention of antigens in lymphoid tissues, such splenic follicles. Based on these findings, it appears that the complement system mainly helps to concentrate foreign antigens at specific immunological locations where cell responses are most effective (Papamichail et al., 1975).

By means of effector and memory B cells, in addition to antibodies generated by B cells, the humoral component of the adaptive immune response ensures the protection of extracellular regions. Pathogens are neutralized and opsonized during this phase, which also gives immunological memory to avoid reinfection. Several factors, including the strength of antigenic stimuli and the availability of helper T-cell support, interact intricately to determine the efficacy of this response. Throughout B-cell development, complement effectors contribute to humoral immunity and have several impacts on B-cell biology (Carroll, 2008). B lymphocytes and follicular dendritic cells (FDCs) include complement receptors (CRs), the most important of which are CR1 (CD35) and CR2 (CD21), which enhance B-cell immunity. This collaboration between the phagocytic system and complement happens when the latter binds to the opsonin of the former. B-cell coreceptor complexes (CD21-CD19-CD81) consist of CR2, a signaling protein CD19, and a tetraspan protein CD81. When this complex interacts with antigen coated with complement opsonins, such as C3d, it raises the signal conveyed through the B-cell receptor (BCR), which includes surface immunoglobulin. This results in a multi-order reduction in the activation threshold of B-cells. So, the complement system is like a "inherent enhancer" that helps the humoral immune response progress (Dempsey et al., 1996).

This change in B-cell signaling can affect how cells work in different contexts. B cells first express the CD21-CD19-CD81 coreceptor when they migrate from the bone marrow to the periphery. The transitional stage is an important time for choosing B1 cells and removing B cells that react to the body's own tissues. The B1 cell population is long-lived and physiologically distinct; it is responsible for producing natural antibodies and has a very selective repertoire for conserved antigens like nuclear antigens. It seems that the complement system is involved in B1 cell selection and maintenance. Animals lacking CR2 show a different spectrum of natural antibodies, proving this point. Damage during ischemia or reperfusion is significantly reduced in these animals, even if their IgM levels are normal. Furthermore, these animals show a decrease in B1a cell numbers and a general impairment in antibody production (Ahearn et al., 1996).

Testing animals deficient in either complement components or CRs reveals the complement's roles in humoral immunity. For the immunological response to thymus-dependent and thymus-independent antigens, it is essential to have an intact complement classical pathway (C1q, C3, or C4), according to research (Carroll, 2008). Both proteins are expressed

by the CR1/2 gene in mice, and animals missing this gene often have similar impairment, suggesting that both receptors are responsible for driving pro-humoral responses (Carroll, 2004). Mice deficient in CR1/2 and C3, for example, had significantly reduced IgM (and IgG) levels, could not convert to IgG, and had trouble absorbing antigens from T-independent type II polysaccharide antigens. Keyhole limpet hemocyanin and bacteriophage ΦX174 are examples of T-dependent antigens. Herpes simplex virus, West Nile virus, and Streptococcus pneumoniae are bacterial and viral diseases (Haas et al., 2002). The importance of complement in several areas of B-cell biology for the establishment of a robust antibody response has been highlighted in these and other research.

The Role of the Complement System in Adaptive Immunity Specifically Pertains to T-Cell Immunity

Relationship of supplement in the other side of adaptable barrier, the Safe framework microbial response, would be usual considering the astonishing variety of activities mediated by supplement that influence the period of convincing humoral reactions. Certainly, the responsibilities of normal protection from lymphocyte mediated safe responses were laid out in Janeway's conception of the 'adjuvant effect' as the outcome of the effect of the inalienable invulnerable system on got invulnerability, approximately twenty years ago (Janeway, 1989). Instantly, it was suggested that supplementation was significantly more important in B-cell research, as a large number of enhancement/CR-lacking mice showed clearly typical lymphocyte responses (Da Costa et al., 1999). Regardless, a more condensed role for supplement was suggested by the finding that CD4 and CD8 lymphocyte planning was reduced in C3-lacking mice following pneumonic influenza exposure. Multiple studies have shown that supplements play an essential role in lymphocyte safety responses to viruses and alloantigens (Li et al., 2008). A crucial area of study in comprehending the roles of supplement in regulating flexible safe responses is the components of this impact that are not overly shown as those related to humoral resistance. Using a DAF-lacking mouse model, we were able to depict the logical occupancy of supplement in lymphocyte opposition. DAF necessitated prolonged supplement permission in a number of in vivo conditions, which certainly made it possible to improve and more clearly observe the prospective directing influence of supplement on lymphocyte protection against than in regular mice. As an illustration, research has demonstrated that animals missing DAF have a more favorable TH1 response, as seen by increased production of interferon-γ and IL-2 and reduced expression of IL-10 in response to reinitialization with antigen. The DAF−/− mice also exhibited increased pathology in the multiple sclerosis mouse model and preliminary safe framework encephalomyelitis (Heeger et al., 2005; Li et al., 2008).

In addition, a model of lymphocytic choriomeningitis disease (LCMV) tainting shown that DAF−/− mice had a superior white platelet safe response. Everything points to a clear and unequivocal total for protracted white platelet blockage in DAF−/− mice, but the crucial component that contributes to this total is really not yet resolved. Some have hypothesized that in the absence of DAF, the activation of AP supplements on APCs and lymphocytes is prolonged, leading to increased production of local anaphylatoxin. A costimulatory clue to White platelet persistence and order is therefore provided by the occurring responsibility of C3aR/C5aR inside the immunological synapse.

It has also been suggested that ordinary (i.e., wild-type) mouse APCs, where DAF is perfect, undergo AP-mediated C3a production and C3aR responsibility. It was recognized that APCs triggered by C3aR motions include cAMP as a subsequent signal and contribute to the regulation of APC development and antigen uptake. Since there have been some concerning reports on the subject, it is important to do more thorough evaluations to see whether anaphylatoxin receptors are indeed expressed in lymphocytes and master APCs (dendritic cells), since this might cast doubt on these notions. In many animal models, C5aR has been shown to be the primary regulator of the supplement's effect on white blood cell blockage. In one study, researchers found that after infecting mice with influenza type A, those treated with C5aR miscreants produced less CD8 lymphocytes that express antigens (Fang et al., 2007). This is in line with the discovery that when infected with LCMV in the presence of C3 or C5aR, DAF-lacking mice display a reshaped naĎve and memory CD8+ lymphocyte response. Another piece of evidence is the finding that animals without designated C5aR had a reduced ability to aspirate when exposed to Pseudomonas aeruginosa in a pneumonic pollutant, even while neutrophils are clearly present (Höpken et al., 1996). Also, in mice, C5aR mediates a synergistic link with Cost-like receptor (TLR)-4, leading to a more robust inflammatory response when both normal safe receptors are activated at once, as compared to when either one is activated alone (Zhang et al., 2007). Given that the TLR structure, like enhancement, detects directed pathogenic topics and is frequently activated simultaneously with the enhancement system, suggesting that these two effectors of the regular safe system may interact with each other, potentially influencing White platelet safe responses, this association makes sense (Hawlisch and Köhl, 2006).

Conclusion:

Inflammation and the immune response to some bacterial infections are both aided by the complement system. Damaged immunological responses associated with autoimmune diseases and reactions to incompatible blood transfusions can also activate complement. The main role of the complement system is to defend the host against inflammation and infections by attracting innate immune cells (chemotaxis) and improving their ability to phagocytose (opsonization).The complement system is an essential part of the innate immune response, serving as the first line of defence against infections. It can be activated through three distinct pathways: classical, lectin, and alternative, all of which converge on the core component C3, resulting in a cascade of responses that improve phagocytosis, promote inflammation, and, eventually, pathogen lysis. To prevent damage to host tissues, the complement system must be

properly regulated; numerous regulatory proteins ensure that its activity is modulated appropriately. A comprehensive comprehension of the mechanisms and regulatory mechanisms governing the complement system yields essential knowledge regarding its impact on both well-being and pathology. Such knowledge lays the foundation for the development of therapeutic strategies targeting disorders that are influenced by complement activity.

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Chapter 51

Management of Escherichia coli (E. coli) Infections Using Alternative and Conventional Medicines

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ABSTRACT

Escherichia coli (*E. Coli*) is a human large intestine's major inhabitant. Most of the strains of *E. coli* are harmless and non-pathogenic but strains with DNA encoded enterotoxin plasmid and invasion factors can cause diseases and infections in humans and becomes pathogenic. Such strains like enteroaggressive, enterotoxigenic, enteropathogenic, enterohemorrhagic, enteroinvasve, cell diffusive and cell detaching are known to be causes of various diseases and illness around the world like. Additionally, these strains possess the ability to secrete Shiga toxins that is the major cause of hemolytic uremic syndrome (HUS) and haemorrhagic colitis. Avoid eating raw meat, consumption of raw milk, use pure or boiled water free from any contamination and avoid vegetables grown in unhygienic conditions i.e. contaminated water. Upon suspicion of any of the infections, families are advised to contact doctors as soon as possible to avoid serious circumstances. These diseases are major burden to healthcare system. Multidrug-resistant *E. coli* is of great concern these days worldwide because of spread of its genetic material such as plasmid, leading to increase in the prevalence. The activity reviews about the management of the infections caused by *E. coli* and how to effectively treat the illnesses.

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INTRODUCTION

E. coli is a gram -ve bacillus bacterium, found commonly in the intestines of warm-blooded organisms and inhabitants of human flora. Most of the strains are harmless but can cause serious food poisoning. They are associated with various intestinal and extraintestinal diseases and thus lead to vast spectrum of diseases that may range from mild self-limited GI inflammation to septic shocks and to severe life-threatening renal failure. This writing will review about the epidemiology, etiology, pathophysiology, diagnosis, and management of *E. coli* infections. *E. coli* is subdivided into two main categories.

1. Intestinal pathogenic *E. coli*
2. Extraintestinal pathogenic *E. coli*

Etiology of *Escherichia coli*

E. coli are commensals that are a part of human flora. The human can easily be exposed to *E. coli* in hospital facilities like floor and in long-term patient care centre but does not cause any disease because of the lack of virulence. Enteroaggressive *E. coli* has been found to be the major cause of travellers' diarrhea in latin Africa, Asia, and America (Adachi et al., 2001). Moreover, when strains of *E. coli* are exposed out of intestine like lungs, genitals colon they can cause pneumonia, bacteraemia, septicaemia, uncomplicated colitis and UTIs (Mylotte et al., 2002). Several strains of *E. coli* like Shiga toxin producing *E. coli* (STEC) are found to be the cause of bloody diarrhea and sometimes lead to development of hemolytic-uremic syndrome (HUS). They are leading causes of thousands of illnesses and ultimately death of patients in United States (Talan et al., 2001).

These strains are equally contributing towards diseases in animals such as bovine mastitis, a, udder tissue parenchymal inflammation in animals that contributes to the abnormalities in milk and pathological changes in the glandular tissues caused by MPEC (Mammary Pathogenic *E. coli)* that serves to resist the host innate defence in mammary gland (Goulart and Mellata, 2022).

The most common way of exposing to *E. coli* infection is by consuming vegetables that are grown in contaminated water reservoirs and by consuming food like ground beef because when they are slaughtered bacteria from the ground

get into the meat, when such food is consumed by human, they cause intestinal diseases. Community acquired UTIs are majorly caused by *E. coli* among elderly women and 1 in 3 infections in adults is caused by gram positive bacteria (Ronald, 2002).

Intestinal Pathogenic *Escherichia coli*

Epidemiology, Pathophysiology, Diagnosis and Treatment of *E. coli* infections

E. coli is the non-pathogenic inhabitant of human intestine flora alongside they are found to be causes of various central nervous and gastrointestinal and urinary tract systems infections. Such pathogens are recognized by the presences of the O antigens and H antigens which are attributed by having repeated units of polysaccharides in LPS of the outer membrane and flagellum, respectively (Nataro and Kaper, 1998). These pathogens are easily and predominantly found to cause diarrhea in infants and various other diarrheagenic diseases.

Some strains of *E. coli* that are found to cause infections both in human and animals are as follow:

1. Mammary Pathogenic *E. coli* (MPEC)
2. Enteroaggressive *E. coli* (EAEC)
3. Enterotoxigenic *E. coli* (ETEC)
4. Enteropathogenic *E. coli* (EPEC)
5. Enterohemorrhagic or Shiga Toxin producing *E. coli.* (EHEC/STEC)
6. Enteroinvasive *E. coli* (EIEC)
7. Diffusively Adherent *E. coli*

Mammary Pathogenic *E. coli* (MPEC):

Epidemiology

These pathogens are found to cause bovine mastitis in animals and are categorised into two types, (Oliveira et al., 2013) the clinical mastitis characterized by redness, pain on palpation, edema and hardening of the mammary gland upon examination and visible abnormalities in udder and milk appearance as clots, flakes, blood discharge and clear serum found to be associated with mastitis. *E. coli* is associated mastitis exhibits symptoms like cold extremities, diarrhea and dysstasia in animals with acute infection of 10-30 days duration but can reappear with lactation (Goulart and Mellata, 2022). From 1996-2014 the incidence of the clinical mastitis was increased from 13% to 25% in united states dairy forms (USDA, 2016). The subclinical mastitis was 15-40%, more common than clinical mastitis and cannot be detected via visual inspection and is commonly caused by *E. coli* and *Klebsiella pneumonia* (Seegers et al., 2003).

Pathophysiology of MPEC

A type of extraintestinal pathogenic *E. coli* has acquit VFs that helps colonization of its special niches within the environment of the mammary gland that include microbes, soluble and antimicrobial in milks that is lactoferrin and lactoperoxidase thus leading to occurrence of bovine mastitis (Goulart and Mellata, 2022; Koshiishi et al., 2017).

Diagnosis

Clinical mastitis can be diagnosed by evaluating the visible abnormalities in udder and milk and for subclinical mastitis the reduction of milk. Other diagnostic approaches that help to evaluate mastitis are the estimation of the SCC that is the indicator of the mammary gland health and quality of milk (Adkins and Middleton, 2018). Microbial culture from the composite sample is also a tool for evaluating the presence of *E. coli* in clinical mastitis. CMT is also a test that helps evaluate bovine mastitis which involves factors like lactation number, stress, yield of milk, season and breed that can disturb SCC values and its results can be false positive or negative (Swinkels et al., 2021).

Conventional Treatment

Herd management and good environmental hygiene are crucial factors for avoiding mastitis. Fluid therapy and treatment with steroids and NSAIDs like dexamethasone considered as first line of treatment and parenteral fluoroquinolones administration like marbofloxacin or third generation cephalosporin like ceftiofer is recommended for the management of severe mastitis (Krömker and Leimbach, 2017). Another approach for management of the infection is through vaccination with *E. coli* J5 strain (Suojala et al., 2013).

Alternative Treatment

The plants extracts are gaining interest for development of therapy for mastitis as compared to antimicrobial therapy they have an advantage that is no resistance induction even after exposure for long time. Plants extracts like baicalein a flavone extract from *Scutellaria lateriflora* claims to attenuate inflammatory response suppressing TLR4 mediated pathways and thus LPS-induced mastitis in mice (He et al., 2015). Similarly, thymol extracted from thyme is found to have anti-inflammatory effect on mammary gland of mice (Liang et al., 2014). Other plants extracts like resveratrol, curcumin, terpeneless compounds are found to have properties like inhibition of mitochondrial-related cell death, attenuate the activity of myeloperoxidase thus treat duct of mammary gland in mice and possesses antimicrobial activity against MRSA, respectively, ultimately management of bovine mastitis (Cheng and Han, 2020).

Entero-aggressive *E. coli* (EAEC)

Epidemiology

Infections are recognized by their pattern of adherence to HEp-2 cells in *E. coli* separated from Chilean children suffering from diarrhea. They are associated with foodborne diarrhea, 2nd most common cause of traveller's diarrhea and endemic diarrhea in the US (Kaur et al., 2010; Nataro and Kaper, 1998), persistent diarrhea including diarrhea in healthy adults and adults with HIV infections. According to meta-analysis report of 41 studies it has been found that these pathogens found to be the major factors contributing to acute diarrheal illnesses in children and adults in developing regions (Huang et al., 2006).

Pathophysiology

The pathogenesis compromises of three stages adherence to the mucosa of intestine using aggregative adherence fimbria factor, then increase the mucous covering EAEC leading to the formation of thin layer over the surface of enterocytes (cells of intestine) and then toxin release alongside inflammatory response activation and intestinal secretions. The summary of the process is the AggR which encodes plasmid that is transcriptional activator helping in activation of several virulence factors including AFF, adhesins, enterotoxin Pet, SheT-1, SheT-2, EAST-1, and surface active dispersin that contributes to AFF-mediated colonization induction. According to studies, virulence factors like EAST-1 and CVD432 are markers associated with chronic diaarhea in children (Asif Sofi, 2023; Govindarajan et al., 2020).

Diagnosis

EAEC has characteristic aggregative adherence of the stacked-brick when cultured in Luria broth in Hep-2 at 37 degree Celsius for 3 hours thus helping in identification of the strains (Nataro and Kaper, 1998). The Hep-2 positive strains were positive for antiaggregating protein transporter gene by PCR. Using biofilm assay it is easy to screen them (Albert et al., 1993).

Conventional Treatment

The antibiotic therapy for management of EAEC infection is based on the individual and local susceptibility pattern of antibiotics because it is self-limiting infection. In most of the countries the EAEC strains are susceptible to fluroquinolones, nalidixic acid, rifaximin and azithromycin due to integrons including dfrA5, aadA1a and oxa5 contributing to resistance of antibiotics (Gassama et al., 2004). The EAEC association with persistent disease make it vulnerable to manage it only with rehydration therapy and vaccination as preventive strategy adopted for the management of infections (Mayer and Wanke, 1994). The lactoferrin from human milk and bovine lactoferrin are found to manage and reduce the complications of the diarrheal symptoms in multiple studies where it inhibits the "stacked-bricked" aggregative adherence in cultured tissue cells (Ochoa et al., 2006).

Alternative Treatment

According to studies where authors elicit the use of extracts from oregano (carvacrol) and Hb extract (brazilin) against EAEC. The author concluded that carvacrol (10mg/ml) is the most effective agent against EAEC by altering the aggregative adhesion pattern. Studies says that there were changes in the adhesion related genes (AggR) and oxidative stress-related genes thus interfering target sites in *E. coli* leading to adhesion reduction (Ortiz et al., 2021).

Enterotoxigenic *E. coli* (ETEC):

Epidemiology

These strains are divided into two types based on colonization factors i.e. type IV pili and chaperone-usher pili. These strains are common in areas where there is improper sanitation facilities or consuming food grown in contaminated water and causing diseases like watery diarrhea, traveller's diarrhea and dehydrating illness in children and infants (Zhang et al., 2022).

Pathophysiology

These strains are divided into two types based on colonization factors which are type IV pili and chaperone-usher pili. These strains are common in areas where there is improper sanitation facilities or consuming food grown in contaminated water. These strains are characterized by the ability to produce heat liable (LT) and heat stable (ST) enterotoxins encoded on the plasmid. The LT is heteroheximer made up of single A subunit and pentameric B subunit and is like cholera toxin (CT). The LT at the surface of intestinal epithelial cells binds via GM-1 ganglioside, which is followed by toxin uptake, removal of biologically active subunit A. The LT is ADP- ribosylating toxin transferring ADP-ribose to targe molecule. LT-A catalyzes a series of reaction forming cAMP similarly, ST forms cGMP. These cAMP and cGMP activates intracellular protein kinase leading to phosphorylation followed by alteration ion channels including chloride channels of cystic fibrosis transmembrane regulators and inhibition of sodium and hydrogen exchangers leading to accumulation of salt and water causing diseases like watery diarrhea (Govindarajan et al., 2020).

Diagnosis

The diaarhea associated with ETEC is characterized by copious watery diarrhea. This can be identified by stereotyping and serological identification of the specific colonization factors thus distinguishing LT and ST and genes identification encoding VFs. The diseases is self-limiting thus in outbreak situation virulence testing is impractical (Natividad et al., 2015).

Conventional Treatment

Vaccination is recognized as the most effective method for controlling and preventing ETEC infections after adopting all types of hygienic measurements. The recent studies on vaccines have ST as major target focusing to remove high toxicity, finding a protein carrier to increase immunogenicity and to reduce the immunological cross-activities (Zegeye et al., 2019). The antimicrobial molecule like histatin-5 has been found to inhibit colonization and adhesion of ETEC thus preventing the spread of infection (Brown et al., 2018).

Alternative Treatment

The dietary *Macleaya cordata* by ETEC induced oxidative stress and by enhancement of the immunological functions plays a crucial role in the prevention and management of the ETEC (Guan et al., 2019). Other agents including icariin and its derivative and polyphenols extracts found to be effective in preventing ETEC infection by inhibition of p38 MAPK leading to reduction in the inflammation and oxidative stress and by blocking LT conjunction and receptors, respectively (Verhelst et al., 2013).

Enteropathogenic *E. coli* (EPEC):

Epidemiology

The strains found to be the primary causative agent of watery diarrhea in developing countries, common among both infants and children. These agents can easily be seen in localities where sewage and sanitation systems are not efficient causing several diarrheal diseases. These are sources of communicable infections and had been a cause of sporadic and endemic outbreaks (Ochoa and Contreras, 2011). The EPEC is atypically causing 78% of cases in children less than 15 years of age with dirrhea and is the most important pathogen which on average 5-10% cause paediatrics diarrheal illness and has 10-20% on average prevalence rate. The Studies conducted in Brazil 2001-2002 found 92% of EPEC strains as atypical (Mare et al., 2021; Ochoa et al., 2008).

Pathophysiology

These strains attach to the enterocytes present at the epithelium of small intestine with the help of bundle-forming pilus thus forming A/E lesions followed by the effacement of microvilli at the surface, rearrangement of F-actin and at the point of adherence the growth of cuplike pedestal outgrowth. Outer membrane have protein colonization factors that include intimin, Tir, mitochondrial-associated proteins (MAPs), T3SS, EspF and 20 other secretory toxin at chromosomal island of locus of enterocytes effacement (LEE) (Govindarajan et al., 2020; Stevens and Frankel, 2014) contributing for adherence and events like, microvilli effacement by EspF and T3SS-dependent Tir insertion leading to Tir-intimin interaction where the interaction serves to produce responses like gathering of the cytoskeleton protein around the bacterial cells, host cells phospholipase phosphorylation and polymerization of the actin for anchoring of the bacteria to host cells (Dean et al., 2006). The EspF is also involved in the protein-protein interactions and intestinal barrier functions disruption. Thus, resulting in the effective microvilli effacement, water and electrolyte absorption and secretion disruption and wherein some studies reports that EPEC serves to inactivates the SGLT-1 that is involved in the intestinal fluid uptake disturbing water leading to dirrhea (Dean et al., 2005).

Diagnosis

It is based on the stool frequency and character. Diagnosis is based on the presence or absence of abdominal pain, vomiting, change of stool character, bloating and increased frequency of stool. Duration is within in 2 weeks (Ye et al., 2010).

Conventional Treatment

The *Staphylococcus aureus* associated diarrheal infection can be treated with vancomycin or linezolid. For patients with *Clostridium difficile* associated mild-moderate diarhhea the original antibiotic therapy must be stopped and if there is severe case it is recommended to take metronidazole or vancomycin. For *Klebsiella pneumoniae* enteritis 1–2-weeks imipenem therapy is recommended, and this infection is common in children less than 2 years of age. When the infection is caused by EPEC they will have mucous, blood and pus in the stool and with severe fever antimicrobial therapy is recommended for 5-7 days which include third generation cephalosporins, amikacin and imipenem (Chen et al., 2018).

Alternative Treatment

Thymol, cinnamic acid, and its derivatives, sanguinarine chloride, hopeaphenol, fusaric acid and butyric acid are found to be inhibitors of T3SS disturbing their action leading to treatment of infection (Pendergrass and May, 2019).

Enterohemorrhagic or Shiga Producing Toxin *E. coli.* (EHEC/STEC)

Epidemiology

According to CDC reports *E. coli* strain O157:H7 is found to be the cause of 63000 cases per year and 2100 patients have been hospitalised due to these infections in the USA. According to report by the foodborne disease active surveillance network, this strain is one of the 9 pathogens that are transmitted via food. And report by the world health organization says that approximately 2.8 million of the cases of the STEC are reported by 2014. STEC is common among both adults and children in Norway whereby this is the leading cause of hemolytic-uremic syndrome in adults of 60 years and children of less than 5 years. HUS is 2nd most common cause of chronic kidney infection and estimated incidence rate calculated is around 0.5 cases per 100000 children (Bahgat et al., 2023; Jenssen et al., 2016).

Pathophysiology

These can lead to the production of bloody diarrhea due to expression of Shiga toxin-1 and Shiga toxin-2 (Govindarajan et al., 2020; Melton-Celsa, 2014). Both toxins consist of bacterial AB protein toxin having A subunit and five B subunits that act to inhibit bacterial protein synthesis by targeting and inhibiting eukaryotic ribosomes and these toxins resembles toxin produced by *Shigella dysentriae*. These acts in several ways for example by disruption in the regulation of the ions channel in the mucosa of intestine of host thus leading to the loss of considerable amount of water and ions and ultimately diarrhea. They act as immune modulators thus activating pro-inflammatory and pro-apoptotic response leading to production of disease symptoms. The hemolytic-uremic syndrome (HUS) is the because of the lesion in microvasculature of kidney epithelium. Additionally, these strains encode adhesin such as intimin and possesses plasmid (pO157) activating EHEC-hemolysin a pore forming toxin. Upon attachment these strains cause local damage to the intestine the Shiga toxins enter the host cells and travel to organs where it starts damaging the enterocytes and ultimately cell death, resulting in thrombocytopenia, renal failure or tolerance, HUS triad and macroangiopathic hemolytic-anemia due to production of inflammatory responses followed by coagulation cascade activation and thrombosis (Bahgat et al., 2023; Tarr, 1995).

Diagnosis

The diagnosis is based on the prodromal diarhea history and clinical and lab findings. It is confirmed by serological and microbiological cultures for the presences of Shiga toxins. The other important parameter for diagnosis is TTP where thrombocytopenia and ADAMTS13 activity defects are analyzed using fluorometric and chromogenic assay (Asif et al., 2014).

Conventional Treatment

The HUS therapy is planned in a way that all the possible scenarios like acute kidney injury and systemic complication must be carefully managed and treated. In cases of severe anaemia packed cells are used while in cases of thrombocytopenia along with bleeding i platelet transfusion are administered. The intravascular volume levels maintenance using fluid and electrolyte to overcome acute kidney injury, HUS, and organ failure. In patients with uremia and electrolyte abnormalities renal replacement therapy is necessary. Eculizumab and plasma exchange plays vital role in management of HUS and other critical situations (Harkins et al., 2020; Mühlen and Dersch, 2020; Raina et al., 2018).

Alternative Treatment

White crobe tree (*Prosopsis alba*), ellagitannin from *Quercus infectoria* and *Ziziphus mistol* extract (Pellarín et al., 2013) were found to possess inhibition of cytotoxicity of Stx of *E. coli.* Additionally, bacterial products like lactic acid, linoleic acid, fruit juice and green tea have been found to be effective in the management of the STEC infection in several studies (Mühlen and Dersch, 2020).

Enteroinvasive *E. coli* (EIEC)

Epidemiology

Strain like *Shigella* that is capable to invade mucosa of colon containing invasion plasmid and pathogenicity island (PIs). The strains responsible for invading epitheliium lead to ulcers of mucosa and inflammation. Eating raw/processed and uncooked and vegetable grown in contaminated water are major source of their spread (Maurelli, 2013). About 120000 foodborne illness-related fatalities and 100 million foodborne cases are reported per year (Kumar et al., 2023). There are more than 5 million cases reported in the USA out of which 80% are foodborne (Ranasinghe and Fhogartaigh, 2021).

Pathophysiology

Both the *Shigella* and EIEC have similar virulence factors (VFs) but damage by *Shigella* infection is greater than the EIEC infection. EIEC strains are responsible for causing bacillary dysentery. These strains have pINV plasmid like *Shigella* and possess the same pattern of invasiveness like crossing through the intestinal barrier, invasion of colon mucosa, macrophage invasion, replication and intracellular transfer causing inflammatory colitis (Govindarajan et al., 2020; Ilia and Philippe 2018).

Diagnosis

Advancement in the biotechnology has opened new and rapid diagnostic methods including, physiochemical tests, immunological and high specific-DNA methods, miniaturized biochemical assays, biosensor-based methods, and automatic diagnostic systems (Kumar et al., 2023; Loderstädt et al., 2021).

Conventional Treatment

To prevent foodborne disease outbreaks food safety is the main aim of the system where it is recommended to adopt proper hygiene guidelines. Bismuth salicylate 262mg, 2 tablets 4times a day helps reduction in the illness by 40% but must not be taken for more than 3 weeks. Similarly, rifaximin is the antibiotic drug of choice for the treatment of EIEC associated gastroenteritis. Oral killed vaccine of whole cholera cells in combination with recombinant B subunit of cholera toxin have been found effective against serotype O1 for 4-6 months (Ranasinghe and Fhogartaigh, 2021).

Alternative Treatment

Due to emerging gut dysbiosis and antibiotic resistance, natural agents are being used as alternative therapy and disinfectants. Chitosan and its derivatives which are non-toxic natural antimicrobial agents help in killing microbes through neutralizing the charge over the cell surface (Yan et al., 2021).

Diffusively Adherent *E. coli*

Epidemiology

A study conducted between October 2018 and May 2019 found that out of 309 patient's stools sample 207 patients gave positive results for the presence of *E. coli* making cumulative percentage of 66.9% using cell culture and biochemical analysis (Javadi et al., 2020; Turniak and Sobieszczańska, 2019). A study conducted in 2017 where a total of 327 stools samples are collected compromising both adults and children found out that 40% of the children and 39% of the adults carried *E. coli* strains where DAEC is the 2nd most prevalent strain (Spano et al., 2017).

Pathophysiology

These strains are recognized by the pattern of diffusive adherence to Hep-2 cells monolayer. They possess fimbria like adhesins F1845 and DAF (surface anchoring proteins), both bind and clusters to form cellular extrusion around the bacterial cells thus inducing cytopathic effects (Meza-Segura et al., 2020). The DAEC infections impair activities by reduction of brush-boarder sucrase-isomaltase and dipeptidyl peptidase IV leading to enteric diseases. The DAEC also express MICA over intestinal epithelium that is potential factor for causing inflammatory bowel diseases (Govindarajan et al., 2020).

Diagnosis

The strains isolated are subjected to PCRs where they are analysed for the presence of specific virulence markers. Other methods involved in the identification and diagnosis of strain are the Hep-2 adherence assay, stool culturing and biochemical tests (Javadi et al., 2020; Spano et al., 2017).

Conventional Treatment

Several studies have observed the resistance of DAEC from antibiotics like cotrimoxazole, sulfonamide, doxycycline, tetracycline, ampicillin and cefotaxime whereas imipenem, nitrofurantoin and gentamicin are found effective in management of DAEC associated infections (Javadi et al., 2020).

Alternative Treatment

The Echeveria extract has been found to be bacteriostatic in nature and effective against all pathotypes that cause diarrhea (Olivas-Quintero et al., 2022).

Extraintestinal pathogenic *Escherichia coli*

When intestinal *E. coli* is exposed outside of intestinal mucosa it leads to extraintestinal illnesses and causes long term hospitalization and is due to unavailability of the hygienic environment and proper sanitation system. The lower respiratory tract infection and ventilator associated pneumonia are the most common community acquired illnesses (Sievert et al., 2013). After pneumonia UTIs are at the 2nd most prevalence rate in the US in terms of ambulatory care visits and hospitalization of the patients. Bacteria go ascending to the urethral proximities cause UTIs. It is common among women than men. Eight percent hospitalization cases and 0.9% emergency and outpatient cases are reported in the US related to UTIs and is considered the 2nd most common healthcare associated infection. Bacteraemia targets adult urinary tract and 2-6% of adult patients undergo transrectal prostrate biopsy due complicated bacteraemia (Hsieh et al., 2019).

Multidrug-resistant *E. coli*

The antimicrobial resistance is one of the major concerns these days worldwide. Continuous migration of the people from one country to the other and inappropriate and increased consumption of antimicrobial agents like penicillin are

some of the key factors which contributes to the evolution and resistance of bacteria against such agents. Because of barriers in the outer membrane, *E. coli* gets intrinsically immune to penicillin G antibiotic that is the 1st beta-lactam antibiotic. Some other antibiotics such as quinolones, aminoglycosides are found to be ineffective against *E. coli* in recent studies done in the Europe (Rahman et al., 2020). The beta-lactamases consisting of several enzymes which encode plasmid are produced by *Enterobacteriaceae* especially *E. coli* is the major reason behind the resistance of *E. coli*. This enzyme leads to resistance against penicillin G and cephalosporins and resultantly causes multidrug resistance in gram negative bacteria. ESLBs are the leading cause of resistance against cephalosporins and monobactams. Alteration to 16S rRNA site by the methyltransferase enzymes is of great threat to ineffectiveness of antibiotics because of armA gene that confer resistance to aminoglycosides that are bactericidal and work by inhibiting bacterial protein synthesis. According to reports of recent years the STEC O104:H4 has been found to be responsible for the large number of outbreaks. The serogroups O157 and O26 have been found to involve in HUS and a lot of cases of HUS were emerged according to reports of 2010 (Allocati et al., 2013).

Therapies against Multi Drug Resistance

The novel therapeutic strategies development is on high demand because of the worldwide emergence of resistance against most of the antibiotics and only a few retain activity against such pathogens. Therapies like phage therapy, combination therapy means combining two or more antibiotics and antimicrobial peptide therapy (Cunrath et al., 2019).

Conclusion

The *E. coli* infections are one of the major concerns in these days not only due to its prevalence but also due to the resistance to most of the antibiotics. Several diagnosis and treatment protocols had been proposed to manage infections due to *E. coli*. Drinking of contaminated water and raw meat and consumption of raw milk are associated with HUS (hemolytic-uremic syndrome) due to STEC/EHEC strains. Similarly, consuming vegetables grown in dirty or unhygienic water is also a leading cause of *E. coli* infection. Resistance to the antibiotics is leading to load on health facilities causing difficulties in managing and curing disease. Drugs like penicillin, cephalosporin, aminoglycoside, and quinolone are getting ineffective in treatment and management of the infections due to the resistance of bacteria. Some factors which are contributing towards the resistance of antibiotics are inappropriate use and immigration of the people from one country to the other. Several therapies like phage and combination therapy are practised to combat the resistance of the drugs.

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Chapter 52

Propagation of Signal Transduction by ITAMs in Thymus-Independent Antigens

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ABSTRACT

The thymus-independent (TI) antigens can stimulate the B cells, thus helping to activate the cells without the need of thymus. The response occurs as a result of ITAMs (Immuno-receptor tyrosine-based activation motifs) that are present on a limited type of BCRs. The signals transduction cascades from ITAMs to TI receptors upon IT antigen binding. That will lead to the activation of Src family by which phosphorylated ITAMs will allow the binding and activation of the downstream molecules such as Syk and Bruton's tyrosine kinase (BTK). These kinases will further trigger the signaling ways which helps B-cell proliferation, differentiation, and antibody production. The unraveling of this ITAMs-mediated signaling pathway in TI response is not only imperative for the designing of new immunotherapies targeting B-cells activation and function but is also the key for success of the whole therapeutic intervention.

KEYWORDS
TI antigens, ITAMs, Signal transduction, B cell activation, Antibodies

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INTRODUCTION

The immune system, a marvel of biological complexity, orchestrates the body's defense against pathogens and foreign invaders. Among its intricate mechanisms, the role of signal transduction pathways in coordinating immune responses is paramount. In this comprehensive exploration, we delve into the fascinating realm of thymus-independent antigens and their propagation of signal transduction through immuno-receptor, tyrosine-based activation motifs (ITAMs). As we embark on this journey, we aim to unravel the mysteries surrounding these key players in the immune regulation (Fulop et al., 2020).

The thymus-independent antigens, unlike their thymus-dependent counterparts, can directly activate B-cells without the need for T-cell assistance. This distinct feature highlights their significance in mounting rapid immune responses against a diverse array of pathogens. Within this context, ITAMs emerge as crucial mediators, functioning as molecular switches that initiate and propagate signaling cascades upon antigen recognition. Understanding the structure, function, and regulation of ITAMs is essential for deciphering the intricacies of thymus-independent immune responses (Kurosaki, 1999).

The human immune system stands as a sentinel, tirelessly guarding against the constant onslaught of pathogens, toxins, and foreign invaders. Its remarkable ability to distinguish between self and non-self, and to mount precise and potent responses, is a testament to millions of years of evolutionary refinement. (Laramee, 2013).

In this era of rapid scientific advancement, our understanding of immune signaling has undergone a paradigm shift, revealing the pivotal role played by immuno-receptor ITAMs in shaping the immune responses (Secombes and Wang, 2012). The advent of cutting-edge technologies, coupled with interdisciplinary collaborations, has unveiled the molecular intricacies underlying ITAM-mediated signaling, unlocking new avenues for therapeutic intervention and immunomodulation.

To embark on this journey of exploration, it is imperative to first grasp the fundamental principles governing immune recognition and signaling. The immune system operates on the principle of discrimination, distinguishing between harmless self-antigens and potentially dangerous foreign invaders (Janeway, 1993). Central to this discrimination process are specialized receptors expressed on the surface of immune cells, which survey the extracellular milieu for signs of danger.

The thymus-independent antigens represent a unique class of immunogens capable of eliciting immune responses in the absence of T-cell assistance. Unlike the thymus-dependent antigens, which require the participation of T-cells for efficient activation, thymus-independent antigens can directly engage B-cells and initiate signaling cascades leading to their activation and differentiation. This distinct mode of activation enables rapid and robust immune responses, particularly against pathogens with repetitive or polymeric surface structures (Vos et al., 2000).

At the forefront of thymus-independent immune responses are ITAM-containing receptors, which serve as molecular conduits for signal transduction. The ITAMs are characterized by tandem arrays of tyrosine residues within their cytoplasmic tails, act as molecular switches that toggle between inactive and active states upon receptor engagement. The phosphorylation of tyrosine residues within ITAMs triggers a cascade of downstream signaling events, culminating in cellular activation and effector function (Newton and Dixit, 2012).

The structural diversity of ITAM-containing receptors underscores their versatility in recognizing a wide array of antigens and pathogens (Crocker et al., 2007). From Toll-like receptors sensing microbial components to B cell receptors recognizing antigenic determinants, ITAM-mediated signaling bridges the gap between innate and adaptive immunity, integrating signals from the extracellular environment to mount effective immune responses.

Negative regulators such as phosphatases and inhibitory receptors act as checks and balances, preventing excessive activation and autoimmunity (Afonina et al., 2017). Dysregulation of ITAM signaling has been implicated in a myriad of autoimmune disorders, highlighting the importance of understanding the intricate regulatory mechanisms governing immune responses. (See Fig. 1)

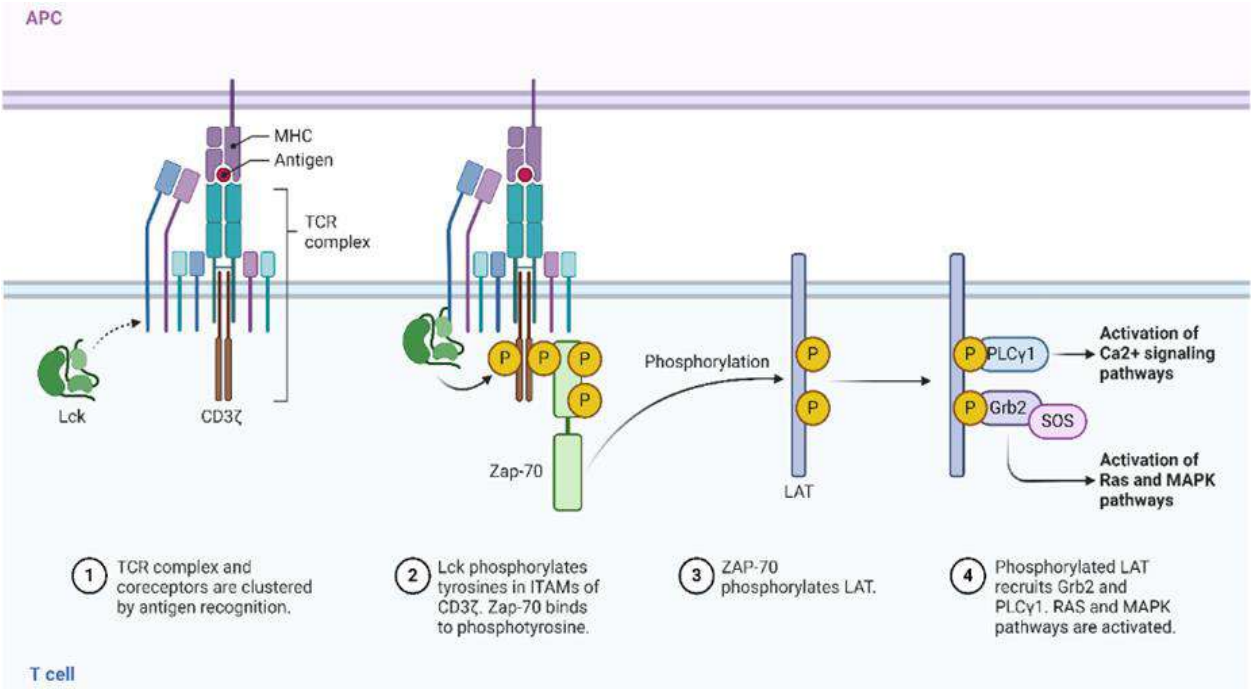

Fig 1: TCR Downstream Signaling

Through a multidisciplinary approach encompassing molecular biology, immunology, and biochemistry, we aim to shed light on this enigmatic yet essential aspect of immune function. Join us as we navigate the intricate pathways of immune signaling, unraveling the mysteries of ITAMs and their role in shaping the immune landscape (Berger and Johnston, 2015).

The immune system, an intricate network of cells, tissues, and signaling molecules, serves as the body's frontline defense against invading pathogens. Central to its function is the recognition and response to antigens, molecules that trigger immune reactions. Within this vast landscape of immune recognition, thymus-independent antigens stand out as a fascinating subset capable of eliciting immune responses without the need for T-cell assistance (Lee and Oh, 2024).

The thymus-independent antigens encompass a diverse array of molecules, ranging from bacterial cell wall components to polysaccharides and repetitive protein structures. What distinguishes these antigens from their thymus-dependent counterparts is their ability to directly engage B-cells and trigger immune responses without the requirement of T-cell help. This unique feature enables the immune system to mount rapid and robust defenses against a wide range of pathogens, particularly those with repetitive or polymeric surface structures (Foster, 2005).

At the molecular level, the recognition of thymus-independent antigens by B-cells initiates a cascade of signaling events culminating in cellular activation and effector functions. Central to this process are immuno-receptor, ITAMs, conserved motifs found within the cytoplasmic tails of various immune receptors, including B-cell receptors (BCRs). The ITAMs serve as molecular scaffolds for signaling proteins, orchestrating the transmission of activation signals upon antigen binding (Dustin and Cooper, 2000).

The structure of ITAMs is characterized by tandem arrays of tyrosine residues flanked by conserved amino acids,

forming docking sites for signaling molecules upon phosphorylation. Upon engagement with thymus-independent antigens, the BCRs undergo conformational changes that lead to the phosphorylation of ITAMs by Src family kinases. This phosphorylation event serves as a molecular switch, initiating the recruitment and activation of downstream signaling molecules, including kinases, adaptors, and effector proteins (Schaeffer and Weber, 1999).

The signaling pathways activated by ITAM-mediated signaling are diverse and multifaceted encompassing key players such as phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinases (MAPKs), and nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) (Chow and Chin, 2020). These pathways converge to regulate critical cellular processes, including proliferation, differentiation, and antibody production, ultimately shaping the magnitude and quality of the immune response.

Moreover, the ITAM-mediated signaling plays a pivotal role in bridging the gap between innate and adaptive immunity (Guilliams et al., 2014). By integrating signals from pattern recognition receptors (PRRs) and BCRs, the ITAM-containing receptors facilitate the coordination of innate and adaptive immune responses, ensuring effective pathogen clearance and long-term immunity.

By elucidating the fundamental principles governing immune recognition and activation, we have set the stage for a deeper dive into the molecular mechanisms underlying immune responses in subsequent chapters. Join us as we unravel the complexities of ITAM signaling and its role in shaping the immune landscape in response to thymus-independent antigens.

Structure and Function of ITAMs in Immune Signaling

In the intricate tapestry of immune signaling, the immunoreceptor ITAMs emerge as key players, orchestrating a myriad of cellular responses upon engagement with thymus-independent antigens. In this chapter, we delve deeper into the structure and function of ITAMs, unraveling the molecular intricacies that underlie their role in immune signaling.

At the molecular level, ITAMs consist of conserved motifs found within the cytoplasmic tails of various immune receptors, including B-cell receptors (BCRs), Fc receptors, and Toll-like receptors (TLRs). These motifs are characterized by tandem arrays of tyrosine residues flanked by conserved amino acids, forming docking sites for signaling molecules upon phosphorylation(Bonifacino and Traub, 2003).

The structure of ITAMs confers versatility and specificity to immune receptor signaling. The tandem arrangement of tyrosine residues allows for cooperative binding of signaling proteins, amplifying and propagating activation signals upon receptor engagement. Additionally, the conserved nature of ITAM motifs ensures functional redundancy across different immune receptors, enabling the integration of signals from diverse antigenic stimuli.

Upon engagement with thymus-independent antigens, such as polysaccharides or repetitive protein structures, BCRs undergo conformational changes that lead to the phosphorylation of ITAMs by Src family kinases. (Schaeffer and Weber, 1999).

One of the primary functions of ITAM-mediated signaling is the activation of key signaling pathways that regulate immune cell responses. The phosphoinositide 3-kinase (PI3K), for example, plays a crucial role in cell survival, proliferation, and differentiation, while mitogen-activated protein kinases (MAPKs) regulate gene expression and cytokine production. Additionally, activation of nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) promotes the transcription of genes involved in inflammation and immune responses.

The spatiotemporal regulation of ITAM signaling is essential for maintaining immune homeostasis and preventing aberrant activation. The negative regulators, including phosphatases and inhibitory receptors, act as checkpoints to dampen excessive immune responses and prevent autoimmunity. The dysregulation of ITAM signaling has been implicated in various autoimmune disorders, highlighting the importance of tight regulatory control over immune receptor activation. (Fig. 2).

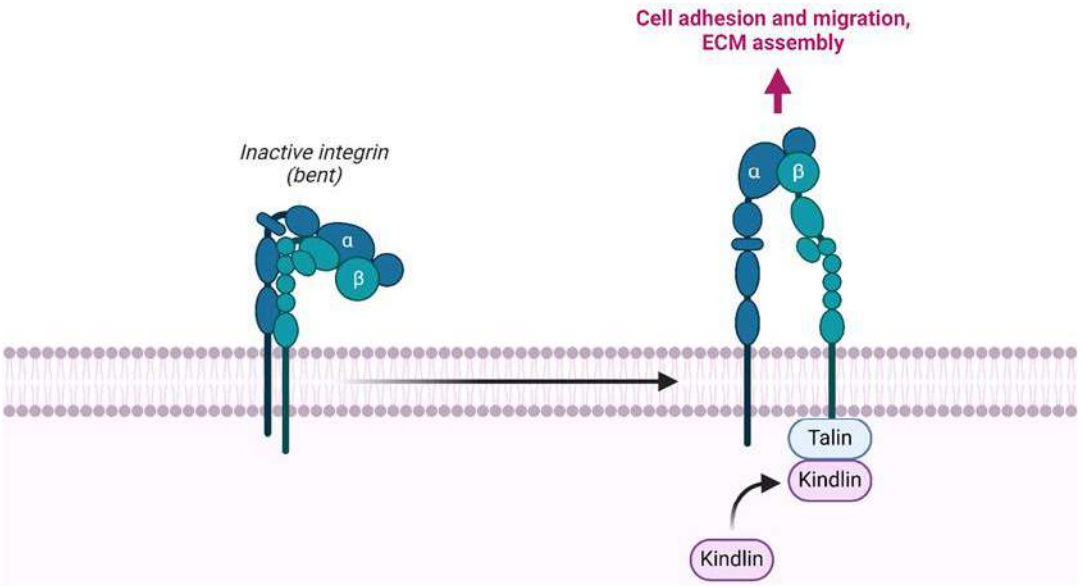

Fig 2: TCR Downstream Signaling

Furthermore, the structural diversity of ITAM-containing receptors allows for the integration of signals from both

innate and adaptive immune receptors, bridging the gap between early innate immune responses and the subsequent adaptive immune responses. This integration ensures the coordination and effectiveness of immune responses against a wide range of pathogens (Medzhitov, 2007).

Signaling Pathways Initiated by Thymus-Independent Antigens

Within the intricate landscape of immune signaling, the activation of signaling pathways initiated by thymus-independent antigens plays a pivotal role in shaping the magnitude and quality of the immune response. In this chapter, we delve into the diverse array of signaling pathways triggered by the engagement of immuno-receptor ITAMs with thymus-independent antigens, unraveling the molecular mechanisms that underlie the immune cell activation and effector functions.

Upon recognition of thymus-independent antigens by immune receptors such as B-cell receptors (BCRs) or Toll-like receptors (TLRs), a series of signaling events is initiated, culminating in cellular activation and immune responses. Central to this process is the phosphorylation of ITAMs within the cytoplasmic tails of immune receptors, which serves as a molecular switch to recruit and activate downstream signaling molecules.

One of the key signaling pathways activated by ITAM-mediated signaling is the phosphoinositide 3-kinase (PI3K) pathway. The PI3K activation leads to the generation of phosphatidylinositol (3, 4, 5)-trisphosphate (PIP3), which serves as a docking site for proteins containing the pleckstrin homology (PH) domains, such as Akt. The Akt activation promotes cell survival, proliferation, and metabolic reprogramming, ensuring the robustness of the immune response.

In addition to the PI3K pathway, the mitogen-activated protein kinase (MAPK) pathway also plays a crucial role in immune cell activation. MAPKs, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK, are activated downstream of ITAM signaling and regulate gene expression, cytokine production, and cell differentiation. The coordinated activation of MAPK pathways ensures the appropriate cellular responses to thymus-independent antigens (Tedgui and Mallat, 2006).

Furthermore, activation of the nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) pathway is essential for the transcriptional regulation of genes involved in inflammation and immune responses. The NF-κB activation leads to the expression of pro-inflammatory cytokines, chemokines, and adhesion molecules, facilitating immune cell recruitment and activation at the site of infection.

The signaling pathways initiated by thymus-independent antigens are tightly regulated to prevent excessive immune activation and maintain immune homeostasis. The negative regulators, including phosphatases and inhibitory receptors, act as checkpoints to dampen signaling cascades and prevent autoimmune responses. The dysregulation of these signaling pathways has been implicated in various autoimmune disorders, highlighting the importance of tight regulatory control over immune receptor activation.

Moreover, the integration of signaling pathways initiated by thymus-independent antigens with those triggered by other immune receptors, such as pattern recognition receptors (PRRs) or co-stimulatory receptors ensures the coordination and effectiveness of immune responses against diverse pathogens. This integration allows for the amplification and fine-tuning of immune responses, optimizing the clearance of infections and the maintenance of immune homeostasis (Rooks and Garrett, 2016).

Role of ITAMs in Immune Cell Activation and Differentiation

In the intricate orchestration of immune responses, the role of immuno-receptor ITAMs extends beyond mere signal initiation; they serve as crucial mediators in the activation and differentiation of immune cells. This chapter delves into the multifaceted functions of ITAMs in modulating immune cell responses, shedding light on their indispensable role in shaping the immune landscape.

Upon engagement with thymus-independent antigens, ITAM-containing receptors on immune cells undergo phosphorylation, triggering a cascade of signaling events that culminate in cellular activation and effector functions. Central to this process is the activation of downstream signaling pathways, including the phosphoinositide 3-kinase (PI3K) pathway, mitogen-activated protein kinase (MAPK) pathway, and nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) pathway (Lai et al., 2013).

The activation of these signaling pathways plays a pivotal role in regulating immune cell activation and differentiation. For instance, PI3K activation promotes cell survival and metabolic reprogramming, ensuring the persistence and functionality of activated immune cells. Meanwhile, MAPK signaling regulates gene expression and cytokine production, contributing to the polarization of immune cell responses towards specific effector functions (Flavell et al., 2010).

Moreover, ITAM-mediated signaling influences immune cell differentiation by modulating the expression of lineage-specific transcription factors and effector molecules. In B-cells, for example, ITAM signaling drives the differentiation of naive B-cells into antibody-secreting plasma cells or memory B-cells, depending on the context of antigen encounter. Similarly, in myeloid cells, ITAM signaling regulates the differentiation and activation of macrophages, dendritic cells, and granulocytes, shaping their effector functions and immune regulatory properties (Geijtenbeek and Gringhuis, 2009).

Furthermore, ITAMs play a critical role in the formation of immunological synapses between immune cells and antigen-presenting cells (APCs), facilitating efficient antigen recognition and signal transduction. (Smith-Garvin et al., 2009).

The regulatory mechanisms governing ITAM-mediated immune cell activation and differentiation are tightly

controlled to maintain immune homeostasis and prevent aberrant immune responses. Negative regulators, including phosphatases and inhibitory receptors, modulate the strength and duration of ITAM signaling, fine-tuning immune cell responses to meet the demands of the microenvironment. (See in Fig. 3)

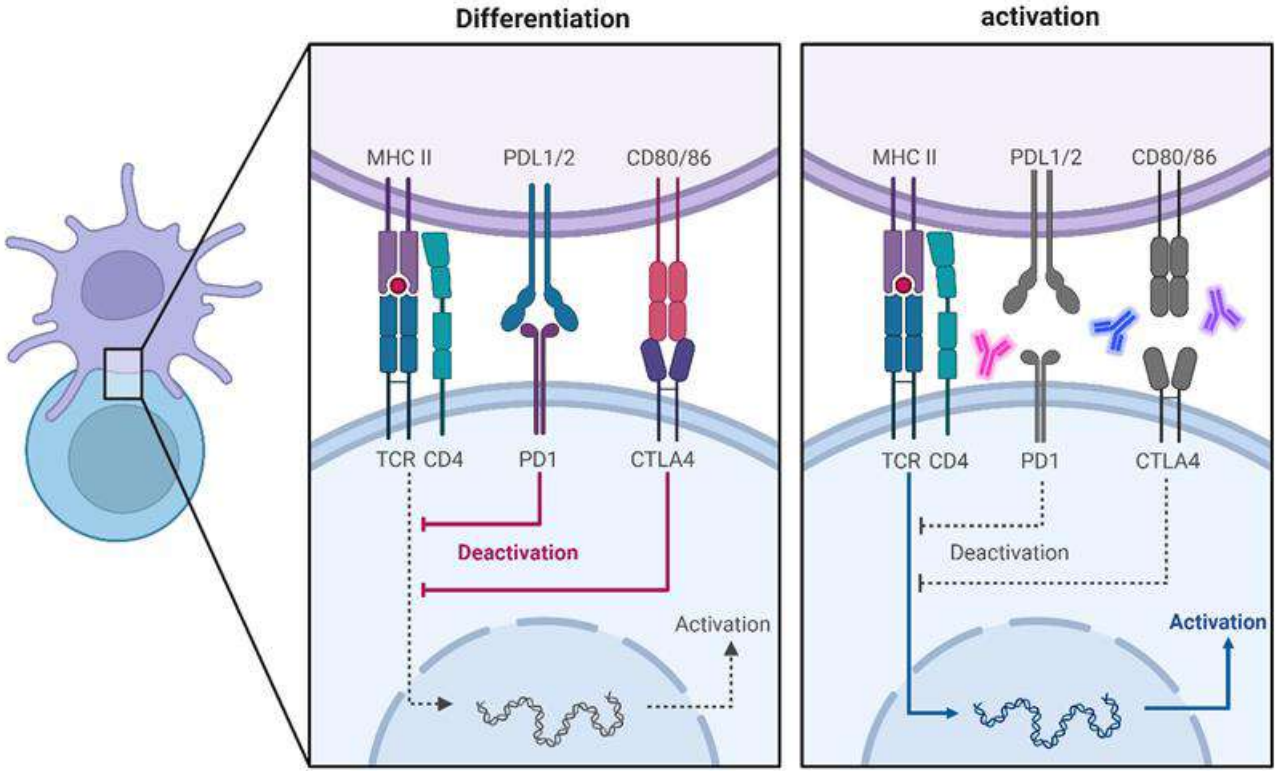

Fig. 3: Role of ITAMs in Immune Cell Activation and Differentiation

Regulation of ITAM-Mediated Signaling in Thymus-Independent Responses

In the intricate landscape of immune signaling, the regulation of immuno-receptor ITAMs emerges as a critical aspect governing the balance between effective immune responses and immune tolerance. This chapter delves into the diverse array of regulatory mechanisms that fine-tune ITAM-mediated signaling in the context of thymus-independent responses, elucidating how dysregulation can lead to immune dysfunction and pathology.

However, the strength and duration of ITAM signaling must be tightly regulated to prevent excessive immune activation and maintain immune homeostasis (Dal Porto et al., 2004).

One of the key regulatory mechanisms controlling ITAM-mediated signaling is the activity of protein tyrosine phosphatases (PTPs), which counteract the actions of protein tyrosine kinases (PTKs) responsible for ITAM phosphorylation. The PTPs, such as SHP-1 and SHP-2, dephosphorylate ITAMs and downstream signaling molecules, dampening immune cell activation and preventing hyper-responsiveness (Takai, 2002). Receptors like Fcγ RIIB and CD22 contain immuno-receptor tyrosine-based inhibition motifs (ITIMs) that recruit phosphatases upon activation, resulting in the attenuation of ITAM signaling and suppression of immune responses.

Furthermore, the spatial and temporal organization of ITAM-containing receptors at the immunological synapse is crucial for regulating signaling strength and specificity. The membrane micro-domains, such as lipid rafts, facilitate the clustering of ITAM-containing receptors and downstream signaling molecules, enhancing the signal transduction efficiency and promoting immune cell activation (Palacios and Weiss, 2004).

The co-stimulatory receptors, such as CD28, amplify ITAM-mediated signaling and promote immune cell activation, whereas inhibitory receptors, such as CTLA-4 and PD-1, dampen ITAM signaling and induce immune tolerance. (Pegram et al., 2011).

The dysregulation of ITAM-mediated signaling has been implicated in various autoimmune disorders, including rheumatoid arthritis, systemic lupus erythematosus, and autoimmune thrombocytopenia. The aberrant activation of ITAM-containing receptors or impaired regulation of ITAM signaling pathways can lead to hyper activation of immune cells, tissue damage, and loss of self-tolerance (Nimmerjahn and Ravetch, 2008).

Diseases and Disorders Associated with Dysregulated ITAM Signaling

In the intricate landscape of immune regulation, dysregulation of immuno-receptor ITAMs can have profound implications for immune homeostasis, leading to the development of various diseases and disorders (Van Limbergen et al., 2014). This chapter explores the link between dysregulated ITAM signaling and the pathogenesis of autoimmune disorders,

immuno-deficiencies, and inflammatory conditions, shedding light on the underlying mechanisms and potential therapeutic interventions.

The autoimmune diseases, characterized by the aberrant activation of the immune system against self-antigens, often involve dysregulated ITAM signaling pathways. In conditions such as rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis, the hyper activation of ITAM-containing receptors on immune cells leads to excessive inflammation, tissue damage, and loss of self-tolerance. The aberrant ITAM signaling can result from genetic mutations, environmental triggers, or dysregulation of negative regulatory mechanisms (Shaffer III et al., 2012).

Furthermore, the dysregulated ITAM signaling has been implicated in primary immunodeficiency disorders, where impaired immune cell activation and function predispose individuals to recurrent infections and susceptibility to pathogens. The mutations affecting ITAM-containing receptors or downstream signaling molecules can compromise immune cell development, maturation, and effector functions, leading to immuno-deficiency syndromes such as X-linked agammaglobulinemia and common variable immunodeficiency (Bonilla and Geha, 2003).

The inflammatory conditions, including allergic reactions, asthma, and inflammatory bowel diseases, are also influenced by dysregulated ITAM signaling. In these disorders, aberrant activation of ITAM-containing receptors on immune cells contributes to the exaggerated inflammatory responses and tissue damage characteristic of these conditions. The targeting ITAM signaling pathways presents a potential therapeutic strategy for mitigating inflammation and ameliorating disease severity (Bonilla and Geha, 2003).

Understanding the molecular mechanisms underlying dysregulated ITAM signaling is essential for developing targeted therapies aimed at restoring immune homeostasis and ameliorating disease pathology. Small molecule inhibitors targeting ITAM-containing receptors, downstream signaling molecules, or negative regulators of ITAM signaling represent promising avenues for therapeutic intervention in autoimmune diseases, immuno-deficiencies, and inflammatory conditions (Van Limbergen et al., 2014).

Moreover, the immunomodulatory therapies aimed at modulating ITAM signaling pathways hold potential for managing autoimmune diseases and dampening excessive immune responses. Biologic agents targeting specific components of ITAM-mediated signaling cascades, such as monoclonal antibodies or recombinant proteins, offer precision and specificity in targeting dysregulated immune pathways while minimizing off-target effects (Manzari et al., 2021).

Therapeutic Approaches Targeting ITAM Signaling in Immunotherapy

In the realm of modern medicine, the burgeoning field of immunotherapy holds immense promise for revolutionizing the treatment of various diseases, including cancer, autoimmune disorders, and infectious diseases (Leach et al., 2019). In this chapter, we explore the therapeutic potential of targeting immuno-receptor ITAMs and associated signaling pathways in immunotherapy, elucidating novel approaches for modulating immune responses and enhancing therapeutic outcomes.

The cancer immunotherapy, in particular, has witnessed remarkable advancements in recent years, with immune checkpoint inhibitors and chimeric antigen receptor (CAR) T-cell therapy emerging as groundbreaking treatment modalities. Targeting ITAM signaling pathways presents a promising strategy for enhancing the efficacy of these immunotherapies by modulating immune cell activation and effector functions (Feng et al., 2019).

One approach to targeting the ITAM signaling in cancer immunotherapy involves the development of small molecule inhibitors that selectively block ITAM-containing receptors or downstream signaling molecules. By inhibiting the aberrant ITAM signaling in tumor-infiltrating immune cells, these inhibitors can enhance anti-tumor immune responses and overcome immune evasion mechanisms employed by cancer cells (Harjunpää et al., 2019).

Furthermore, combination therapies incorporating the ITAM-targeted agents with existing immunotherapies, such as immune checkpoint inhibitors or CAR T-cell therapy, hold potential for synergistically enhancing therapeutic outcomes. By simultaneously targeting multiple checkpoints in the immune response, these combination approaches can overcome resistance mechanisms and improve patient responses to treatment (Sharma and Allison, 2015).

In addition to cancer immunotherapy, targeting ITAM signaling pathways holds promise for the treatment of autoimmune disorders, where dysregulated immune responses contribute to disease pathology. By modulating ITAM-mediated signaling in autoreactive immune cells, therapeutic agents can dampen excessive inflammation and restore immune tolerance, providing relief for patients suffering from autoimmune conditions (Horwood et al., 2012).

Moreover, the development of personalized immunotherapy approaches tailored to individual patient profiles offers the potential for precision medicine in the treatment of immune-mediated diseases. By profiling patients based on their immune cell phenotypes and signaling profiles, clinicians can identify optimal therapeutic strategies targeting specific dysregulated pathways, including ITAM signaling, to achieve personalized treatment outcomes (Gong et al., 2021).

Future Perspectives: Advancements and Challenges in Understanding ITAM Signaling

As we stand at the forefront of scientific discovery, the field of immuno-receptor ITAMs continues to unravel its mysteries, offering unprecedented insights into immune regulation and therapeutic intervention. In this final chapter, we gaze into the crystal ball of future prospects, exploring the advancements, challenges, and potential breakthroughs that lie ahead in understanding and harnessing ITAM signaling.

Advancements in technology, such as single-cell sequencing, high-resolution imaging, and computational modeling,

hold promise for unraveling the complexities of ITAM-mediated signaling with unprecedented resolution and granularity. By dissecting the signaling dynamics at the single-cell level, researchers can elucidate the heterogeneity and plasticity of immune cell responses, providing novel insights into the regulation of ITAM signaling in health and disease (Graham and Xavier, 2020).

Moreover, the integration of multi-omics approaches, including genomics, proteomics, and metabolomics, offers a holistic view of ITAM signaling networks, uncovering novel biomarkers and therapeutic targets for immune-mediated diseases. By leveraging big data analytics and machine learning algorithms, researchers can identify predictive signatures of ITAM pathway dysregulation and stratify patients for personalized therapeutic interventions (Sweatt et al., 2019).

In parallel, the development of innovative therapeutic modalities, such as gene editing technologies and nanoparticle-based drug delivery systems, opens new avenues for precisely modulating ITAM signaling in a targeted and spatiotemporal manner. By engineering immune cells with enhanced ITAM signaling capacity or delivering therapeutics directly to immune cell subsets, clinicians can tailor treatment strategies to specific disease contexts and patient profiles (Larson and Maus, 2021).

However, amidst the excitement of technological advancements and therapeutic innovations, challenges remain on the horizon. Unraveling the crosstalk between ITAM signaling pathways and other immune regulatory networks poses a daunting task, requiring interdisciplinary collaborations and integrative approaches to decipher the intricacies of immune regulation comprehensively (Zitnik et al., 2023).

Furthermore, translating basic research findings into clinical applications requires overcoming barriers related to drug development, regulatory approval, and patient access (Grimshaw et al., 2012). Collaborative efforts between academia, industry, and regulatory agencies are essential for bridging the translational gap and bringing promising ITAM-targeted therapies from bench to bedside.

Conclusion: Unveiling the Mysteries of ITAM Signaling

In the intricate tapestry of immune regulation, immuno-receptor ITAMs stand as key orchestrators, shaping the destiny of immune cells and the outcome of immune responses. Through our exploration across the chapters, we've embarked on a journey through the molecular intricacies of ITAM-mediated signaling, unraveling its profound implications for immune homeostasis, disease pathogenesis, and therapeutic intervention.

From the fundamental principles of ITAM structure and function to the intricate web of signaling pathways initiated by thymus-independent antigens, we've gained a deeper understanding of how ITAMs serve as molecular switches, toggling between immune activation and inhibition. We've delved into the role of ITAMs in immune cell activation, differentiation, and regulation, uncovering their multifaceted functions in shaping immune responses to diverse antigens and pathogens.

Furthermore, we've explored the regulatory mechanisms that fine-tune ITAM signaling, ensuring the delicate balance between protective immunity and immune tolerance. From protein tyrosine phosphatases to inhibitory receptors and membrane microdomains, a myriad of checks and balances govern ITAM-mediated immune responses, preventing aberrant activation and maintaining immune homeostasis.

As we peer into the future, the horizon is aglow with promise and potential. Advancements in technology, therapeutic modalities, and translational research offer unprecedented opportunities for harnessing the power of ITAM signaling in revolutionizing the landscape of immune-mediated diseases. From personalized immunotherapy approaches to innovative drug delivery systems, the possibilities are limitless in our quest to unlock the full potential of ITAMs for the betterment of human health.

Yet, amidst the excitement of scientific discovery and therapeutic innovation, challenges remain. Bridging the translational gap, deciphering the complexities of immune regulation, and overcoming regulatory hurdles require concerted efforts and collaborative partnerships across disciplines and sectors. Only through collective endeavor can we translate our knowledge into tangible solutions that benefit patients and society at large.

In closing, our exploration of ITAM signaling has illuminated the intricate workings of the immune system, revealing the beauty and complexity of its regulatory networks. As we bid farewell to these pages, let us carry forth the torch of curiosity and inquiry, ever vigilant in our pursuit of knowledge and ever hopeful in our quest for healing. For in the mysteries of ITAM signaling lie the keys to unlocking the secrets of immune regulation and unleashing the power of the human immune system.

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Chapter 53

Effects of Combining Vaccines and Immunostimulants on Protection of Fish against Infectious Diseases

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ABSTRACT

Infectious diseases pose an increasing threat to the sustainability and productivity of aquaculture/100mil from a global perspective. Despite the proven success of vaccines and immunostimulants at enhancing fish disease protection, an integrating approach to utilize both vaccines and immune stimulatory agents represents a more promising strategy for eliciting strong- broad range host immune responses. Chapter four discusses vaccines associated with immunostimulants for protection against infectious diseases in fish. Starting with fish immune systems and mechanisms of vaccine/immunostimulant action, it then fleshes out the scientific side why these approaches should work better together or at least as additives to each other and discusses from a mechanistic perspective why synergy might occur (or not), including broader pathogen coverage; longer duration protection. Significant data from many studies of a variety of types have revealed successful vaccine immune-stimulant combinations in different fish species, such as salmonids, cyprinids and marine fish including teleosts and flatfish which are protected with increased survival or specific antibody production characteristics for the prevention against viral, bacterial and parasitic pathogens through humoral response(s), cellular mediated immunity; etc. This review summarizes challenges and critical factors affecting efficacy, such as timing, route of administration, dose optimization, and compatibility. Discussion includes novel delivery systems, adjuvants and emerging vaccine/immunostimulant development trends Haslbeck and Brodte reviewed potential applications in sustainable aquaculture practices focusing on preventive health management, decreased reliance therapeutants such as antibiotics, chemotherapeutics (i.e., causes) and environmental sustainability. The potential for future research and collaborations are discussed, such as identifying new immunostimulant sources optimized delivery systems omics-based mechanistic insights and multi-sector working from researchers' industry regulators.

KEYWORDS
Vaccines, Immunostimulants, Fish, Infectious Diseases

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INTRODUCTION

Over the past decades, a phenomenal increase in aquaculture has been achieved to meet the human demand for fish protein as it is essential also due to declining stocks of wild caught fishes. Nonetheless, the practice of intensive aquaculture has also presented an absence potential for infectious diseases to emerge and cause major threats towards fish health as well as a measure loss in production and economic viabilities. Vaccination for fish has become important in the fight against these diseases, providing a cost effective and ecofriendly method of disease prevention (Nasr-Eldahan et al. 2021).

Fish Vaccination in Aquaculture: There is no overstatement as to why fish vaccination must be done. The high et record of mortality, growth depression and deterioration in quality of products as a consequence to infectious diseases on livestock is grossly economic damaging. Vaccination not only saves fish from suffering and death but also reduces the frequency of antibiotic treatments (that can lead to increased antimicrobial resistance, as well as negative environmental

impacts) In addition, such programs can improve the sustainability of aquaculture through by insuring fish health and welfare, minimizing potential disease outbreaks as well helping to secure a continuity in supply for products from fisheries (Imtiaz et al. 2023).

These vaccines are recognized for their potential benefits but there remain challenges in developing an effective vaccine that can be deployed across the myriad of fish species. Secondly, fish immune system is greatly different from mammals regarding innate as well adaptative cells and responses with a direct influence of environment factors such temperature and stress. Furthermore, some of the fish species have low antibody responses to these conventional vaccines leading to challenge in obtaining prolonged protection. In addition, the delivery of vaccination to fish is an issue in aquatic environment (Sahoo et al. 2021).

Combining vaccines with Immunostimulants: Potential potential benefits of this kind hybrid design, to solve the full achieving business enterprise promoting indium due fish vaccines efforts which in general have largely been unsuccessful. Immunostimulants: Chemicals that can boost the immune system and might help to achieve a stronger response following vaccination. The use of immunostimulants in combination with vaccines provides an opportunity to address some deficiencies associated with current fish vaccine approaches, particularly low antibody levels and duration (Priya and Kappalli2022). Studies have been conducted to study the possible advantages and disadvantages of combining vaccines with immunostimulants in fish. An example of this is the coadministration of immunostimulants with viral or bacterial vaccines, results in an increase in antibody production, improved protection against challenge with the pathogen and a lengthening of duration immunity. In addition, use of immunostimulants can be beneficial with delivery methods such as immersion or oral vaccination by increasing the uptake and presentation of vaccine antigens to fish immune components (Vinay and Bedekar 2022).

In addition, the immunostimulants can also control a range of functions of fish immune system such as activation and proliferation in immune cells or production cytokines and other mediators involved in immune responses to regulate inflammation process. This study thus offers new insights to optimize the immune response against fish pathogens by fine-tuning the choice and combination of immunostimulants/ adjuvants with particular vaccines (Ching et al. 2021). Although vaccines and immunostimulants can be developed in combination for these purposes, it is critical to understand that the effectiveness as well as safety of this approach may differ by fish species, vaccine types and immunostimulants used while targeting a particular disease. The mechanisms, formulations and administration protocols of combination strategies should be understood before translating into clinical practice; it requires more research studies to investigate the efficacy as well as safety profile (Du et al. 2022). Immune system (innate and adaptive) of fish: The immune response is a complex defence mechanism, which has been evolved to protect the body from pathogenic organisms or allergens/antigens in man as well as in phylogenetically less advanced organism like teleost. The innate immune response, also referred to as the ancestral immunity of fish (as in other vertebrates), is highly conserved and plays a significant role in antiviral defense. The immune response has two key components: the innate that is non-specific and involves immediate, first-line defense mechanisms against a pathogen. These include physical barriers like the skin, scales and mucous membranes as being the first line of defense to prevent entry or movement through tissues. Moreover, the innate immune system is composed of cellular components (macrophages/neutrophils/natural killer cells) and humoral factors -complement proteins/antimicrobial peptides/acite phase protein (Mokhtar et al. 2022).

In the case of adaptive immune system offers pathogen specific and long-term protection. It depends on lymphocytes activities which contain B cells and T cells. The B cells produce the antibodies that bind to specific pathogens: and neutralize them, mark for destruction or block their interaction with host. T cells, such as helper and cytotoxic T-cells facilitate maintaining the immune response by serving to other immunity-enhancing processes like activating different white blood cells or patrolling body areas where they might instantly attack infected host tissues (Pieren et al. 2022). Immune responses against bacterial, viral and parasitic pathogens: Fish use a wide range of immune mechanisms to kill bacteria, viruses or parasites. AbstractBackground: The innate immune system is essential for the rapid recognition and response to bacterial pathogens. Macrophages and neutrophils are phagocytic cells that eat up invading bacteria, while antimicrobial peptides cause conditions completely different from those necessary for bacterial growth. The adaptive immune response is important as well and includes B cell-secreted antibodies against surface antigens that can opsonize bacteria for phagocytosis, or neutralizing bacterial toxins (Simón et al. 2021).

Natural immunity recognizes viral structures of a pathogen in the case of eno-viral pathogens and induces an antiviral response, for example by producing type I interferons or other cytokines (Munang'andu et al., 2022). In viral infections, the adaptive immune response is required for the elimination of viruses and cytotoxic T cells are crucial in killing virus-infected cells while antibodies produced by B cell can neutralize viable particles thus block there binding to host celles (Carty et al. 2021).

Innate and adaptive immunity responses are associated with parasitic infections. In particular, features of innate immunity as the initial response including phagocytic cell activity and plasma components such as complement products or antimicrobial peptides aim at eliminating these sensors in an early stage. Adaptive responses produce antibodies through B cells for targeted binding of these antigens to facilitate phagocytosis or alter their life cycle, with the aid of T cells that can kill infected host cells and coordinate the immune response (Dimitriu et al. 2020).

Factors Influencing the Immune Response in Fish

Environmental and physiological factors can affect the immune response in fish, including changes to how effectively the immune system works as well as how resistant a host is toward infection with pathogens.

Environmental Factors

Temperature – Fish are poikilothermic animals, i.e., their body temperature changes according to the external surroundings. A substantial proportion of the immunising immune responses happen in a defined range of temperature, and very simple auto-regulatory mechanisms highlight become apparent (Biswal et al. 2021).

Poor water quality – such as low oxygen levels, or high ammonia and/or nitrite concentrations (which are toxic to fish) plus pollution is linked with reduced immune system function (Paredes-Trujillo and Mendoza-Carranza 2021).

Stress—Handling, transport and crowding are all stressors that can reduce the effectiveness of a fish's immune response to invading pathogens (Masud 2020).

Physiological Factors

- Developmental stage: The immune system of fish undergoes developmental changes, with larvae and juveniles exhibiting less robust immune responses compared to adult fish (Auclert et al. 2024).
- Nutritional status: Good nutrition is necessary to keep our immune system in optimal shape so take steps early on. Insufficient intake of certain vitamins, minerals or essential fats can cause immune suppression (Noor et al. 2021).
- Genetics: Genetics Genetic factors are related to the status of immunocompetence and reactivity in fish species or strains (Flores-Kossack et al. 2020).

A better understanding of the basis for variation in fish immune response is central to devising disease management practises and refining vaccination protocols. Environemental factors, husbandry practices and the diet need to kept in tiptop shape so as not jeopardize the immune status of a fish that may be dealing with miniscule numbers of pathogens (Natnan et al. 2021).

Types of Fish Vaccines (inactivated, live-attenuated, subunit, DNA)

In this perspective, several vaccines have been developed and used in fish aquaculture directed against infectious diseases. These include

Inactivated vaccines: These are made by killing the pathogen (bacteria or virus) that causes a disease and, because they are dead, they cannot cause illness but remain capable of navigation requirements an immune response. Inactivated vaccines have been use in aquaculture for the safety and promote humoral immune response as well (Ghattas et al. 2021).

Live-attenuated vaccines: contain live, but weakened form of the organism altered either by genetic mutation or multiple passages to reduce its virulence while retain viable and immunogenic capability that can allow replication with sustain both humoral and cellular immunity (Munang'andu et al., 2022). Live-attenuated vaccines are capable of eliciting potent and durable protective immunity; however, there are few reports on their applications for fish vaccination because of safety issues (Mondal and Thomas 2022).

Subunit Vaccines: Subunit vaccines are made from the pieces of pathogens — like proteins, polysaccharides or peptides that have a high degree of antigenic specificity but lack pathogenicity. These vaccines are typically safer than inactivated or live-attenuated vaccines but can need the addition of adjuvants or immunostimulants to improve their immugenicity (Verma et al. 2023).

DNA vaccines: DNA based vaccine like plasmid (small piece of circular, double-stranded DNA) encoding antigens proteins from pathogen. Once administered, the plasmid DNA is taken up by host cells and then expresses encoded antigens that can stimulate both humoral and cell-mediated immune responses. As one of the next-generation vaccine technologies, DNA vaccines have numerous advantages including simple production and capacity to activate diverse immune responses; however they are effective in fish (Ben Hamed et al. 2021).

Vaccine Delivery Methods (injection, immersion, oral)

The delivery of fish vaccines can be accomplished by a variety of methods, each with its own advantages and disadvantages:

ntraperitoneal or intramuscular injections: These injection methods are reliable dosing techniques that can successfully deliver the vaccine antigen. Injectable methods, however are labour intensive for both the fish and operator with potential to become unwieldy on a large scale (Li et al. 2021).

Immersion: in immersion vaccination fish get exposed to a vaccine solution and then the antigens will be taken up via their gills, skin or oral routes. Although more stressful to the fish, this method is easier to apply in mass vaccination programmes and efficacy can be influenced by variables such as water quality, size of the fish or vaccine stability (Du et al. 2022).

Oral: In oral vaccination delivery form, a vaccine is associated in fish feed for ingestion and further afterwards processed by the digestive system of fed fish leading to induction of mucosal as well systemic immune responses. This process is easy and less crowded however, it may limit by the antigen degradation or poorly uptake (Gómez et al., 2021).

Advantages and Limitations of Different Vaccine Approaches

Different varieties of vaccines and the ways they are administered come with their own benefits, but also drawbacks:
Inactivated vaccines: Pros : Safe, stable and can induce a humoral immunity. Drawbacks: May not activate cellular immune responses, need adjuvants/immunomodulators for best efficacy.
Live-attenuated vaccines: The Good Broad and durable immunity both humoral and cellmediated. Frustrations: Necessary safety concerns, potential reversion to virulence, short shelf-life.
Subunit vaccines: Pros Safe, RI targeted; may be multivalent. Downsides: May need to be used with adjuvants/immunostimulants, may not produce enough protection.
DNA-based vaccines: They have many advantages:Immunity they generate is broadProduction of such vaccine platforms could be easy and scalableThey are stable. Limitations: variable efficacy in fish, genomic integration.
Injection delivery: Advantages: Precise dosing, effective antigen delivery. Limitations: Labor-intensive, stressful for fish, impractical for large-scale application.
Advantages: Immersion delivery Suitable for mass vaccination Not stressful. Restrictions: Variability due to environmental conditions, possible antigen degradation.
Route: Oral Advantages- convenient, non-stressful, mass immunization suitable. Conclusions: Antigen degradation, low-uptake and possible feed ingredient interference.

The type of vaccine and the delivery strategy can vary depending on pathogen, fish species, production system or even because of economic reasons. Therefore, the strategy of vaccination for aquaculture must be implemented according to their benefits and disadvantages in order to provide maximum immunity (Su et al., 2021).

Immunostimulants in Aquaculture

Recently, Immunostimulants have been investigated as promising alternative to improve the immune response and protection against diseases in fish along with conventional vaccinating method of aquaculture. These compounds have been demonstrated to modulate and enhance different elements of the immune system in fish, which may lead them towards improved protection against infectious diseases, enhanced survival rates as well better growth rate and overall performance (Vijayaram et al. 2023).

Types of Immunostimulants (Bacterial Derivatives, Plant Extracts, Synthetic Compounds): There are numerous sources from which immunostimulants used in aquaculture can be derived:

Bacterial Derivatives

Bacterial lipopolysaccharides (LPS): LPS are found in the cell wall of gram-negative bacteria and best known as immune-modulating agent able to stimulate innate immunity response fish (Vijayaram et al. 2022).

Peptidoglycans - These are the cell wall components present in both gram-positive and negative bacteria, so can elicit immune cells functional activities accompanied by cytokine production observed in fish species (Jayathilaka et al. 2024).
Bacterial DNA: bacterial DNAs contain unmethylated CpG motifs which can interact with pattern recognition receptors on immune cells and function as immunostimulants (Zhou and Deng 2021).

Plant Extracts

Plant extracts/ herbs and spices -Ginger, turmeric garlic; Extracts from handful of other plants have shown to possess immunostimulatory properties in fish (Elumalai et al. 2020). Polysaccharides: Polysaccharide immunostimulants are the long chains of carbohydrate molecules that comes from plant sources such as mushrooms, yeast and algae (Khanjani et al. 2022).

Synthetic compounds

Cytokines and chemokines: Fish-derived cytokine (interleukins, interferons), naturally occurring glycoproteins may be synthesized into Industry standard single-chain or multichain synthetic format as well in combination for multifunctional use has been reported to exhibit immunomodulatory effects against the target organism (Abachi et al. 2023).

Immunomodulatory Drugs Synthetic immunostimulants, including levamisole isoprinosine and imiquimod act to increase immune function have been developed for the treatment of humans as well as being investigated in fish (Kumar et al. 2022).

Factors Affecting Immunostimulant Efficacy

The efficacy of immunostimulants in aquaculture can be influenced by various factors, including:

Dose and Duration- The ideal dose can make dissimilarity as per type of immunostimulant, fish species or environmental factor (Ching et al. 2021). Inappropriate dosages or treatment durations may lead to suboptimal or adverse effects.
Route of administration: The delivery mode (oral, injection, immersion) can influence the bioavailability and efficacy of immunostimulants as well. The immunostimulant needs to be processed by digestive processes, absorbed and distributed throughout the body of fish before it can reach its target immune cells or tissues (Harshitha et al. 2023).

Environmental Dependence Temperature, pH and salinity are parameters affecting the stability of some

immunostimulants. Moreover, environmental stressors such as handling, transportation and crowding may affect the immune response in fish rendering animals unable to reap benefits from immunostimulant treatment (Rajasekar et al. 2020).

Nutritional status: He response to an immunostimulant may be influenced by the nutritional condition of fish (Kord et al. 2021). It is important to be aware that if a fish does not have enough of the basic nutrients, vitamins or minerals necessary for various metabolic processes then its immune capacity willbe impired and the possibilities from an administrationof immunostimulants significantly lowered.

Combination with other treatments: Immunostimulants can be used in combination or synergistically with vaccines, probiotics, and/or another therapeutic agent to have a desirable and optimized efficacy of treatment. It is also important to take this difference into account when integrating immunostimulants with disease management programs (Abdolalipour et al. 2022).

Despite the obvious potential of this category in increasing disease resistance and overall health, there remains an urgent need for strict screening before any feed ingredients are produced as drugs with immunostimulant properties to safely raise Atlantic salmon or other fish species. Furthermore, developing a greater insight into the immunological pathways and toxicity profile in relation to other treatments will be key for an informed deployment of immunostimulants as sustainable part of aquaculture practices (Kumar et al. 2023).

Rationale and Potential Benefits

The strategy using vaccines and immunostimulants together tries to take advantage of the synergetic effects between these two factors, with potential limitations hindered that appears when each was used independently. Thus, vaccines are intended to stimulate targeted responses against the specific pathogen it has been manufactured for; however certain fish species during vaccination can give suboptimal response which leads up-to incomplete or short-lived protection. Immunnostimulants may, however act by modifying or enhancing several aspects of the biochemical pathways constituting both innate and adaptive immunity that could improve fish immune competence in general (Quiros-Roldan et al. 2024).

Potential Synergistic or Antagonistic Effects

Therefore, when combining vaccines and immunostimulants it is important to take into account that these two treatments can have a synergistic or an antagonistic effect on the immune response resulting a significant booster of overall protection against infectious diseases (Nanishi et al. 2022).

Synergistic Effects

Enhanced antibody production: In a study by Mohammadian et al. (2021), The co-delivery of a Streptococcus iniae vaccine and chitosan nanoparticles immunostimulant enhanced cellular uptake, potentiated the antibody response to stimulation as determined by ELISA titers with improved protection against streptococcosis when compared to delivery of vaccines alone

Augmented cell-mediated immunity: Andresen et al. (2021) showed that combining an inactivated viral vaccine with a immunostimulant like polyinosinic:polycytidylic acid (poly I:C) which worked to increase the activation of cytotoxic T cells and improve clearance of virus was effective for Atlantic salmon Oncorhynchus mykiss [Salmo salar] as stated by

Antagonistic Effects

Interference with immune responses: A study by Jain et al. (2022) showed that Labeorohitareported suppressed antibody responses and reduced protection afforded by co-administration of an inactivated Aeromonas hydrophila vaccine with glucan immunostimulant versus the vaccine alone suggesting a possible interference between both.

Adverse reactions: Adverse events: In several cases, a mixture of various immunostimulants and vaccines may increase the incidence of adverse reactions or side effects. For example, the co-administration of a DNA vaccine and an adjuvant such as poly I:C in fish might result in exaggerated inflammatory responses or tissue damage (Gong et al. 2022).

More extensive investigation, and in situ experiments are required to further assess the synergistic or antagonistic effects of specific combinations of vaccine-immunostimulants. This includes the evaluation of immune responses, protection levels and safety profile recoverable bacteria as well in general efficacy endpoints (in target fish species / production conditions). Also, it is important to investigate whether the treatment could be further optimized when considering dose escalation, different routes of administration and timing for combined therapy to ensure maximal benefits with minimal side effects (Wicha et al. 2021 Through exploring and understanding the vaccine-immunostimulant interactions by carefully reconstituting vaccines, effective strategies can be tailored for disease prevention and control in aquaculture which could lead to overall sustainability of an industry with high profitability. Antibody production and cellular immune responses: Vaccines with immunostimulants are also able to considerably influence both humoral (antibody mediated) as well as cellular immune responsiveness of fish (Ali et al. 2023).

Factors Influencing Efficacy

Fish Species and Life Stage

- Therefore, vaccine-immunostimulant combinations must be developed according to the fish species and

developmental stage.

- Since different fish species vary in their immune response and susceptibility to pathogens, the optimization of vaccine doses at immunostimulant adjuvants concentrations as well as method of administration is indeed essential.

For example, Pothiraj et al. (2024) using an inactivated Aeromonas hydrophila vaccine combined with levamisole as immunostimulant succeeded to boost up the protection level in a freshwater carp, the rohu (Labrohita).

Nevertheless, strains of other species being salmonids or marine fish may have different optimal doses and administration route.

- Life stage is also an important factor in that the immune system of fish is still developing early on.

Larvae and juvenile are susceptible to viral infections as they have less efficient immune responses than adults (Buchmann 2022).

Siwicki et al. (2020) successfully vaccinated with a Yersinia ruckeri bacterin adjuvated in Biomus® to the immunostimulation of adult rainbow trout against enteric redmouth disease

Yet the most effective mix and dose for younger life stages of a species may be different.

Environmental Conditions (temperature, salinity, stress)

- Various environmental factors including water temperature, salinity and stress levels can greatly influence the immune response of fish towards vaccines-immunostimulants mixtures.
- Fish being poikilothermic belong to the category of ectotherms, as such their immune responses are highly influenced by temperature (Pandey et al. 2021).
- Meanwhile in marine or brackish water species, salinity may also affect the immune response and bioavailability (Du et al. 2022).
- The efficacy of vaccine-immunostimulant combinations may be reduced due to suppression of the immune system under stress factors such as handling, transport and overcrowding (Diro et al. 2021).

Dosage and Administration Routes

- The dose of each vaccine and immunostimulant, as well the routes can effect on how much frequent time that we use this association for future work.
- Dosing matters, with both underdosing and overdosing potentially having a detrimental effect on efficacy or safety (Sun et al. 2022).
- The method of administration, such as injection, immersion and oral delivery can affect the availability/bioavailability (BA), uptake kinetics and distribution of vaccine/immunostimulant components (Mičúchová et al. 2022).

These factors should be taken under consideration during the development and assessment of vaccine–immunostimulant combinations for specific fish species, production systems and disease challenges. In aquaculture context, 30 such combined strategies require optimization and validation through controlled experiments as well as field trials in order to ensure their efficacy (functional trait), safety and practical applicability.

Practical Considerations and Challenges

Compatibility and Stability of Vaccine-immunostimulant Combinations

For an effective and stable combined formulation, compatibility between vaccine components with immunostimulants is necessary (D'Amico et al. 2021). Certain immunostimulants can interact with vaccine antigens or adjuvants such as plant extracts, and microbial derivatives which could result in effects contrary to their intended functions impacting stability of the vaccines and also response (Nooraei et al. 2023).

Regulatory Aspects and Safety Concerns

- The use of vaccines and immunostimulants in aquaculture are regulated to ensure the safety for target species, consumers and environment (Wright et al. 2023).
- Combining vaccines with immunostimulants would typically involve having to undergo additional regulatory evaluations and approvals as the potential interactions, interacting synergistic/cumulative effects have not been assessed for (Zhao et al. 2023).
- There are potential safety concerns in using subtances that act as immunostimulants if derived from plant or microbial sources, which can cause adverse reactions and other long-term effects (Kazi et al. 2023).

Cost-effectiveness and Commercial Viability

- In terms of production cost, commercial interest and industrial viability a combined strategy for vaccine-immunostimulant development should also be taken into account from the perspective of aquaculture producers (D'Aniello et al. 2024).
- The future economic feasibility may be predicted only if the production costs of vaccines and immunostimulants as well as their formulation, storage addition to administration perform an intrinsic role in this concern (Hossain et al. 2024).
- However, if the immunostimulants are derived from natural sources or come along with complicated extractions steps it may also increase production costs and make products less economically viable (Hasted et al. 2021).

- For the near future, researchers and industry stakeholders will need to judiciously match hygienic benefits that can result from combined strategies with their cost-effectiveness and market conditions so we could expect a step-by-step view on practical implementation of these two approaches (Stenzinger et al. 2023).

These practical perspectives and challenges are crucial to the successful development and implementation of vaccine-immunostimulant cocktails in aquaculture. These synergistic efforts in disease prevention and control will require concerted collaboration among researchers, regulatory bodies, and industry partners to overcome these challenges.

Summary of Key Findings

In some fish species, such as salmonids, cyprinid and marine fish, administration of vaccines together with immunostimulants like a glucan derivative from fungi cell wall or plant extracts have been shown to improve survival rates in vaccinated animals by increasing the amount of antibodies produced against specific antigen challenge straight after vaccinations along strong cellular immune responses.

Together, the use of these interventions has proven effective against an array of viral, bacterial and parasitic pathogens reinforcing their astuteness in disease management.

Potential Applications in Sustainable Aquaculture

- We have determined the optimal timing and routes of delivery (which are important for specific combinations to get maximal protection yet avoid any interference/stronger side effects).
- The inclusion of vaccine-immunostimulant combinations in the aquaculture will further help to develop sustainable disease management strategies, denying massive amount of chemotherapeutics and antibiotics used.
- This combined practice enhances the protection that naphtalnedione application provides leading to increased productivity, less economic losses and more profitability for aquaculture corporations.

Future Research Opportunities and Collaborations

- More-depth researches in new resource and the action mechanisms of immunostimulants are raised to design more potential targeting formulations.
- The bioavailability, stability and targeted delivery to specific immune cell populations may be improved when combining vaccines with immunostimulants by optimizing the adjuvants or vehicle systems for vaccineimmunostimulant combinations.
- Omics-based approaches, such as transcriptomics and proteomic analyses offer important clues for unraveling molecular mechanisms of the synergistic effects in these interventions to guide future rational design strategies.
- Engagement of researchers with industry partners and regulatory agencies, working collaboratively across these stakeholders to facilitate translation from modelling into practice is important for safe and effective application within aquaculture settings.

Considering the current benefit of dispersing vaccine-immunostimulant combinations into aquaculture, which comes at a time when fish farming is expanding rapidly together with novel challenges and risks: this would be an approach to reinforce sustainable disease resistance processes while improving fish health. The success of these integrated strategies should only support further research on, and the formation of interdisciplinary collaborations around knowledge transfer about replicable models to ensure that they are applied universally in aquaculture.

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Chapter 54

Beyond the Barn: Immunization in the Modern Age of Animal Husbandry

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ABSTRACT

Immunization is an important concept in animal husbandry and epidemiology. A sufficient number of animals in a herd should be immune to infectious diseases. Therefore, it is very critical to maintain herd immunity for long-term disease control. As we are now in the era of science and technology, many advancements are being made in immunization practices. Biotechnology, nanotechnology, and artificial intelligence are the main areas that revolutionize herd immunization practices. These technologies aided previous practices and now personalized vaccines or drugs are being used to combat infectious diseases and also helping to control or eradicate the diseases from the herd. Bioinformatics approaches improve the study of host-pathogen interactions also with the involvement of genomic approaches, targeted vaccine designing and bioavailability of vaccines have been improved. Now modern ways are used to deliver and monitor the response of vaccines which make animal husbandry practices easier and improve the health status of animals.

KEYWORDS
Herd Immunization, Livestock Vaccination, Animal Husbandry, Technological Advancements, Next Generation Vaccines, Immunogenicity

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INTRODUCTION

Animals, like other organisms, have a defense system against different susceptible pathogens called natural immunity. One is innate immunity which includes humoral and cell-mediated while the other is adaptive immunity. Innate immunity is nonspecific because some components do not have antigen recognition receptors while some components have pattern recognition receptors. Adaptive immunity is specific; first, it recognizes the pathogen and then eliminates the pathogen. Mainly lymphocytes are involved in adaptive immunity, their response is delayed but it improves over time (Kennedy, 2010).

Antibodies that are produced from B-cell antigen receptors in response to antigens have two distinct regions one is the constant region and the second is the variable region (Janeway Jr et al, 2001).

Technological Advancements in Immunology

Role of computational biology in vaccine design

Vaccines and drug designing are complex procedures while now with advancements in bioinformatics and computational biology, vaccine designing and drug development are easier things to do. Through computational biology, an organism's biology is thoroughly and systematically analyzed. Information and data regarding genomics, transcriptomics, proteomics, and metabolomics are comprehensively used to understand biology, and now this helps in vaccine development. Proteomics along with genomics play an essential role in vaccines in designing potent immunogenic protein vaccines. Different biomarkers are predicted by the use of a systematic simulation-based meta-analytical framework (Sunita et al, 2020)

Subunit Vaccine Designing

A subunit vaccine is a vaccine having antigenic epitope which is required to initiate and produce immunological response in the organism. Computational approaches such as molecular dynamic simulations, molecular docking, modeling, and protein-protein interaction studies help determine immunodominant epitopes that are effective in initiating immune response (Arya and Bhatt, 2021). *In-silico* studies are utilized for recognizing and analyzing B-cell and T-cell epitopes. Both B and T-cell epitopes interact with humoral and cell-mediated immunity and then produce an immune response. Subunit vaccine designing has three important steps (i) prediction of effective epitope with *in-silico* studies (ii) synthesizing epitope-based vaccine (iii) evaluation of vaccine. Bioinformatics tools are used to map B or T-cell epitopes and then find high-affinity molecules for further studies. These approaches minimize the cytokine storm, immune tolerance, and an easy and fast approach to develop subunit vaccines (Parvizpour et al, 2020).

Immunoinformatics

Immunoinformatics is an emerging field in bioinformatics that is related to immunological studies of vaccine responses produced by the immune system. Several databases are available that provide information about the immune system and this information is used to analyze and simulate infection, immune response, and vaccinal immune response. Nowadays most infections are newly emerging and most are re-emerging. These are due to behavioral changes, environmental changes, or intermediate factors. The advanced knowledge of pathogen-immune system interactions, their genome, and proteome is beneficial for controlling the potential emergence and re-emergence of infection and disease. As our understanding of the relationship between innate and adaptive immunity increases, new effective vaccine targets and approaches are being utilized. Thus, disease control through pathogen genome sequencing enables the identification of novel vaccine targets, design, and development. (Oli et al, 2020).

Next-generation Vaccines

The last three decades have witnessed the development of numerous software programs, methodologies, and databases for the analysis and prediction of vaccine characteristics, owing to advancements in genome sequencing and biomedical computing, enhancing research and development in the field (Salemi et al, 2021). The realm of design for bio-modalities such as proteins, peptides, and vaccines has widened with advances in pharmaceutical sciences. Vaccines, essential for controlling infectious diseases, come in a multitude of types, including subunit, polysaccharide, conjugate, recombinant protein, live attenuated and inactivated, and toxoid vaccines. Moreover, new varieties such as DNA and mRNA vaccines are being produced (Yajie et al., 2021). There now exist eight major vaccine categories (Fig. 1), each with its own origin, composition, and immunogenicity.

Nanoparticle-based Vaccines

Nanotechnology's potential to control attributes like size, surface coating, and antigen loading efficiency has sparked interest in developing nanoparticles as potential vaccines (Gheibi Hayat andDarroudi, 2019). Nano vaccines are centered on subunit vaccines, that offer focused immune reactions with few antigens compared to conventional immunizations including inactivated pathogens (Zhang et al., 2019). Subunit vaccines, on the other hand, usually lack pathogen-associated molecular patterns (PAMPs) for significant immunogenicity, and therefore demand added adjuvants or nanomaterial delivery systems for maximum effectiveness (Tandrup Schmidt et al., 2016). By providing essential antigen shielding until they reach antigen-presenting cells, nanomaterials limit unwanted systemic immune reactions (Liu et al., 2020). Several nanomaterial delivery systems have been established as nano vaccines, such as liposomes, inorganic nanoparticles, composite, and metallic and polymer-based nanoparticles (Cai et al., 2020).

Vectored Vaccines

Using genetically modified viruses, recombinant viral vector vaccines serve in veterinary medicine to deliver important antigens. Both humoral and cell-mediated (CD8+ T cell) responses are triggered by these vaccinations, which are equally safe as inactivated subunit vaccines (Miller et al., 2022). Canarypox and fowl pox virus vectors were among the first to be explored, and then adenovirus vectors for different diseases and antigens linked to tumors. In an attempt to generate larger immune responses, chimeric recombinant vector vaccines broaden these concepts by integrating genes and antigens from various disease types into a single vector (Aida et al., 2021).

DNA and RNA Vaccines

DNA and RNA-based vaccines, that leverage injection-based plasmid DNA delivery, are an innovative form of vaccination. These polynucleotides can now be subjugated, presenting alternatives to traditional vaccination approaches owing to developments in molecular biology (Ferraro et al., 2011). Adjuvants are not required for DNA vaccines to elicit humoral and cell-mediated immune responses through the use of plasmids encoding antigens. Ease of engineering, viable broad-spectrum combination vaccines, and safe administering to immunocompromised persons are their primary benefits (Chapman andRybicki, 2019). DNA vaccines work as pathogen-associated molecular patterns (PAMPS), imitating the biosynthesis of intracellular pathogen proteins and evoking robust CD8+ T cell responses mediated by MHC-I. Antigen presented on MHC-I by transfected somatic cells triggers a CD4+ T-cell response, whereas antigen presented on MHC-II complexes triggers CTLs cross-primed by dendritic cells (Lee et al., 2018).

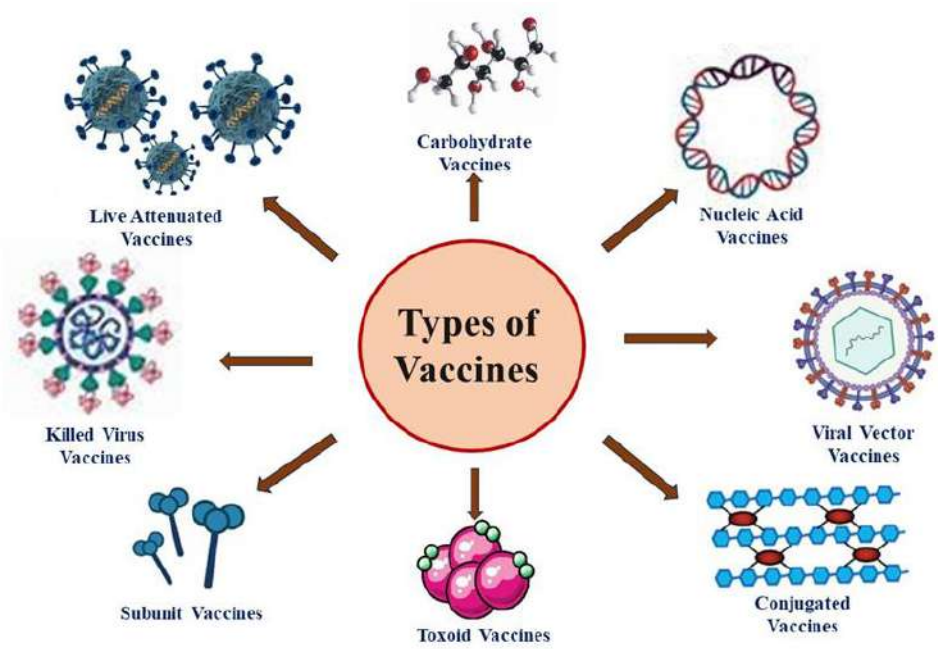

Fig. 1: Types of Vaccines

Advances in Vaccine Delivery

Microneedle Patches

Recent studies in humans and animals as well as in vitro research have shown that limiting antigen dosages for vaccines is essential for enhancing T-cell responses. A potential alternative is intradermal administration through microneedle (MN) patches. The cutaneous layer is momentarily penetrated by MNs, which are hundreds of microns in size, permitting minimally invasive transcutaneous administration. By lowering healthcare waste and needle-related hazards, MN vaccination has demonstrated better immunological responses than hypodermic injections, boosting vaccine efficacy and safety. According to a recent study, MNs are beneficial for disrupting the stratum corneum painlessly and allowing transcutaneous distribution of specific vaccine constituents (D'amico et al., 2021).

Oral Vaccines

Non-intrusive vaccinations—such as vaccines delivered orally or nasally—are encouraged due to their reasonable price and simplicity of use. By triggering the production of protective antigen-specific secretory IgA in the gastrointestinal mucosa, oral vaccines strengthen mucosal immunity and thwart pathogen invasion. Moreover, oral vaccination successfully prevents the dissemination of invasive mucosal conditions by inducing systemic immunity and IgA-mediated immunity at mucosal sites (Yuandong et al., 2021).

Aerosol Vaccination

Aerosol delivery offers substantial lung concentrations, elevates efficacy, and lessens off-target effects, whilst productively engaging local lung immunity when provided through inhalation. It can enter the bloodstream and produce systemic effects while offering easy access to lymph node drainage for the activation of the immune system. Overcoming formulation limitations and coming up with efficacy predictions in the course of preclinical and clinical evaluations are challenges (Emma et al., 2023).

The characteristic features of different types of vaccines are discussed in Table 1

Genomic Insights into Host-Pathogen Interactions and Vaccine Responses

A significant worldwide health threat, transmissible diseases are triggered by sophisticated host-pathogen interactions and pathogen transformations. Comprehending clinical manifestations is essential for efficient medication and immunization. RNA sequencing and multi-omics profiling are examples of high-throughput approaches that provide an understanding of disease diversity. Extensive insights into host-pathogen interactions are offered by integrative genomics techniques. To better comprehend the impact and severity of diseases, functional microbiomes can be explained using a holo-transcriptome-based technique (Mehta et al., 2022).

Table 1: Characteristic Features of Different Categories of Vaccines

Categories	Administration	Handling	Utility	Safety	Stress	Compliance	Therapeutic Onset
 **Injectables**	Requires Needle	Cold Chain	Limited	Inadequate	Piercing	Sub Optimal	Fast

 **Microneedle Patches**	Microneedle	Room Temperature	Admirable	Adequate	Non-Piercing	Fair	Fast
 **Nano-Vaccines**	Injectable/ Aerosol	Depends on Formulation	Admirable/ Limited	Adequate	Piercing /Non-Piercing	Optimal	Fast
 **Oral Vaccines**	Oral	Cold Chain	Admirable	Adequate	Non-Piercing	Excellent	Slow

Digital Solutions for Immunization Management

The good health and well-being of animals crucially depend on livestock vaccination. It ensures the long-term viability of the farms and their adherence to food safety regulations. Manual management and record keeping of vaccination in livestock is met with several challenges. Some of these are depicted in Fig. 2A. The use of digital record-keeping software contributes significantly to the efficiency of data collection and management. Fig. 2B contains some of the key features provided by this software.

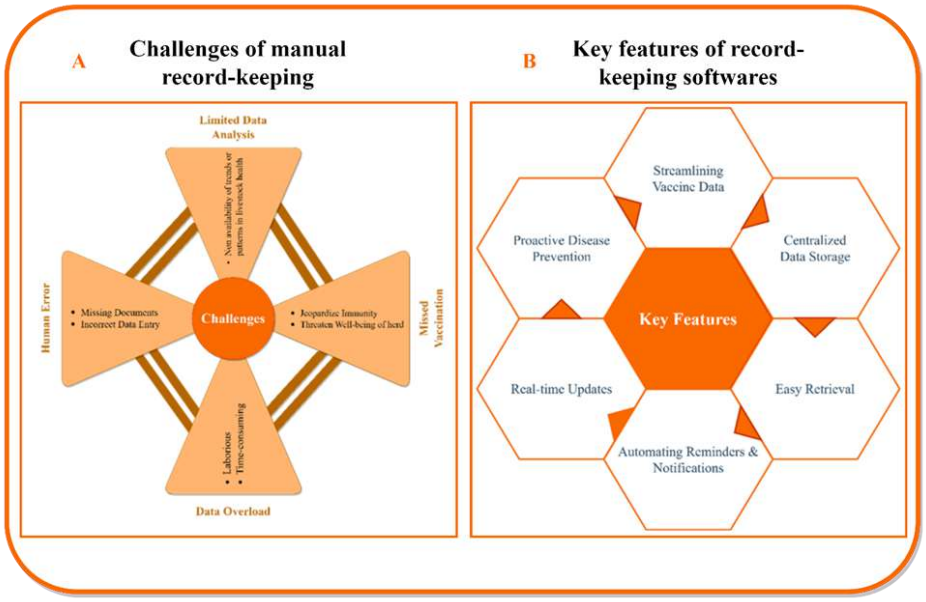

Fig. 2A: Challenges in manual record-keeping of livestock vaccination. B: Key features of livestock vaccination record-keeping softwares (Data source (El Idrissi et al., 2021))

The world population is estimated to increase up to 9.8 billion by the year 2050, which also raises concern about increasing food production by 70% to meet dietary needs. Transformation of the agriculture and food sector by incorporating technological advances and innovation is expected to play a critical role in ensuring food safety and security. Integrating science and technology into animal husbandry and livestock farming has resulted in the intensification of farming systems by improving animal health, and product quality and reducing the burden of diseases (El Idrissi et al., 2021). The deployment and use of digital technologies, like mobile applications, cloud computing, artificial intelligence, blockchain, etc., have spectacularly transformed every sector of the economy including animal health, welfare, and production. The accuracy of data collection, timeliness, improved veterinary practices, disease surveillance, and animal health monitoring are some of the opportunities offered by the recent developments in information and communication technologies (ICTs) and innovations. These advances can positively affect the quality and performance of veterinary services owing to a better, more efficient, and timely decision-making aptitude (El Idrissi et al., 2021). Integrating information from different digital technologies empowers the digital network for real-time monitoring and predictions. An overview of some of these digital technologies is as follows:

Internet of Things (IoT)

IoT allows sensors and electronic appliances to connect via the internet by using nanotechnology and quantum to achieve incredible storage, computing speed, and sensing. Various wireless technologies that can be implemented for IoT

applications include ZigBee, Wi-Fi, Sigfox, RFID, NB-IoT, etc.

Artificial Intelligence

It is a combination of training, perception, and problem-solving in a system by using various models such as artificial neural networks, machine learning, support vector machines, heuristics analysis, etc.

Machine Learning

It is a modern application of AI that supports the existence of giving machines entrance to more data for refining the human design and learning for themselves by gathering data, pre-processing and wrangling data, analysis, model training, and testing.

Cloud Computing

It is an efficient alternative to owning and managing computer applications and resources by providing hosted services over the Internet through various cloud models. It also lowers operating costs and improves data access efficiency, security, and Quality of Service (QoS).

Drones

These human-operated, airborne vehicles can operate a broad range of missions such as surveillance, patrolling, and protection and can travel thousands of kilometers.

Blockchain

It is a novel technology that serves as a distributed ledger system and provides great protection against the manipulation of information. It involves the collection of transactions in the form of a database that is further verified and documented in a centralized ledger (Singh et al., 2022).

Vaccination Tracking

Vaccination is critical in protecting livestock from infectious diseases and ensures productivity and overall health. However, for larger populations, tracking of vaccination can be challenging which may lead to potential gaps in vaccination schedules and disease risk. The emergence of IoT technology has revolutionized vaccination tracking by offering innovative solutions that can improve disease management and herd health. The challenges of manual record-keeping methods can be addressed by IoT vaccination tracking that involves equipping each animal with a wearable sensor or a smart tag containing unique identification data. These tags continuously collect data on the type of vaccine administered, dosage used, and vaccination date and automatically transmit it to a centralized database through IoT-connected devices. These vaccination records are securely stored and easily accessible through cloud-based storage systems, ensuring data integrity. These data provide comprehensive insights into disease prevalence and vaccination trends, enabling veterinarians to identify potential outbreaks, adopt pre-emptive disease management strategies, and implement required containment measures (Celeritas). IoT-based tracking ensures real-time and accurate vaccination data that can lead to improved herd health and reduced disease outbreaks. It is time and cost-effective, unlike manual record keeping which is prone to errors and is labor intensive. IoT system allows remote monitoring of vaccination data from multiple farms or locations and can provide complete traceability of each animal's immunization history. It improves the implementation of necessary biosecurity measures and enhances food safety (Celeritas).

Blockchain Technology

An agricultural supply chain is a complex network that is responsible for the market distribution of agricultural products. Advancements in modern technologies, such as blockchain, can potentially improve food security by increasing transparency, lowering transaction costs, and supplying real-time data. The blockchain is like a distributed database consisting of encrypted blocks or public ledgers that record all transactions or digital events shared by the parties involved without allowing any data manipulations and can be verified at any time in the future. A blockchain-enabled system is expected to improve speed, responsiveness, security, efficiency, and visibility for all the involved parties including government agents, customs officers, traders, and consumers. Due to its robust and decentralized abilities, it was originally designed for use in global financial systems and was later on expanded to include supply chain tracking of medications, plant germplasm, animal feed ingredients, and vaccinations. The implementation of blockchain technology in livestock is still in its early. In the animal health sector, input supply chains including medicine and vaccination face a large number of challenges from record keeping and traceability of vaccines/ ingredients to production, storage, and transportation of vaccines as per the required standards. Therefore, registration and data harvesting in the vaccine supply chain irrespective of the complexity of the process are preferred. In blockchain technology, each stakeholder can connect to a single central system and can easily share information and data. Advanced reporting and data analysis can use this combined data for the safe and efficient production of medicines, vaccines, and other inputs (Makkar andCosta, 2020).

Eco-Friendly Immunization Practices

Vaccination is a preventive weapon in the toolkit of strategies for controlling disease on farms (Jonathan M E Statham, 2022). Over the last few decades, sustainability and environmental awareness have received much attention in various sectors, especially in the vaccine-manufacturing unit. Vaccine manufacturers can mitigate the environmental concerns associated with their production through the implementation of sustainable production technologies and eco-friendly packaging supplies (Bae et al., 2009).

Sustainable Vaccine Production Methods

Due to the extensive testing, examination, and regulatory authorization requirements, vaccine development requires an extended time. Although vaccines offer a reasonable means of improving health by reducing the rates of infection-related mortality, still no vaccine provides life-long or complete protection. Continuous progress in the fields of vaccine research and development is indispensable to combat emerging infections and mutant strains. Existing vaccine manufacturing methods include subunit vaccines, cell-based vaccines, virus-like particles, and recombinant DNA technologies (Fig. 3). These vaccines are associated with challenges like virulence regaining risk, limitations in immunocompromised individuals, production hazards, storage concerns, low immunogenicity, and expensive production of larger stocks. These limitations can be overcome by sustainable and eco-friendly immunization platforms such as nonthermal plasma-based vaccination (NTP) and plant-based vaccines. NTP technology ensures vaccine integrity by effectively inactivating the pathogen without excessive heat treatment. Plant-based vaccines are safer and scalable and reduce the risk of contamination as they are independent of pathogen replication. These advances suggest sustainable development technologies for efficient and accessible vaccine manufacturing ensuring an eco-friendly vaccination era (Kaushik et al., 2024).

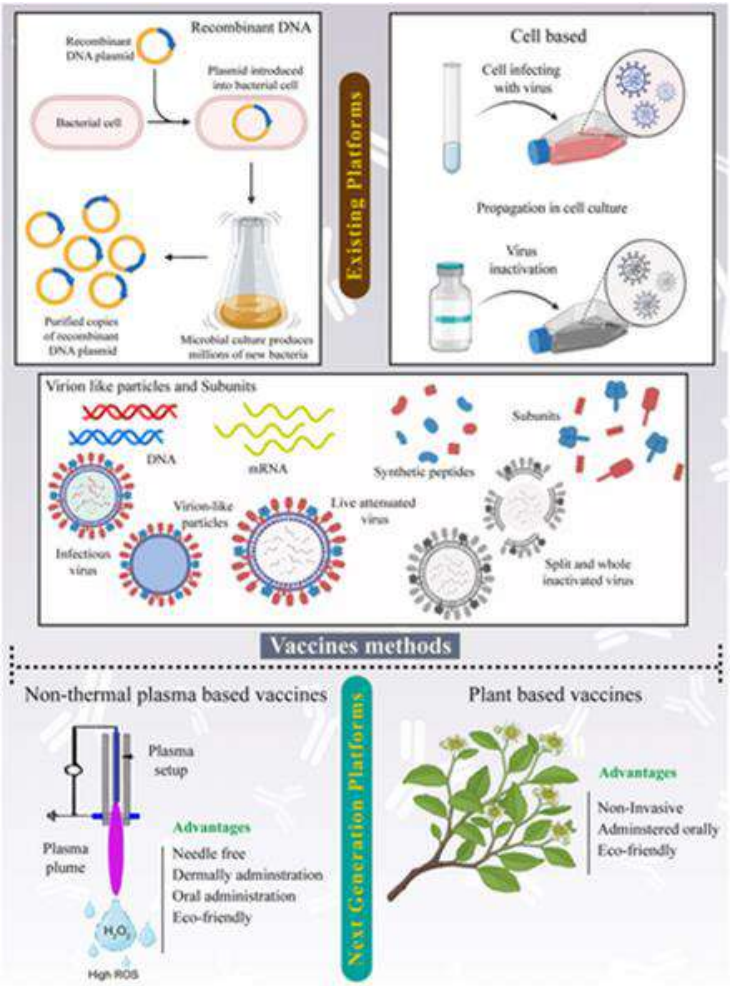

Fig. 3: Schematic illustration of cutting-edge eco-friendly vaccine manufacturing techniques (Kaushiket al., 2024)

Eco-friendly Packaging Materials

Although massive-scale vaccination against a potential pandemic pathogen offers a containment method, it also leads to the production of an increased amount of medical waste such as glass, plastic, rubber residues, syringes/ needles, gloves, and masks. Concerns arise on how to overcome a potential pandemic without risking the environment. The primary emphasis of a sustainable vaccination model is to redefine the packaging process. For over a century, borosilicate glass has been the most widely used packaging material. The use of temperature-controlled, sustainable, multi-material thermal insulations, renewable plant components, and paper offers an eco-friendly alternative to EPS foam (expanded polystyrene) and disposable plastic that requires more than 350,000 tons of packaging material

annually. Environment management solutions can reduce pollution and waste linked to the entire life cycle of vaccine packaging from its production, transportation, and storage to waste management strategies. To accomplish this, the reuse or recycling ability of materials is of utmost importance besides the reduction in carbon emissions and the addition of a renewable energy source in the production line. Optimization of the distribution process using foldable packaging is also necessary to ensure the requirement of small storage space in cargo. Technologies for the replacement of traditional glass vials containing freeze-dried vaccine pellets with blister packs enclosing effervescent vaccine tablets can reduce the water footprint and impact on climate change by 70% and 80% respectively. Syringes made of either glass, plastic, or stainless steel are an important component in vaccine packs. Therefore, consideration of an alternative material for the manufacturing of syringes is also essential in a sustainable vaccination model. The most commonly used plastic for syringe production is polypropylene, which is recyclable and safe, but only 1% is recycled, and the rest is dispatched to landfills. During incinerations, toxic additives present in polypropylene like cadmium and lead release poisonous vinyl chlorides and dioxins. To overcome this problem, Cambridge Consultants created pre-filled syringes called Syreen syringes. These syringes contain COP plastic (cyclic olefin polymer) that is metal oxide residue-free and produces less ash upon incineration. These syringes eliminate the need for secondary packaging and thus reduce the packaging weight and volume by 30% and 50% respectively (Phadke et al., 2021).

Impact of Immunization on environmental Sustainability and biodiversity conservation

Healthy animals require fewer resources and produce more eggs, milk, and meat. According to WHO, diseases directly result in a 20% loss in animal production worldwide. Improving the well-being and health of livestock ensures the maximum utilization of invested resources. Recently, an Oxford Analytica report stated that cattle beef productivity can improve by 50% as a result of a 60% global vaccination rate. It is also reported that good husbandry practices and improved animal health can result in a reduction in emissions by 18-30% (Australia). Vaccines have the potential to improve the spillover control and conserve biodiversity. Evolutionary impacts and the ecological complexity of imperfect immunity provide many obstacles to wildlife vaccination. However, understanding the ecology and physiology of the system by use of wildlife vaccination for spillover prevention or conservation is not ignorant (Barnett andCivitello, 2020).

Global Health Security and Pandemic Preparedness

Importance of Animal Immunization in Preventing Zoonotic Disease Outbreaks

Scientists estimate that zoonosis is responsible for 60% of human infections. Many of these diseases have high fatality rates and have the potential to spark epidemics and pandemics. Each year, zoonotic infections affect animal output and food security and cause about 2.7 million fatalities and 2.5 billion illnesses in humans. Zoonotic diseases have the potential to impact global food security, human, animal, plant, and environmental health, as well as economic stability. Animal vaccinations limit the spread of certain zoonotic illnesses to people, manage diseases in companion animals, and guarantee a safe food supply by keeping livestock populations healthy. Vaccinating domestic animal species to stop disease transmission to humans and vaccinating wild animal species to halt disease transmission to domestic animals and humans have been two strategies against zoonotic illnesses (Carpenter et al., 2022).

Vaccination is a cornerstone of disease control in veterinary medicine. A key tool in this arsenal is the DIVA (Differentiating Infected from Vaccinated Animals) vaccination. These vaccines, lacking antigenic epitopes or proteins in the dominant field strain, play a crucial role in disease eradication and control. Despite the current inability of these vaccines to distinguish between vaccinated and diseased animals, their objective is highly beneficial. Alongside DIVA vaccinations, serological assays like ELISA (Enzyme-Linked Immunosorbent Assay) can identify infected and immunized animals, further enhancing disease control efforts (Erdem andSareyyüpoğlu, 2022).

Vaccinations for animals against disease can be obtained at a reasonable cost. They can also improve food production efficiency and lessen the spread of zoonotic and food-borne illnesses to humans. Without vaccinations to stop epizootics in animals used for food production, the cost of producing enough animal protein to feed the 7 billion people on Earth would increase significantly (Roth, 2011). Without vaccines, livestock diseases that cause severe economic losses to farmers, communities, and nations would become more common. Without efficacious vaccinations for use in animals, zoonotic illnesses like brucellosis and leptospirosis would be far more common in people (Aleem et al, 2022). If both methods are available, producers may control certain diseases with vaccines or antibiotics based on cost. For example, vaccination or medicines combined with excellent management methods can reduce swine ileitis caused by *Lawsonia intracellularis* (Roth andSandbulte, 2021).

One Health Approach

To promote the health of people and animals, the One Health strategy is utilized to reduce antimicrobial resistance infections, improve food safety and security, control and prevent zoonotic disease outbreaks, and perform cooperative disease surveillance. The One Health concept fortifies the network for early response and detection of zoonosis, the diagnostic laboratory systems, the disease monitoring system, and the data-sharing mechanism with all stakeholders by encouraging strong collaboration among pertinent sectors. This strategy unquestionably improves the workforce for zoonotic disease prevention and control. It guarantees efficient and well-coordinated public health emergency preparedness, whereby all tactics effectively contribute to decreasing zoonotic illnesses. By addressing common health threats like zoonoses, antibiotic resistance, food safety, and security issues, the One Health approach generally strongly

supports international health security through its effective multi-sectoral collaboration, coordination, and information communication at the interface between relevant sectors (Erkyihun andAlemayehu, 2022).

Addressing ethical considerations and public acceptance of new vaccine technologies

Over the years, the development and application of vaccines have significantly reduced diseases. Through altering DNA, RNA, proteins, and sugars, many new vaccine types have been introduced due to advancements in genetic engineering and our growing understanding of immune protection (Gebre et al., 2021). The development of attenuated mutants, the expression of putative antigens in living vectors, and the direct manufacture and purification of antigens in novel systems have all significantly advanced vaccination research. Today, vaccinations cover both infectious and noninfectious diseases (Yousaf et al, 2024). The abundance of novel vaccines makes it possible to target and immunize previously unvaccinated populations to treat and eradicate infectious organisms from their natural reservoirs. However, in addition to modern diseases like the human immunodeficiency virus and ancient ailments like malaria, a potent vaccine remains elusive, presenting a more significant challenge to science (Yadav et al., 2020).

Many immunogens used in veterinary medicine are still created with traditional technology, such as attenuated vaccines. Nonetheless, the creation of biotechnological instruments has led to their application in developing vaccines. These "modern" vaccine methods reduce the spread of infectious diseases and boost immunity against ectoparasites. There are several methods for creating a vaccine that can induce acquired immunity (Asrar et al, 2023). The most common techniques involve using the pathogen in its entirety, either attenuated or inactive, or they can even rely on subunits like isolated proteins or self-assembling structural molecules, also known as virus-like particles, nucleic acids, or viral vectors. Utilizing synthetic peptides, which are created by computational prediction studies specifying potential sequences containing immunogenic determinants, is another technological advancement (Barbosa et al., 2022).

Challenges and Opportunities

The assessment of rationally gene-deleted live attenuated vaccine candidates for target host-pathogen exposure entails determining their genetic stability and potential reversion to virulence. In particular instances, appropriate biocontainment facilities are needed for the manufacturing of these vaccines. The reason behind the development of TAD (Transboundary animal diseases) and zoonotic veterinary vaccines is that all licensed high/maximum biocontainment facilities have complex engineering and procedural controls for the security of the facility and the safety of the staff (Aleem et al, 2022). These controls must be constantly validated and maintained by a sizable team of highly skilled facility technicians and engineers. There is a lack of immunological reagents and assays tailored to veterinary species to fill in the gaps in our understanding of disease pathophysiology and host defense immunity (Smith et al., 2021). Through immunization, veterinary livestock disease models offer the chance to directly test and assess EID (Emerging Infectious Diseases) and zoonotic human vaccine candidates for safety and efficacy. If funding supports the desired result, there are also new chances to coordinate the concurrent research of vaccination candidates for humans and animals. While there is still more to be done to strategically execute this notion among government organizations responsible for public health and animal health, there are several benefits to developing human and veterinary vaccines for zoonotic illnesses and TADs simultaneously (Brake et al., 2020).

Conclusion

This chapter covers immunological advances with an emphasis on animal immunization. It commences with the difference between innate and adaptive immunity, emphasizing the function of antibodies and lymphocytes. The formulation of vaccines has been completely transformed by technological developments in bioinformatics and computational biology, which have made it possible to create powerful immunogenic protein vaccines and subunit vaccines through in-silico work. The importance of immunoinformatics and next-generation vaccines, such as vectored and nanoparticle-based vaccinations, in improving vaccine safety and effectiveness are highlighted. Additionally, the chapter discusses novel approaches to vaccine delivery, such as aerosol immunization, oral vaccinations, and microneedle patches. By increasing data reliability and real-time monitoring, digital tools such as blockchain, IoT, and AI are revolutionizing immunization management and boosting food safety and animal health. The environmental friendliness of sustainable vaccine production techniques is highlighted, with particular emphasis on plant-based and nonthermal plasma-based vaccines. The chapter emphasizes how important animal vaccination is to limit the spread of zoonotic diseases and promote the One Health strategy for global health security. Together with potential advantages and challenges associated with vaccine development, ethical considerations and public acceptability of novel vaccination technologies are discussed. To maintain food security and sustainability, the chapter concludes with an emphasis on the importance of integrating science and technology into animal husbandry.

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Chapter 55

Antimicrobial Activity of Aromatic Herb Essential Oils against *Pseudomonas aeruginosa*

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ABSTRACT

The emergence of multidrug resistance such as *Pseudomonas aeruginosa* is due to the misuse of antibiotics. This has become a significant health concern and calls for the development of novel approaches to combat these challenges. Promising antibacterial activities have been shown by essential oils extracted from aromatic plants, providing a natural and potentially efficient remedy against resistant bacteria. This chapter examines the antibacterial properties of essential oils derived from several aromatic herbs, with a focus on their ability to combat *Pseudomonas aeruginosa*. The chemical makeup of these essential oils, their modes of action, and the effectiveness shown in both in vitro and in vivo investigations are all included in the discussion. Among the important plants that were studied were Sage Oil (*Salvia officinalis*), Thyme (*Thymus vulgaris L.*), Tea Tree Oil (*Melaleuca alternifolia*), Eucalyptus Oil (*Eucalyptus globulus*). The chapter also discusses the implications for clinical applications and the possibility of synergistic effects when conventional antibiotics and essential oils are combined. This chapter intends to contribute to the development of new, natural therapies in the fight against multidrug-resistant *Pseudomonas aeruginosa* by elucidating the antibacterial potential of aromatic plant essential oils.

KEYWORDS
Pseudomonas aeruginosa, Salvia officinalis, Thymus vulgaris L., Melaleuca alternifolia

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INTRODUCTION

P. aeruginosa is a monoflagellated, motile, aerobic, non-spore-forming, rod-shaped, non-fermenting. The genus *Pseudomonas* was initially defined by Migula in 1894, and *P. aeruginosa* was the species type of that genus. *P. aeruginosa* was first isolated from green pus by Gessard in 1882 (Urgancı et al., 2022). Since *P. aeruginosa* is a non-fastidious microbe, it doesn't need any particular growing environment. The majority of non-selective media, such as Mueller-Hinton, Nutrient agar, Luria-Bertani, blood agar, etc., are suitable for its growth; however, some media, such as cetrimide agar and King-A and King-B media, are designed expressly to facilitate the selective propagation of *Pseudomonas*. Although pseudomonads develop best at 37°C, they can withstand temperatures as high as 40°C (Gajdács et al., 2019).

P. aeruginosa has phenotypic traits that include a distinct odor (characterized as flower-like, "grape juice", or "fresh tortilla"), β-hemolysis (on blood agar), and color of the colonies (in the right culture media), which enable rapid organoleptic identification (Clark et al., 2015). *P. aeruginosa* has phenotypic traits that include a distinct odor (characterized as flower-like, "grape juice", or "fresh tortilla"), β-hemolysis (on blood agar), and color of the colonies (in the appropriate culture media), which enable rapid organoleptic identification (Hall et al., 2015; Abbas et al., 2022).

The bacterium is significantly more genetically versatile than other bacteria. It has a relatively large genome, which allows it to grow in a variety of environments, produce a wide range of virulence factors, and exhibit antibiotic resistance to most currently prescribed antibiotics (Cillóniz et al., 20016; Nguyen et al., 2018; Raman et al., 2023). It is a common microorganism found on the surfaces of fruits, vegetables animals, plants, and insects and in water, soil, and sewage (Kaszab et al., 2021). *P. aeruginosa* is mostly responsible for nosocomial infections, including bloodstream, pneumonia, urinary tract infections (UTIs), wounds, and bones and joints (Morin et al., 2021; Sekhi, 2022; Sathe et al., 2023). *P. aeruginosa* is recognized to be linked to lower respiratory tract infections among individuals with cystic fibrosis. It also causes community-acquired infections such as gastrointestinal, skin, soft tissue, and otitis externa (Morin et al., 2021).

Pathogenicity of *Pseudomonas aeruginosa*

Several virulence factors, some of which are essential components of *P. aeruginosa's* cell structure, contribute to the pathogenicity of the bacteria. However, a variety of other virulence factors are also produced and released, contingent upon the conditions surrounding the infection (Azuama et al., 2020; Shaw and Wuest, 2020). The ability of *P. aeruginosa* to adapt to a variety of natural environments and harsh (in vivo) conditions is one of its most essential traits, which is consistent with the genus's ideal metabolic diversity (Chauhan et al., 2023: Abdulhaq et al., 2020).

The primary pathogen-produced virulence factors and hallmark abilities are as follows: (1) biofilm formation factors; (2) iron acquisition systems and factors controlling iron homeostasis (3) extracellular invasive enzymes and secreted toxins; (4) toxic secondary metabolites; and (5) bacterial motility and attachment factors (Chadha et al., 2022).

Table 1: Virulence factor of *P. aeruginosa* and their mechanism in bacterial pathogenesis

Category	Factor	Description/Role
Toxic Secondary Metabolites	Pyocyanin	Generates reactive oxygen species (ROS), induces apoptosis in neutrophils
	Hydrogen Cyanide	Inhibits aerobic respiration, binds to host metalloproteins
Extracellular Invasive Enzymes and Toxins	Exotoxin U (ExoU)	Potent phospholipase causes rapid cell death
	ExoS, ExoT	Disrupt the actin cytoskeleton, inhibit phagocytosis and cell migration
	ExoY	Elevates intracellular cAMP, disrupts actin cytoskeleton
	Exotoxin A (ToxA)	Inhibits protein synthesis, induces apoptotic cell death
	LasB (Elastase B)	Breaks down elastin, collagens, and immune components
	LasA (Elastase A)	Degrades staphylococcal peptidoglycan
	Protease IV (PIV/PrpL)	Cleaves fibrinogen, plasminogen, immunoglobulins
	Alkaline Protease (AprA)	Inhibits neutrophil chemotaxis and degrades complement proteins
	Phospholipase C (PlcH)	Hydrolyzes phospholipids, causes hemolysis
	Phospholipase D (PldA, PldB)	Target PI3K/Akt pathway, promote bacterial internalization
	VgrG2b	Targets microtubules, enhances bacterial internalization
	TplE (Phospholipase)	Induces endoplasmic reticulum stress and autophagy
Motility and Attachment Factors	Type IV Pili	Includes type IVa (involved in twitching motility, retractile due to PilT ATPase) and type IVb (non-retractile, aids attachment to surfaces) pili
	Flagellum	Drives swimming motility in liquid and swarming motility on semi-solid surfaces
	CUP Pili	Five systems (CupA to CupE) crucial for attachment to host surfaces
	BapA, LecA, LecB	Facilitate attachment to solid surfaces and host cells
Biofilm Formation Factors	Exopolysaccharides (Alginate, Pel, Psl)	Critical components of biofilm matrix, contribute to mucoid phenotype
	Extracellular DNA (eDNA)	Integral to biofilm structure
	Rhamnolipids	Aid in biofilm formation, maintenance, and dispersion; provide protection from phagocytes
	Glycine Betaine	Enhances resistance to osmotic stress and survival during lung infections
Iron Acquisition Systems and Factors	Pyoverdine	High-affinity siderophore, sequesters iron from host proteins
	Pyochelin	Functions with pyocyanin, induces oxidative stress
	Heme Acquisition Systems (Phu, Has, Hxu)	Transport heme into periplasm, activate virulence
	Bacterioferritins (BfrA, BfrB)	Store excess iron, preventing toxicity
	Superoxide Dismutase (SodB), Catalase (KatA)	Protect against oxidative damage from excess iron
	Small RNAs (PrrF1, PrrF2)	Regulate response to iron toxicity, critical during infection

Motility and Attachment Factors

The flagellum and type IV pili are essential for *P. aeruginosa* movement. These appendages regulate the bacterium's movement (Table 1) and play a key role in several virulence processes, such as finding a favorable surface and permitting cell-surface contact, changing from a planktonic to a sessile lifestyle, and encouraging the development of biofilms (Geiger and O'Toole, 2023). The bacterial cell is propelled by hydrodynamic forces through the utilization of polar protein complexes called flagella, which enable swimming and swarming motility on semi-solid surfaces. Type IV pili are classified into two primary subfamilies: type IVa and type IVb. Type IVa pili are responsible for driving twisting motility on solid surfaces, and they work in tandem with flagella to produce swarming motility (Geiger et al., 2024). Because of the activity

of PilT ATPase, type IVa pili, which comprises several PilA pilin subunits, are retractile (Little et al., 2024). Flp pilin subunits are the building blocks of type IVb pili, better known as tight adherence (Tad) pili. Since tad pili are non-retractile, they primarily help *P. aeruginosa* adhere to abiotic and biological surfaces. This process is essential for the appropriate colonization and invasion of host tissues as well as the production of biofilms (Webster et al., 2022). The primary adhesins, also known as chaperone-usher pathway (CUP) pili, are essential to *P. aeruginosa* adhesion to host surfaces. Five distinct CUP systems are produced by this bacterium (from CupA to CupE) (Böhning et al., 2023). Additional proteins required for *P. aeruginosa* adherence to host cells and attachment to solid surfaces include the lectins LecA and LecB and the biofilm-associated protein BapA (Böhning et al., 2023).

Biofilm Formation Factors

The development of persistent infections is largely dependent on *P. aeruginosa's* capacity to form a biofilm. Although other species in the host are expected to contribute to *P. aeruginosa's* ability to form biofilms, the majority of experimental data on virulence factors and their regulation came from studies of biofilms from *P. aeruginosa* monocultures (Fernández-Billón et al., 2023). Due to its ability to produce the clinically significant mucoid phenotype when this EPS is overproduced, alginate is regarded as a significant virulence factor of *P. aeruginosa* (Chung et al., 2023). Mucoid cells develop into compact microcolonies that provide defense against immune system attacks and drugs, allowing *P. aeruginosa* to persist over an extended period of time in chronic infections (Muggeo et al., 2023). Additionally involved in *P. aeruginosa* biofilm production at various phases are extracellular secondary metabolites called rhamnolipids. These comprise the initial interactions between cells and surfaces in addition to the upkeep and diffusion of the biofilm structure (Ragno, 2024). Rhamnolipids are considered important virulence factors because they operate as protective agents against phagocytes in biofilms and because they promote the active dispersal of cells from biofilms, hence promoting the colonization of new niches (Skariyachan et al., 2018). Furthermore, when integrated into host membranes, rhamnolipids' amphiphilic qualities might cause host cell tight junctions to break (Wargo, 2013).

Extracellular Invasive Enzymes and Toxins

Extracellular invasive enzymes are essential because they degrade a variety of host connective tissues and immune components early in *P. aeruginosa* colonization of host tissue. Extracellular proteases play a crucial role in the pathogenicity of this bacteria. Examples include protease IV (PIV, also known as lysyl endopeptidase PrpL), elastase A (LasA, staphylolysin), elastase B (LasB, pseudolysin), and alkaline protease (AprA, aeruginolysin) (Galdino et al., 2017). LasB displays elastinolytic activity on human elastin and degrades collagens and different components of the innate and adaptive immune defense, including interferon-γ (IFN-γ), interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), and the surfactant proteins A and D (SP-A and SP-D), which are essential for the phagocytosis of pathogens (Sánchez-Jiménez et al., 2023). In addition to increasing phagocytic evasion, the alkaline protease AprA also inhibits neutrophil chemotaxis and degrades complement proteins and cytokines, such as IFN-α, TNF-γ, and IL-6. *P. aeruginosa* invasion and tissue damage are facilitated by the PIV/PrpL protease's cleavage of fibrinogen. This protease can also hydrolyze plasminogen, complement proteins, immunoglobulin, and host antimicrobial peptides. The proteases LasA, LasB, and PIV/PrpL are secreted to the extracellular media by the *P. aeruginosa* Xcp type II secretion system (Xcp-T2SS), while AprA is secreted by the type I secretion system (T1SS) (Galdino et al., 2017).

P. aeruginosa produces phospholipases, also known as lipolytic esterases, which are an important class of extracellular invasive enzymes. The pathogen uses these enzymes, among other things, to hydrolyze eukaryotic phospholipids (Table 1). This includes the phospholipase C PlcH released by Xcp-T2SS, which has a predilection for phospholipids that include choline, such as phosphatidylcholine and sphingomyelin (Ejike et al., 2023). Phosphocholine and ceramide, which are produced by hydrolyzing sphingomyelin and phosphatidylcholine, respectively, are involved in significant signal transduction cascades in mammalian cells (i.e., growth, differentiation, death, proliferation, and inflammation). Additionally, PlcH produces hemolysis in erythrocytes and humans due to its hemolytic action. The ceramidase CerN expressed in the plcH gene cluster increases its activity (Sánchez-Jiménez et al., 2023). Significantly, PlcH suppresses angiogenesis, or the growth of new blood vessels, and is extremely deadly to endothelial cells.

P. aeruginosa produces toxins and enzymes that cause cytotoxicity and other types of cell death in target host cells, which is one of its main pathogenicity strategies (Wood et al., 2023). Strong cytotoxic A2 phospholipase ExoU (for extotoxin U) can rapidly kill eukaryotic cells by inducing necrosis and a loss of plasma membrane integrity (Muggeo et al., 2023; Song et al., 2023). ExoU killing appears to target epithelial barriers and phagocytes, which encourages bacterial persistence and spread. ExoU is released by *P. aeruginosa* via the type III secretion system (T3SS), a needle complex also referred to as the injectosome that injects toxic effectors directly into the cytoplasm of host cells (Gil-Gil et al., 2023). ExoS, ExoT, and ExoY are among the several toxins that T3SS releases. The bifunctional proteins ExoS and ExoT are composed of an N-terminal GTPase-activating protein (GAP) domain and a C-terminal ADP-ribosyltransferase (ADPRT) domain (deWeever, 2023). By preventing phagocytosis and rupturing epithelial barriers that encourage bacterial dispersion, ExoT ADPRT domain activity suppresses cell motility, adhesion, and proliferation. It targets a more limited and particular subset of host proteins. It has long been believed that ExoS's sole roles are cytotoxic and phagocytic, however new research has revealed that this toxin also encourages *P. aeruginosa* internalization into eukaryotic cells and preserves the intracellular niche (Kroken et al., 2022). The last T3SS-secreted toxin that is known to be involved in infection and host colonization is

ExoY, an adenylate cyclase that increases the intracellular cAMP content in eukaryotic cells and induces the differential regulation of many cAMP-regulated genes. The action of this enzyme results in disruption of the actin cytoskeleton, inhibition of bacterial absorption into host cells, and enhancement of endothelial permeability.

Pseudomonas aeruginosa produces exotoxins and exoenzymes that are injected into eukaryotic cells using a variety of secretion systems, including the type VI secretion system (T6SS). In particular, the phospholipase D enzymes PldA and PldB, which are released by H2- and H3-T6SS, respectively, target the host PI3K (phosphoinositide 3-kinase)/Akt pathway. This allows *P. aeruginosa* to internalize into epithelial cells (Sana et al., 2016). Furthermore, P. aeruginosa produces lipoproteins that have the potential to act as pro-inflammatory lipotoxins, which could cause CF patients' lungs to react to inflammation more exaggeratedly. Examples of this include the osmoprotective lipoprotein OsmE and the lipoaccharide transport proteins A and B (LptA and LptB, respectively), which increase the manufacture of interleukin-8 in human macrophages and host epithelial tissues.

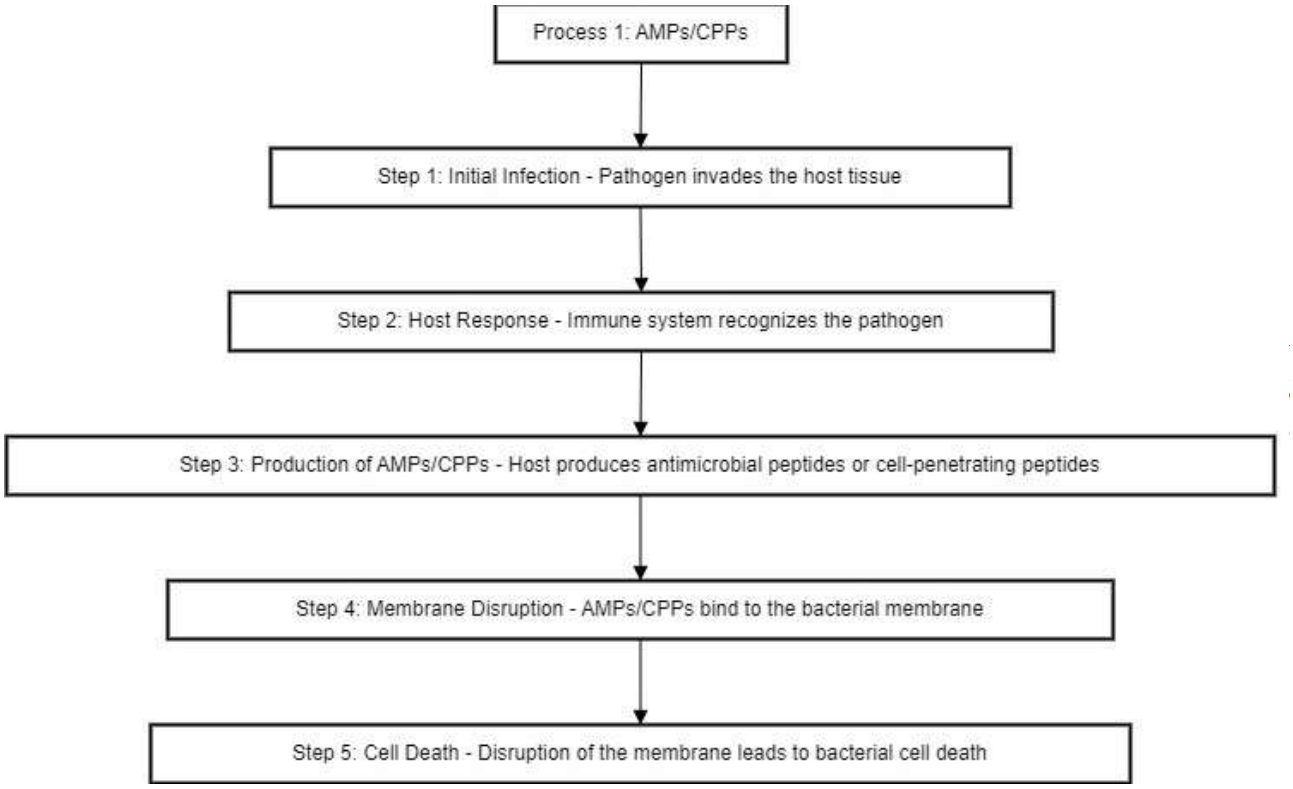

Fig. 1: Pathogenesis of AMPs/CPPs: Initial Infection: Pathogen invades the host tissue.Host Response: Immune system recognizes the pathogen. Production of AMPs/CPPs: Host produces antimicrobial peptides or cell-penetrating peptides. Membrane Disruption: AMPs/CPPs bind to the bacterial membrane. Cell Death: Disruption of the membrane leads to bacterial cell death.

Toxic Secondary Metabolites

Additionally, *P. aeruginosa* produces harmful secondary metabolites that cause cytotoxicity in host cells (Table 1). Phenazines are redox-active chemicals that are produced by this pathogen and are well-known for contributing significantly to both virulence and antibiotic resistance. Particularly noteworthy is pyocyanin, a pigment generated from phenazine that can cause neutrophils to undergo apoptosis by producing reactive oxygen species (ROS) that harm mitochondria (Neve et al., 2024). In addition, *P. aeruginosa* develops hydrogen cyanide, particularly in low-oxygen environments and at high cell densities. By attaching itself to the Fe3+ of the respiratory chain's cytochrome oxidase, this hazardous metabolite prevents the host cells from engaging in aerobic respiration (Besse et al., 2023). Furthermore, cyanide can attach to other metalloproteins due to its structural resemblance to oxygen, which can hinder various cell processes.

Iron Acquisition Systems

All living organisms require iron as a redox cofactor of enzymes for numerous vital processes. The main ways that human iron is kept in cells are either in ferritin or in hemoproteins when it combines with heme. The body uses 'nutritional immunity' during infection to restrict the amount of iron available to pathogens by sequestering it and increasing the production of iron-scavenging molecules such as hemopexin and haptoglobin (Ullah and Lang, 2023). *Pseudomonas aeruginosa* produces the HasAp hemophore and the siderophores pyoverdine and pyochelin, which chelate iron and heme, respectively (Otero-Asman et al., 2019). Its three heme acquisition systems are called Phu, Has, and Hxu. Heme is

transported into the periplasm by TonB-dependent transporters (TBDTs) such as PhuR and HasR, but HxuA, albeit having a lesser function, triggers virulence when it detects heme. Pyoverdine gives the pathogen an advantage during infection because of its strong affinity for iron, which enables it to outcompete host proteins including lactoferrin and transferrin. Pyoverdine-defective *P. aeruginosa* strains exhibit decreased virulence in animal models, which is indicative of this (Gi et al., 2015) While less effective in binding iron, pyochelin is more cost-effective to make and is the recommended option in situations with mild iron deficiency. It causes persistent tissue damage by inducing oxidative stress and inflammation in conjunction with pyocyanin.

Pyoverdine gives the pathogen an advantage during infection because of its strong affinity for iron, which enables it to outcompete host proteins including lactoferrin and transferrin (Kümmerli et al., 2023). While less effective in binding iron, pyochelin is more cost-effective to make and is the recommended option in situations with mild iron deficiency. It causes persistent tissue damage by inducing oxidative stress and inflammation in conjunction with pyocyanin (Kümmerli et al., 2023). The bacterium uses catalase (KatA) and superoxide dismutase (SodB) to reduce oxidative stress and bacterioferritins (BfrA and BfrB) to store excess iron (Lin et al., 2023).

Clinical Relevance of *P. aeruginosa*

P. aeruginosa can spread, avoid immune reactions from the host and harmful antibiotics, create toxins and exoenzymes that harm host cells, and effectively adapt to any environment (Sanya et al., 2023). *P. aeruginosa* is the most common source of infections when considering the global epidemiological aspects of NFGNB (Pourcel et al., 2020). Despite having several virulence characteristics, pseudomonads are not regarded as extremely pathogenic when compared to other bacteria, such as *Streptococcus pyogenes* or members of the Enterobacterales order (Rasoo et al., 2016). Still, they might be accountable for a variety of disease presentations, and these diseases frequently show up as persistent, difficult-to-cure infections. *P. aeruginosa* frequently develops multisite infections as well. The majority of those impacted are immunocompromised patients (Kreitmann et al., 2024). Since it is frequently found in intensive care units (ICUs) and surgical theaters where the widespread use of antimicrobials has allowed for the selection of these microorganisms *P. aeruginosa* is primarily thought to be an opportunistic, nosocomial Gram-negative pathogen, accounting for 13–19% of hospital-acquired infections in the US (Hammoudi Halat and Ayoub Moubareck, 2024; Parcel et al., 2018). Due to *P. aeruginosa's* capacity to survive on a wide variety of inanimate surfaces and to disseminate by aerosol, almost all healthcare facilities have recorded outbreaks and intrahospital infections (Tarafdar et al., 2020). *P. aeruginosa* can only temporarily colonize the digestive tract under normal conditions (however immunocompromised patients may experience an increase in this rate). However, 8–20% of nosocomial infections and outbreaks are linked to people who have colonized the area. Strict adherence to environmental cleaning plans, hand hygiene practices, and infection control measures is essential to preventing nosocomial outbreaks. It's also crucial to identify and eradicate potential infection reservoirs. Clinical presentations may include pneumonia (primarily ventilator-associated pneumonia, or VAP; 10–30%), skin and soft tissue infections related to burns and surgeries (8–10%), "hot tub" folliculitis, "swimmer's ear" otitis externa, eye infections (keratitis), urinary tract infections (UTI; 813.8615%), endocarditis, and bacteremia/sepsis (often secondary to pneumonia or often associated with central line-associated) (Newman et al., 2017; Moradali et al., 2017). *P. aeruginosa* is one of the bacterial pathogens that cause contact lens-associated keratitis. It can cause a corneal ulcer, which is the worst clinical presentation and can occur in 40–60% of cases. This can lead to poor outcomes, fulminant destruction of the cornea, and the loss of eyesight (Khan et al., 2020). The mortality rate from pseudomonad infections, which ranges from 25–39% for pneumonia and 18–61% for bacteremia in hospitalized and immunocompromised patients, is a major concern. These rates may reach 40–70% in the case of MDR isolates (Rojas et al., 2019; Zhang et al., 2020). It was also discovered that cigarette smoke stimulated *P. aeruginosa*, which resulted in the formation of a nfxC drug-resistant phenotype (Xu et al., 2020). *P. aeruginosa* is the most prevalent and well-researched pathogen associated with cystic fibrosis. A mucoid phenotype and a very slimy surface are isolated 3-6 months after *P. aeruginosa* strains with a non-mucoid phenotype first settle in the lungs of CF patients (Armbruster et al., 2021). It has been demonstrated that in CF patients, the age at which *P. aeruginosa* positive first occurred plays a significant role in determining the course of the illness. Between the ages of 0 and 5, the pathogen is present in 10–30% of individuals; after the age of 25, it is prevalent in >80% of patients, and these chronic lung infections are seldom totally cleared. One of the most significant contributing causes to CF patients' deadly pulmonary exacerbations is *P. aeruginosa* (Li and Schneider-Futschik, 2023).

Antibiotic Resistance in *P. aeruginosa*

Generally speaking, the treatment of NFGNB-caused infections is a serious therapeutic challenge for clinicians in both community and hospital settings because of the rising number of isolates that are resistant to many antibiotic classes (Zhen et al., 2020). According to the guidelines set forth by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), isolates categorized as multidrug-resistant (MDR), extensively drug-resistant (XDR), and even pan drug-resistant (PDR) or drug-resistant (TDR) are increasingly showing up in clinical settings (Corona et al., 2023). Antibiotic resistance is primarily caused by two factors: the widespread use of antibiotics in inappropriate contexts and pharmaceutical firms' declining interest in participating in antimicrobial research (Gajdács, 2019; Naeem et al., 2021).

A bacterial species' intrinsic resistance to antibiotics is its inherent ability to reduce an antibiotic's effectiveness through innate structural or functional traits (Baran et al., 2023). Pseudomonas aeruginosa has demonstrated a significant

degree of intrinsic resistance to the majority of antibiotics through mechanisms such as limited permeability of the outer membrane, efflux systems that remove drugs from the cell, and the synthesis of enzymes that inactivate antibiotics, such as β-lactamases (Verdial et al., 2023). Antibiotics cannot pass through the bacterial cell membrane of *P. aeruginosa* and reach their intracellular targets because of the bacterial cell membrane's limited permeability (Huang and Li, 2023). Additionally, the cell membrane of this bacteria has many efflux mechanisms that allow it to expel a variety of drugs. These efflux systems are classified into five primary families: multidrug and toxic compound extrusion, small multidrug resistance, major facilitator superfamily, ATP-binding cassette, and resistance–nodulation–division (Santos et al., 2024; Al-Ouqaili, 2018). In addition to giving drug resistance, these efflux pumps have also added to *P. aeruginosa's* pathogenicity. *P. aeruginosa* can release enzymes that break down antibiotics, specifically focusing on medications such as amikacin, netilmicin, gentamicin, penicillin, streptomycin, aztreonam, kanamycin, neomycin, and tobramycin (Elfadadny et al., 2024).

The bacterium also demonstrates methods of adaptive resistance mediated by polysaccharides and biofilms. *P. aeruginosa* cells have developed phenotypic modifications called biofilms and polysaccharides to shield them against antibiotics (Basit et al., 2021; Chung et al., 2023). As the bacteria that forms biofilms and is least susceptible to medications, *P. aeruginosa* is the main cause of chronic lung inflammation and death in individuals with cystic fibrosis. Additionally, this bacterium acquires antibiotic resistance genes through horizontal gene transfer (HGT) from other bacterial species in the environment, enabling it to evolve resistance to new antibiotics (Michaelis and Grohmann, 2023).

Essential Oils as Potential Tools against AMR

Essential oils (EOs), alternatively referred to as volatile oils, are intricate blends of several volatile, lipophilic chemicals in varying quantities (Yu et al., 2020). Usually, two or three primary components are found in relatively high concentrations (20–70%), with trace levels of additional chemicals present (Xu et al., 2023). Terpenes, which are primarily composed of isoprene units and frequently possess multiple chemical functions like alcohol, phenol, aldehyde, ketone, ether, and hydrocarbon groups, are the major components of essential oils (EOs) (Guimarães et al., 2019). Essential oils (EOs) are secondary metabolites that aromatic plants make to fend against microbes, predators, and harsh weather (Tokgöz, 2024). EOs can be produced by many different portions of the plant and subsequently extracted using a variety of techniques, including solvent extraction (solvent, subcritical water, supercritical CO2), distillation (hydrodistillation, steam distillation, hydro diffusion), solvent-free microwave extraction, and combination techniques (Mahizan et al., 2019). EOs have been shown to have antibacterial, antioxidant, anti-inflammatory, analgesic, antiemetic, and cancer chemoprotective properties in medicinal practice (Man et al., 2019). Additionally, several EOs can be cytotoxic (to kill bacteria, viruses, fungus, protozoa, parasites, and mites), allelopathic, insect repellant, and insecticidal, making them potential substitutes in a range of sectors (Romanescu et al., 2023).

Certain EOs work well against infections that are important to public health. Several EOs have demonstrated in vitro antibacterial action against pathogens on the WHO Priority 1 list (Badescu et al., 2022). Pharmaceutical formulation advances have made it possible to put EOs onto carriers like nanoparticles, greatly enhancing their stability and bioavailability (Nair et al., 2022). But by using spices, the general public can also gain from the antimicrobial EOs' benefits (Li et al., 2022).

Generally speaking, phenols and aldehydes have chemical functionalities that cause antibacterial action, whereas a large percentage of esters, ketones, and terpene hydrocarbons have little to no impact (El-Tarabily et al., 2021). EOs can stop the growth of bacteria by causing membrane proteins to break down and increase cell permeability since they are hydrophobic (Ortega-Ramirez et al., 2020). In many different types of microbes, they can disrupt the expression of genes that code for efflux pumps (*tetA, tetK, pmrA, norA*, *blaTEM*, *blaOXA-23*) (Evangelista et al., 2022). EOs have the potential to impact proton pumps as well, leading to ATP depletion and a decrease in membrane potential. Additionally, EOs can interfere with quorum sensing and prevent the production of biofilms. As a result, they affect gene expression regulation and cell-to-cell communication, two processes that are essential for adaptation in harsh environments (Gurkok and Sezen, 2023). The dilution method (using agar or liquid broth) and the agar diffusion method (using a paper disc or well) are the two most popular in vitro methods for evaluating the antibacterial activity of Eos (Abdollahzadeh et al., 2021).

Aromatic Herbs and their Essential Oils

Aromatic plants' secondary metabolism produces perfumed liquids known as essential oils. Since they are the most important part of the plant, they are referred to as "essential". EOs are concoctions of organic materials made from a variety of plant sources; they give plants their unique scent (Zhang and Piao, 2023). Aromatic herbs are used to extract essential oils (EOs) from a variety of organs, including seeds (caraway, cumin, and coriander), leaves (mint, thyme, sage, rosemary, oregano, basil, celery, and parsley), fruits (anise, fennel, and lemon), flowers (rose and rosemary), bark (cinnamon), cloves or buds (clove and garlic), and rhizomes (ginger) (Mohamed and Alotaibi, 2023). Aromatic plants can produce essential oils (EOs) by combining various organic constituents in the cytoplasm and plastids of plant cells. These pathways include mevalonic acid, malonic acid, and methyl-D-erythrol-4-phosphate (MEP). The resulting compounds are then stored in epidermal cells, secreting fissures, glandular trichomes, or resin canals (da Silva et al., 2021). Depending on the origins of the plants, species, and organs, essential oils have a distinct color and smell. While certain EOs, such green European valerian and blue chamomile, have vivid colors, most are pale yellow or colorless (Ailli et al., 2023). The volatile molecules found in Eos have a crucial role in the ecology because they can shield plants from invading fungi, bacteria, viruses, and insects while also drawing specific insects needed for pollination (Weisany et al., 2024).

Extraction of essential oils

Plant EOs are analyzed using two basic processes: chemical analysis, which takes several minutes, and oil extraction/distillation, which takes several hours (Ashaq et al., 2024). The traditional method used to extract essential oils (EOs) in laboratories is the Clevenger system hydro-distillation, due to the volatile nature of EOs. Although the traditional method used to purify EOs in commercial processes is steam distillation (Karimkhani etal.,20241). Solvent-based EO extraction is widely used in industrial processes, however it is prohibited in the food industry due to the extremely hazardous nature of the organic solvents used (Ghenabzia et al., 2023). To increase the effectiveness, sustainability, and economy of the applicable system, various other techniques have been investigated for the extraction of essential oils (EOs). These techniques include ohmic hydro-distillation and microwave and ultrasonic assisted extraction (Cavallaro et al., 2023). The most important component in ensuring the quality of essential oils is the extraction technique employed, since poor techniques can modify the chemical makeup of aromatic oils, changing both their quality and functionality (Katekar et al., 2023). Additionally, if EOs are extracted using the steam distillation method, the resultant chemicals will always be volatile; but, if solvents are used, the chemical makeup of the extracted EOs will change from that of an equivalent essential oil that is extracted using distillation (Ayub et al., 2023). Selecting the appropriate extraction method based on the properties of each plant material is crucial since the extraction method employed affects the chemical composition of any oil. To preserve consistency in chemical composition, quality, and quantity, the annual extraction of essential oils (EOs) should be carried out under identical conditions each year. These conditions should include employing similar plant parts, a similar extraction technique, and a similar harvesting season. Flowers must be chosen fresh, although plant parts that are collected for extraction can be partially dried, dehydrated, or picked fresh (Saleh et al., 2023).

Biological Activities of Essential oils

Many aromatic plants have been used for centuries in the food business as flavorings and preservatives, as well as as key sources of scent and flavor. Various aromatic plants have medicinal effects, and these effects are mostly caused by EOs (Bolouri et al., 2022). The diverse biological effects of essential oils (EOs) are ascribed not only to their primary constituents, which comprise two or three compounds present in high concentrations, but also to the potent synergistic effects of additional active chemicals. Essential oils are generally used in pharmaceutics for aromatherapy and to enhance the olfactory qualities of pharmaceutical drugs. EOs are used by various traditional systems around the world to treat a wide range of medical issues (Butnariu, 2021). For instance, the essential oils of eucalyptus and clove can treat coughing and bronchitis, respectively, while the essential oils of sage and peppermint can stop the growth of certain bacteria, relieve respiratory congestion, and are well-known carminatives (Kumar, 2016).

Essential oils as Antioxidants

Plants with aromatic properties have phenolic chemicals in their structure, which contribute to their antioxidant action. Among these, flavonoids, phenolic acids, and phenolic terpenes are the most prevalent (Pinto et al., 2021). The removal of free radicals, the formation of compounds with metal ions (metal chelation), and the prevention or reduction of singlet oxygen formation are some of the mechanisms behind the antioxidant action of phenolic compounds. In order to stop free radicals from oxidizing lipids and other biological components, the compounds can supply the hydrogen found in hydroxyl groups in their aromatic rings. Plants primarily include flavonoids and other phenolic chemicals in their leaves, flowers, and woody sections. This is the reason aromatic herbs are frequently employed as remedies, either as essential oil extracts made through processes like distillation and extraction, or as dried leaf and flower parts. The antioxidant properties of aromatic plants differ due to a multitude of factors influencing their chemical makeup (Konfo et al., 2023).

Essential oils as Antibacterial Agents

One significant issue in antimicrobial chemotherapy is the increasing prevalence of antibiotic resistance, which results in inadequate antimicrobial treatment. Antibiotic usage and the ensuing pressure of antibiotic selection are thought to be the primary factors leading to the emergence of various forms of resistant bacteria (Rathore et al., 2023). Plant bioactives, such as EOs, demonstrated potent antibacterial properties against a range of Gram-positive and Gram-negative bacteria (Amin et al., 2023). Several EOs and their major compounds gained widespread recognition due to their strong antibacterial properties. These compounds can be employed as a variety of helpful additives to lengthen food products' shelf lives and ensure the microbiological safety of consumers (Rasheed et al., 2024). Strong antibacterial properties of many EOs have been demonstrated; however, these properties are frequently influenced by the concentration and presence of certain EO constituents, such as terpenoids, alcohols, phenols, ketones, and esters, as well as phenylpropanoids (Zhang and Piao, 2023). The ability of the hydrophobic elements in EOs to interact with the lipids in the cell membranes of microorganisms caused damage to the permeability and solidity of the membrane, which in turn caused high fluctuations in the chain of electron transport, in nutrient uptake, and in the synthesis of nucleic acids and proteins. This, in turn, caused clotting of the cellular contents and inhibited different metabolite enzymes, which ultimately lead to cell death (Bhavaniramya et al., 2019). Following the rupture of bacterial membranes, the bioactive components of EOs can also kill bacteria by penetrating the cell and blocking polysaccharides, RNA, DNA, or proteins (de Sousa et al., 2023). Strong antibacterial properties were demonstrated by several essential oils, such as rosewood, clove, cinnamon, oregano, and lemongrass (Mohamed and Alotaibi, 2023).

Essential oils as Antifungal Agents

Various essential oils and aromatic herbs demonstrated strong antifungal properties against a variety of pathogenic fungi, including yeasts. The target infection and the oil used determine how effective EOs are against it (Corrêa et al., 2023). Effective antifungal activities were demonstrated by the volatile oils of fennel, coriander, and anise against *Candida albicans* at varying concentrations of 1%, 0.5%, and 0.25%, respectively (Hleba et al., 2024). Moreover, lavandula multifida volatile oil demonstrated strong antifungal activity against *Candida albicans* (Alves-Silva et al., 2023). In contrast to *C. albicans* drug-resistant biofilms, several EOs and their constituents exhibited the strongest antifungal effects by inhibiting membrane ergosterol and changing the signaling pathways that stop the yeast from hyphal growth (Augostine, 2023).

Different Important Medicinal Plants Produce Essential oils

For thousands of years, Asian and African civilizations have used medicinal herbs in their traditional medicines. Over the past few decades, there has been a notable rise in public interest in and acceptance of natural medicines in developed as well as developing countries. Approximately 4 billion people worldwide, or 85% of the total population, use herbal medications in place of conventional ones. Additionally, about 25% of all contemporary medications are derived directly or indirectly from medicinal plants (Bhoi et al., 2023).

Research on synthetic pharmaceutical substances indicates that the therapeutic properties of essential oils (EOs) extracted from aromatic and medicinal plants have a wide range of applications. Farmers and researchers have therefore been motivated to develop these chemicals (Swamy and Sinniah, 2016). Medicinal plants that produce EOs are usually found in warm regions.

Of the 3000 recognized essential oils (EOs) produced by different genera of plants, only about 300 exhibit significant commercial potential. Many plants belonging to different families, including the Alliaceae, Lamiaceae, Myrtaceae, Apiaceae, Asteraceae, Rutaceae, and Poaceae families, are capable of synthesizing significant percentages of essential oils and producing them in commercial quantities. These commercial concentrations of essential oils are used in a variety of industries, primarily in the food, pharmaceutical, cosmetic, aromatherapy, agronomy, and polishing sectors (Yingngam, 2023). The most commonly used essential oils with therapeutic value include thyme, oregano, tea tree, eucalyptus, rosemary, lavender, orange, basil, maize mint, lemon, camphor, citronella, eucalyptus, clove, and eucalyptus.

Mechanisms of Antimicrobial Action

Essential oils' antimicrobial properties have been extensively documented. It's still unknown, though, how these essential oils work. Historically, biochemical tests have been the primary means of determining the mode of action of essential oils (Russo and Palla, 2023). These tests are typically not very good at pinpointing the precise causes or pathways that lead to antimicrobial effects because of technical constraints. However, it is thought that an essential oil affects the structure of the bacterial membrane and its transport mechanism.

Membrane Disruption

The modulation of cellular osmotic pressure and the influx and efflux of biomolecules are significant functions of the bacterial membrane (Foster et al., 2024). Therefore, osmotic pressure disruption caused by a weakened membrane will result in intracellular leaking and ultimately cell destruction. The capacity of essential oil to damage bacterial membranes is one of the primary hypothesized mechanisms of action (Angane et al., 2022). The antibacterial activity of EOs is often related to their hydrophobic character, which can have toxic effects on membrane integrity and function (Yap et al., 2021). Research has shown that the EOs' mechanism of action is not independent, but rather linked to a sequence of processes involving the cytoplasm and the cell's outer envelope. Peptidoglycans are present in the cell walls of both Gram-positive and Gram-negative bacteria, and they are crucial for structural integrity and preserving the shape of the cell (Tavares et al., 2020). The phospholipid bilayer that makes up the bacterial plasma membrane serves as a barrier to molecules entering and leaving the cell, which is a general function of the membrane. The thin peptidoglycan and lipid-rich outer membrane of Gram-negative bacteria distinguish them from their Gram-positive counterparts (Garde et al., 2021). Gram-negative bacteria's outer membrane is mostly composed of lipopolysaccharide (LPS), which when combined with porins to form an assembly creates a selective barrier that shields the bacteria from various antibiotics, detergents, and dyes that would otherwise harm the inner membrane (Pandeya et al., 2021). Numerous investigations have revealed that the outer membrane of Gram-negative bacteria functions as an efficient and dynamic barrier to antibiotics (May and Grabowicz, 2018). Consequently, colistin, an antibiotic that targets the outer membrane, is inevitably the last line of treatment for infections brought on by Gram-negative pathogens that are highly resistant to multiple drugs. Nevertheless, EOs offer a viable substitute since they have been demonstrated to have strong effects on both Gram-positive and Gram-negative bacteria in addition to being specific to a certain type of bacteria.

Quantitative measures of potassium leakage, protein leakage, genetic material (DNA and RNA) leakage, and membrane potential are among the quantitative indications of increasing membrane permeability that ultimately result in the loss of cell viability (Khater et al., 2020). High phenolic levels of essential oils (EOs)—such as those containing thymol, eugenol, and carvacrol—were discovered to be the primary causes of the cytoplasmic membrane's breakdown, which allowed protons and other ions to passively flow through (Perumal et al., 2022). Bacterial metabolism heavily depends on the differential between the external and intracellular potentials of the organisms, which is referred to as membrane

potential. The cell membrane's structural damage could be the cause of the drop in membrane potential. The ratio of fluorescent dye intensities inside to outside the cell can be used to measure the potential of the bacterial membrane (Benfield and Henriques, 2020). Following exposure to EOs, depolarized bacterial cells are frequently stained using the membrane potential-sensitive dyes 3,3′-dipropylthiacarbocyanide iodide (DiSC35) and bis-oxonol (Yap et al., 2021). However, it should be noted that modified membrane depolarization may not always result in cell death; rather, it may depend on the extent of modification or whether the cell's functionality was impacted.

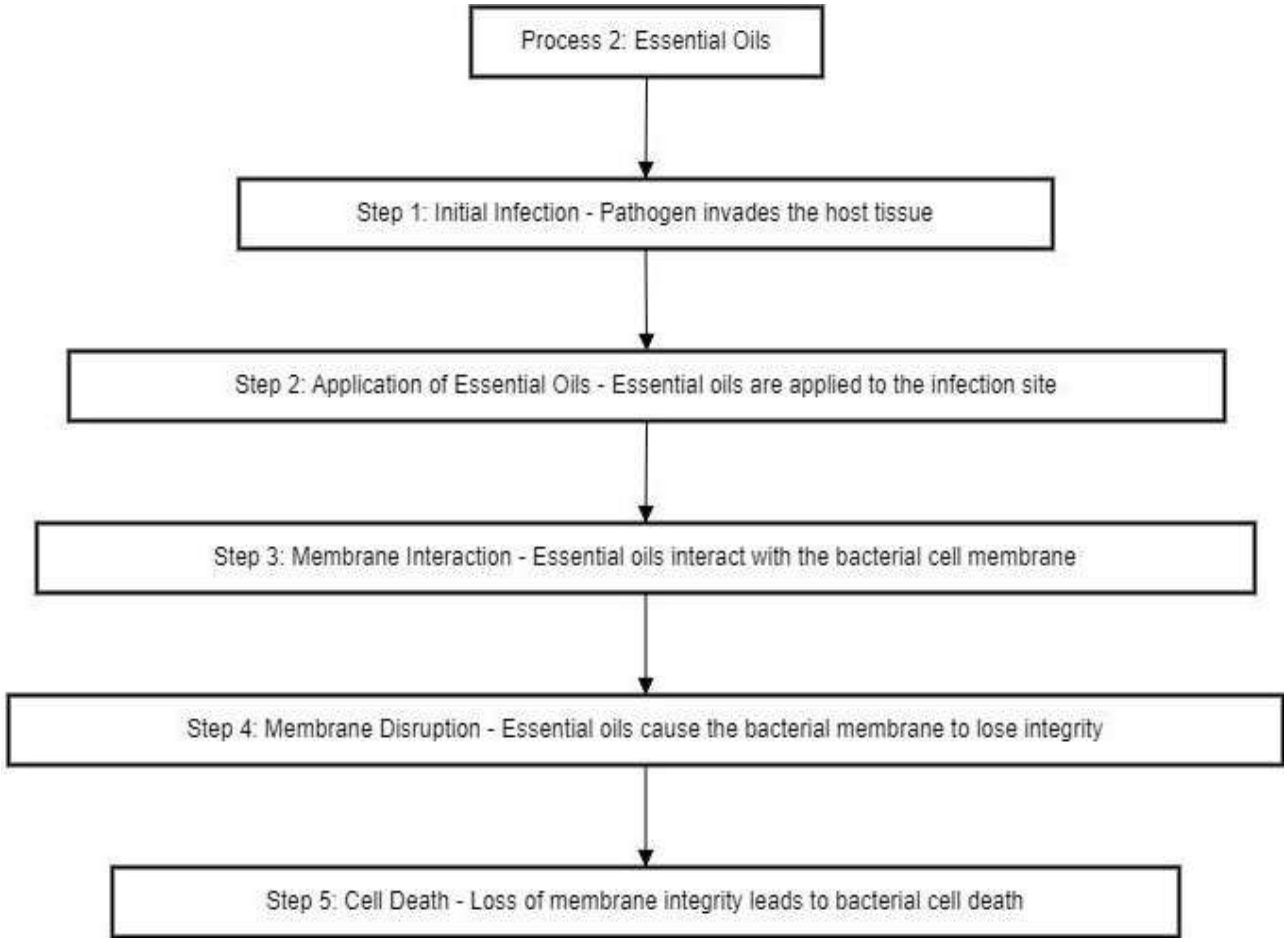

Fig. 2: Pathogenesis of Essential Oils: Initial Infection: Pathogen invades the host tissue. Application of Essential Oils: Essential oils are applied to the infection site. Membrane Interaction: Essential oils interact with the bacterial cell membrane. Membrane Disruption: Essential oils cause the bacterial membrane to lose integrity. Cell Death: Loss of membrane integrity leads to bacterial cell death.

Efflux Inhibition

Impeding the bacterial efflux system is a different perspective on the mechanism of action of essential oil. Specialized channel proteins on the bacterial membrane comprise the bacterial efflux system, necessary for eliminating toxic substances like antibiotics from the intracellular environment (Agreles et al., 2021). Various efflux pumps enable bacteria to thrive in the presence of antibiotics, from compound-specific pumps to general pumps. To combat antibiotic resistance and restore the effectiveness of medicines, it is crucial to inhibit the functioning of these pumps.

Essential oils against *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a well-known bacterium that is resistant to multiple drugs. Essential oils have drawn interest for their possible antibacterial qualities. Research on the efficacy of several essential oils against *Pseudomonas aeruginosa* has been done in vitro.

Tea Tree Oil (Melaleuca alternifolia)

Melaleuca alternifolia leaves are used to make tea tree oil, which is well known for having antibacterial qualities. Its primary active ingredient, terpinen-4-ol, works well against a variety of infections, including *Pseudomonas aeruginosa*, when combined with other terpenes and phenolic chemicals (Haines et al., 2022). Through an increase in fluidity and permeability, terpinen-4-ol damages bacterial cell membranes, causing internal leakage and ultimately cell death. Tea tree oil further suppresses bacterial development by interfering with bacterial enzyme systems, including ATP generation.

According to studies, tea tree oil is useful against persistent infections because it prevents *Pseudomonas aeruginosa* from forming biofilms and can disturb existing biofilms. According to some research, combining tea tree oil with antibiotics like ciprofloxacin or gentamicin may have synergistic effects that boost antibacterial activity. Tea tree oil resistance is possible, although it seems less common than with traditional antibiotics—possibly because of the oil's intricate makeup (Nascimento et al., 2023).

Thyme (Thymus vulgaris L.)

Essential oil (TEO) derived from thyme (Thymus vulgaris L.) is frequently utilized as a substitute treatment for a number of infections, including upper respiratory tract infections (Kowalczyk et al., 2020). TEO has antiviral, antibiofilm, antibacterial, and antifungal qualities as a result of its biological action (Tariq et al., 2019). The chemical makeup of TEO may influence its biological action. Thymol, the main ingredient in EO, has been shown to possess antiviral, antibacterial, antifungal, and antihyperglycemic properties. The antibacterial impact of TEO may be enhanced or altered by its constituents, which include carvacrol, p-cymene, γ-terpinene, β-myrcene, linalool, and terpinen-4-ol (Heghes et al., 2020). The plant's phenophase and the time of oil preparation can change the chemical composition of essential oils (EOs) and hence affect their biological activity (Pandur et al., 2021). Strong antibacterial effects are exhibited by bioactive components including carvacrol and thymol found in thyme oil, which is derived from Thymus vulgaris. Carvacrol and thymol damage bacterial membranes, causing cellular contents to seep out and ultimately bacterial death. *Pseudomonas aeruginosa* has been effectively inhibited in its proliferation and biofilm formation by thyme oil. Combined with drugs such as ceftazidime and ciprofloxacin, it has also shown synergistic effects. To completely comprehend its methods of action against *Pseudomonas aeruginosa* and its possible therapeutic applications, more research is necessary (Ribeiro et al., 2022).

Sage Oil (Salvia officinalis)

Salvia officinalis is a plant that belongs to the mint family. It is angiosperm, a dicotyledonous flower with united petals, in the order Toby Floral, suborder Mangroves, family Mint, and gender Salvia (Pejčić et al., 2020). Evergreen perennial salvia has woody branches, green leaves, and violet-blue blooms. The leaves are gray-green in hue, with creases on their top surface and nearly white, soft fluff on their lower surface. The genus Salvia has some species that have important medicinal properties, such as salvia officinalis. It worked well for relaxation, decreasing blood sugar, and other things. Thujone, 1, 8 cineole, Borneol, Borneol acetate, sesquiterpene, tannins, and phenolic acids are among the compounds found in this plant (Shaaban, 2020). The chemical makeup of sage oil is complex and includes phenolic chemicals, monoterpenes, and sesquiterpenes, which are responsible for its antibacterial properties. It has been demonstrated that these substances have antibacterial properties against a variety of infections, including *Pseudomonas aeruginosa* (Swamy et al., 2016).

Eucalyptus Oil (Eucalyptus globulus)

Along with other terpenes and phenolic compounds, 1,8-cineole, or eucalyptol, is the main ingredient of eucalyptus oil, which is derived from Eucalyptus globulus. By rupturing bacterial membranes, blocking bacterial enzymes, and interfering with cellular functions, 1,8-cineole has antibacterial action (Chandorkar et al., 2021). *Pseudomonas aeruginosa* has been shown to be effectively inhibited by eucalyptus oil, which also decreases the virulence factors like elastase and pyocyanin produced by the bacteria. Because of its broad-spectrum antibacterial activity, it can be used in a variety of clinical settings, such as environmental disinfectants, mouth rinses for dental hygiene, and topical preparations for respiratory infections (Sagar et al., 2022).

Invitro Techniques to Access the Antibacterial Activity

Agar diffusion (using a paper disc or well) and dilution (using agar or liquid broth) are the two most popular in vitro methods for evaluating the antibacterial activity of Eos (Abdollahzadeh et al., 2021). Regarding cost and methodology, agar diffusion methods rank among the most practical approaches. Using the spreading plate approach, a pathogenic bacterium is introduced onto an agar plate in the agar well diffusion method (a precise volume of the microbial solution is dispersed over the surface of agar, through a glass diffuser). The tested solution (such as extract) is poured into a well or hole formed aseptically using a sterile cork borer with a diameter of 6–8 mm, and the area is then incubated at the ideal temperature and humidity. The tested solution will gradually permeate the agar media, stopping the growth of the bacterium. The inhibitory zone's diameter will be assessed afterward. A filter paper disc holding the test solution is placed on the agar medium and inoculated with the tested microorganisms using the agar disc diffusion method (Abdollahzadeh et al., 2021). In general, agar diffusion techniques facilitate the facile testing of several extracts/substances against diverse microorganisms; nonetheless, they cannot elucidate the minimum inhibitory concentration (MIC) or the capacity of a substance/extract to impede or eliminate a merorganization. Using the dilution method, one can precisely count the live cells in a culture of bacteria, fungi, or viruses by creating successive dilutions of concentrated solutions of microbial strains. Every diluted sample is mixed with agar medium that has been liquefied, and then put into a petri dish where it solidifies and contains the bacteria in its matrix. As the germs spread around the agar plate, they can be precisely counted. This technique is used to find a substance's minimum inhibitory concentration (MIC) and its capacity to either kill or stop the growth of the strains under investigation. Additionally, it serves as the benchmark for testing for antibiotic susceptibility. The minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of the chosen AB are

commonly used to characterize bacterial resistance. Nonetheless, there is a great deal of variation in the reported values for MIC and MBC, which may be due to the large variety of bacterial strains, methodological differences, and research design changes (Ayobami et al., 2020). Interestingly, a number of factors that affect the extract's variability (such as the plant component employed and the extraction technique) also affect the antibacterial activity of essential oils.

Synergistic Interactions between the Essential Oil and Antibiotic

The combination of antibiotics with plant extracts affects bacteria that are resistant to them, providing new therapeutic options for diseases caused by pathogens. Synergy therapies have the potential to reduce adverse effects and the active daily dosage of antibacterial agents required for therapy. Combining essential oils with antimicrobials to counteract multidrug-resistant bacteria is one of the newest approaches in this fight. Because new antibiotics aren't coming onto the market and more pathogens are becoming resistant to them, Three distinct outcomes synergistic, additive, or antagonistic can arise from the interaction of antimicrobials in combination. When two antimicrobial chemicals are combined, they produce antibacterial activity that is larger than the sum of their separate antibacterial activity. This is known as synergy. Combining antimicrobials results in an antimicrobial impact that is equal to the sum of the effects of each compound alone, which is known as an additive effect. Two chemicals that operate antagonistically on one another reduce each other's antibacterial activity as compared to when they act separately (Sharma et al., 2020).

A Gram-negative bacterium known for both its quick acquisition of acquired resistance mechanisms and innate resistance to numerous drugs is *Pseudomonas aeruginosa*. Because there are few therapeutic options for *Pseudomonas aeruginosa* infections in clinical settings, these infections present substantial problems. Antibiotics that are frequently used to treat *Pseudomonas aeruginosa* include; Broad-spectrum antibiotics such as carbapenem (Imipenem, meropenem, and doripenem) are frequently used as first-line treatments for severe *Pseudomonas* infections, particularly those that are resistant to other antibiotic classes (Langendonk et al., 2021). They work well against strains of bacteria that are resistant to many drugs and have strong tissue penetration. *P. aeruginosa* is susceptible to the antibacterial properties of cephalosporins, among which ceftazidime and cefepime are fourth- and third-generation antibiotics, respectively. Cephalosporin resistance, particularly to ceftazidime, has increased, nevertheless, as a result of the advent of AmpC and extended-spectrum beta-lactamases (ESBLs). *P. aeruginosa* infections have been treated with fluoroquinolone antibiotics such as levofloxacin and ciprofloxacin. However, chromosomal mutations and efflux pump mechanisms have led to the widespread development of fluoroquinolone resistance. Three aminoglycoside antibiotics that are frequently used in combination therapy to treat *P. aeruginosa* infections include gentamicin, tobramycin, and amikacin. They can aid in overcoming resistance mechanisms and frequently work in concert with beta-lactam antibiotics

It has been suggested that combining essential oils with medicines can improve their antibacterial effectiveness against *Pseudomonas aeruginosa*. Antibiotics and essential oils can work synergistically to promote bacterial susceptibility and overcome resistance mechanisms (Ju et al., 2022). Increased intracellular accumulation of antibiotics, potentiation of antibiotic activity, suppression of resistance mechanisms, and improved permeability of bacterial cell membranes are some of the processes causing these synergistic effects. Carvacrol and ciprofloxacin, thymol and gentamicin, tea tree oil, and imipenem are a few examples of synergistic pairings. These mixtures have shown potential for therapeutic applications by exhibiting increased antibacterial activity against *P. aeruginosa* in vitro. A few examples of synergistic pairings are ciprofloxacin and carvacrol, gentamicin and thymol, and imipenem with tea tree oil. In vitro tests of these combinations have revealed increased antibacterial activity against *P. aeruginosa*, indicating potential for use in clinical settings (Herrera-Espejo et al., 2020).

Practical Applications

The antibacterial properties of essential oils can aid in preventing wound infections. Certain oils are well known for their capacity to heal wounds, including chamomile oil (*Matricaria chamomilla*), tea tree oil (*Melaleuca alternifolia*) and lavender oil (*Lavandula angustifolia*). These oils can be diluted and applied topically to clean wounds to help promote speedier healing and prevent the formation of dangerous bacteria (Gadisa and Usman, 2021).

Essential oils are powerful disinfectants because of their inherent antibacterial qualities. The antimicrobial properties of several oils, including eucalyptus (*Eucalyptus globulus*), lemon (*Citrus limon*), and thyme (*Thymus vulgaris*), have been investigated. They offer a healthy substitute for chemical-based disinfectants on surfaces in households, healthcare facilities, and locations where food is prepared. Research has demonstrated that specific essential oils possess preservation properties, which can aid in prolonging the shelf life of food items. Certain oils, such as those derived from rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*), and cinnamon (*Cinnamomum verum*), has antibacterial qualities that can impede the development of foodborne pathogens and spoilage organisms. By incorporating trace amounts of these oils into food products, chemical preservatives may be avoided by helping to guard against microbial infection and spoiling (Gadisa and Usman, 2021).

Safety and Toxicity Considerations

Since essential oils are quite concentrated, it's best to dilute them before using them to lower the possibility of negative reactions. The oil and the planned use determine the safe dilution ratio. For topical usage, general guidelines advise dilution of essential oils to a concentration of 1–3%in a carrier oil (Hoang et al., 2021). For certain populations, such

as toddlers, pregnant women, or people with sensitive skin, dilution ratios might need to be modified. Individuals may experience negative effects from essential oils, such as sensitization, respiratory problems, allergic reactions, or skin irritation. Before applying any new essential oil, especially on sections of the skin that are sensitive, it is imperative to conduct a patch test to rule out any potential responses (Garg et al., 2021). Certain essential oils have the potential to be harmful if misused or used in significant amounts. Essential oils should not be used internally unless directed by a licensed healthcare professional. Some oils, including eucalyptus and wintergreen (Gaultheria procumbens), have significant concentrations of particular chemical components that are poisonous if consumed. Additionally, as some essential oils can be toxic or hazardous to animals, caution should be used while using them near pets (Bunse et al., 2022).

Conclusion

The study of aromatic plant essential oils' antimicrobial activity against *Pseudomonas aeruginosa* reveals encouraging findings that highlight their potential as strong treatments for bacterial illnesses. A thorough analysis of the literature reveals that these essential oils have significant inhibitory effects against *P. aeruginosa*, a bacterium that is notoriously difficult to treat and resistant to a variety of drugs. critical oils possess a wide range of bioactive components, such as terpenes, phenols, and aldehydes, which play a role in their antibacterial capabilities. These molecules damage bacterial cell membranes, decrease enzyme function, and interfere with critical microbial processes.

The practical applications of essential oils' antimicrobial properties span a wide range of domains, such as food preservation, hygiene, and healthcare. Applying essential oils topically, for example, maybe a safe, effective way to promote wound healing and avoid infections in wound care. Similar to this, essential oils offer effective microbial control without the hazards of toxicity or environmental damage, making them a safer and more ecologically friendly option to synthetic chemical agents in the disinfection space. Furthermore, food safety and quality can be improved in the food business by utilizing the antibacterial and antioxidant qualities of essential oils to prolong the shelf life of perishable food items and prevent the formation of foodborne infections.

Even though essential oils have a lot to offer in terms of antimicrobial applications, it is crucial to use caution and take safety precautions into account when using them. The danger of negative responses must be reduced by following the right dilution and application procedures, especially in people who are allergic to or sensitive to specific plant chemicals. Standardized testing procedures must also be created in order to guarantee correct evaluation of the antimicrobial efficiency of essential oils, making study comparisons easier and boosting the validity of research findings.

Future study should focus on a few key areas to better understand the potential of essential oils as antibacterial agents. While investigation of lesser-known botanical sources may provide novel bioactive chemicals with strong antimicrobial properties, standardization of testing techniques will allow more reliable assessments of antimicrobial activity. Furthermore, studying the synergistic relationships between traditional antibacterial drugs and essential oils may result in the creation of improved formulations with higher efficacy and lower resistance risk.

The study concludes with the noteworthy potential of aromatic herb essential oils as useful tools in the battle against microbiological illnesses, particularly *P. aeruginosa* infections. Essential oils present promising ways to combat antimicrobial resistance, enhance public health outcomes, and promote sustainable practices in food safety, cleanliness, and healthcare by utilizing their natural sources and strong antibacterial qualities. To fully realize the medicinal potential of essential oils and ensure their safe and efficient use in a variety of applications, however, more study must be done as well as careful consideration of safety issues.

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Chapter 56

Vaccination and Immunization: Myths, Facts and Controversies

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ABSTRACT

Infectious diseases pose a serious threat to human life and health. Vaccination is one of the most potent preventative techniques in human history, even when wider treatment or sophisticated options available to control. Prevention is still thought to be the best strategy to combat illnesses. The ethical standards, effectiveness and/or safety of vaccines are mainly having a controversial debate often. Vaccines are not always 100% safe or effective, but the advantages far outweigh the drawbacks. Paradoxically, vaccine safety debates are intensifying even as the number of infectious diseases those can be prevented by vaccination is declining. There are many issues regarding the safety of vaccines, the most prevalent ones are that the sheer quantity of vaccines contain adjuvants like aluminum, preservation agents like mercury, deactivating agents like formaldehyde, production remnants like human and animal DNA fragments and manufacturing residuals like formaldehyde might be too much and weaken or disturb the immune system. Because of this, some people worry that vaccinations are causing a variety of conditions, including autism, diabetes, attention-deficit disorders, hyperactivity and developmental delays. Generally speaking, vaccines are victims of its own success. Controversies have the potential to harm vaccination acceptance, reduce coverage and uptake ultimately endanger the health of both human and animals.

KEYWORDS
Myths, Including autism, Diabetes, Attention-deficit disorders

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INTRODUCTION

Vaccines are among the most effective medical innovations of the modern era. Most ailments that can be prevented by vaccination are unknown to parents in the present era. As a result, more and more people are beginning to doubt the need for vaccinations, particularly in light of the fact that no vaccine is 100% risk-free or without side effects. The advantages and disadvantages of the advised vaccinations should be known to family doctors. As one of the most beneficial and economical public health initiatives, vaccines are widely acknowledged for their substantial impact on lowering the overall mortality and morbidity rates of numerous infectious diseases. Immunizations are unique and special kind of medicine in and of themselves. They are special because of the way they apply a biochemical modification to strengthen the immune system, one of the body's more peculiar physiological systems, in order to fight against illness (Lantos et al., 2010). Every year, millions of people, mostly children, are spared from diseases that may have been prevented by vaccination. Vaccines, like any drug, can have side effects, though. The majority of the time, these side effects are minor and restricted to localized reactions at the injection site as well as the emergence of a low temperature that passes quickly on its own. Rarely, they are severe enough to cause hospitalization for life-threatening conditions, which extreme circumstances can result in death (MacIntyre and Leask, 2003).

Vaccine-related debates involve issues of safety, efficacy, ethics and morality. Vaccines have been shown to have certain hazards in addition to advantages, but comprehensive safety regulations and constant observation guarantee vaccination safety for the greatest number of individuals. Ironically, vaccine safety debates are intensifying even as the benefits of mass immunization are being demonstrated by the steadily declining prevalence of vaccine-preventable diseases. Uncertainty and misinformation can undermine parents' trust in terms of validity and jeopardize their children's health, when facts are lacking or when unofficial sources of information are regarded as equally trustworthy as official ones. Still, the creation of new,

improved vaccinations is not the main problem. The public's growing mistrust of vaccinations and immunizations is more urgent. Many places have serious misleading information about vaccination programs that puts them at risk, including assertions of poor efficacy, chronic sequelae and adverse effects (Spencer et al., 2017).

Vaccines are under attack, despite the fact that they have saved millions of lives and are still going to do so. The primary focus of criticism is vaccine safety. The role of social media cannot be neglected that spreads misleading information and even conspiracy ideas quickly lead to vaccine skepticism. Concerns regarding vaccine safety are common and among the most common ones are those related to adjuvants, additives and neutralizing agents and novel technologies like genetic vaccines. There has been a widespread increase in skepticism about side effects, which could result in a growing mistrust of scientific findings and, consequently, the scientific approach (Loffler, 2021).

This addresses vaccine-related issues and provides an overview of the data that dispels vaccination myths and conspiracy theories. The incorrectly inferred link between vaccines, autism and genetic vaccines should be addressed. Scientific research indicates that vaccine safety is of interest. Moreover, there is no evidence in the literature to back up the claims that vaccines pose a major risk to human life in general. In general, vaccinations rank among the safest and most economical pharmaceuticals and none of the subjects addressed presented a significant risk to health (Morrow and Whithear, 2011).

The recent, misleading connection made between the MMR vaccine and autism is the most well-known example. Such questionable evidence undermines confidence in vaccinations in general and reduces vaccination coverage and acceptance. The onset of autistic symptoms frequently coincides with a child's routine immunization schedule, particularly for the measles, mumps and rubella vaccine (Prymula, 2013).

One of the top 10 dangers to world health, according to the World Health Organization, is vaccine reluctance. Although there are many different factors at play when people decide not to be vaccinated, one of the main ones has been found to be a lack of trust in the safety of vaccines due to worries about side effects. Health care providers, particularly those in primary healthcare, continue to have a significant impact on vaccination decisions. Therefore, it's critical that they be backed with simple access to reliable, fact-based vaccination information (Barclay, 2017).

Adjuvants and Preservatives in Vaccine

Adjuvants have been implicated in the inflammatory reaction after vaccines, which is linked to the formation of fibrosarcoma's at injection site. Preservatives and adjuvant-free vaccines are still commonly used despite efforts to reduce adverse consequences (such as vaccine hypersensitivity reactions and the aggravation of pre-existing autoimmune diseases by mercury-based preservatives). Through direct activation of primitive response (causing local inflammatory reactions and promoting the nonspecific multiplication of lymphocytes), adjuvants are hypothesized to improve the immunological response for tiny peptide and glycoprotein antigens that trigger a poor immune response alone (Geoghegan et al., 2020).

Potential Allergens in Vaccine

Inactivated poliovirus vaccinations, varicella and the measles, mumps and rubella (MMR) vaccine all include trace levels of antibiotics, including neomycin. Consequently, those who have previously experienced an allergic reaction to these kinds of antibiotics should not receive these vaccinations. Certain live viral vaccines, such the MMR and varicella, contain gelatin; those who have previously experienced a gelatin allergy should not receive these vaccines. Despite the fact that the MMR vaccination originated from chick embryonic fibroblast tissue culture, egg allergy does not exclude receiving it. Individuals who experience allergic reactions to egg proteins ought to take the trivalent inactivated influenza vaccine instead of the inactivated vaccine (Prymula, 2013).

Thimerosal in Vaccine

Thimerosal is an FDA-approved organic mercury (ethylmercury, EtHg) preservative that is used in vaccines to guard against bacterial and fungal contamination. The CDC reports that there are currently four quadrivalent influenza vaccines (Afluria, Flucelvax, Flulaval and Fluzone) and one tetanus and diphtheria vaccine (TDVAX) that contain thimerosal. Because thimerosal-containing vaccines are less expensive, more accessible and logistically appropriate for these areas in addition to being safe and efficacious, the World Health Organization is in favor of their ongoing use in developing nations (Bigham and Copes, 2005).

Human and Animal DNA Fragments in Vaccines

Varicella, hepatitis A, rubella and rabies vaccines are among the vaccinations that contain some leftover human DNA since some vaccines are produced using human embryo cell lines. It has been suggested that there may be a safety risk if vaccinated individuals are exposed to such DNA. Recipients of these vaccinations are not at risk from this exposure. There have been several other theories regarding the origins of autism, including MMR in general, aluminum, thimerosal (mercury) and vaccinations against hepatitis B. Studies, however, revealed that these drugs did not induce autism (Offit, 2007).

Manufacturers of vaccines have worked hard to lower the amount of these stabilizers and additives in their products because they know that mercury can be hazardous. According to Prymula (2013), however, they cannot be completely avoided for safety concerns. The delivery of vaccines containing yeast and gelatin may also result in anaphylaxis. Many

vaccinations, such as those for typhoid, influenza, MMR, MMRV, Japanese encephalitis and rabies employ gelatin as a stabilizing agent. Caution is required while the administration the vaccine to children who have previously experienced anaphylaxis in response to this component (Larson et al., 2014).

In prosperous countries, the danger of contracting infectious diseases is steadily declining and a significant number of infectious disorders are currently under control. The Czech Republic's immunization program suggests that burden of this infectious disease has virtually vanished, as is evident. The number of instances and fatalities from universal immunization programs significantly outweighed any side effect dangers when they were first put into place, but today's society only notices the (few) negative side effects. This is a regrettable trend that is increasing and governments in industrialized nations occasionally face pressure to discontinue universal programs. A few decades later, they have to deal with the preventable illnesses resurfacing (Geoghegan et al., 2020).

This anti-immunization sentiment is not new. Since the end of the eighteenth century, when vaccinations first became popular, opponents have put forward a number of justifications for why they are ineffective. Some of the arguments made against vaccines are that they may be harmful since they contain a variety of hazardous compounds, that people should practice good personal hygiene instead, that individuals should employ natural immune modulators and that vaccinations impede the immune system's normal development. Lastly, there have been claims that vaccination regimens overwhelm the immune system and that vaccination laws go against religious or individual liberties (Fleming et al., 2001).

Adverse Reactions to Vaccine

Injection site discomfort, edema and erythema are frequently experienced local reactions to vaccinations. There may also be systemic responses, such as rash, fever, irritability and sleepiness (Principi and Esposito, 2016). Temperature and injection site responses are linked to higher doses of the acellular pertussis vaccination (DTaP), tetanus toxoids and diphtheria toxoids. The human papillomavirus (HPV), quadrivalent meningococcal conjugate vaccine (MCV4), tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (Tdap) vaccines can all cause syncope, especially in teens. Children who get antipyretics may have a diminished antibody reaction to some vaccine antigens, however some side effects may be mitigated by giving acetaminophen at the time of immunization or shortly after (Spencer et al., 2017).

Myths about Vaccines

A portion of myths are based on fact and most can be traced back to their original source. For instance, eczema; which was actually a legitimate contraindication to the smallpox vaccine, became a legendary contraindication to other vaccinations, as well as to other allergies (Löffler, 2021). Few of the commonly prevailing myths about vaccination ahs been discussed below:

Vaccination Link to Allergy

The smallpox vaccine is actually contraindicated in cases with eczema. In the past, there have been recommendations regarding contraindications to measles vaccine and some brands of rubella vaccine, such as intolerance to eggs or rabbit fur. A family or personal history of allergy to certain animal products is mentioned in several package instructions as a reason why receiving the measles and rubella vaccines was not recommended. The measles, mumps, rubella, influenza and yellow fever vaccines are still mentioned as contraindications if a person has severe hypersensitivity or anaphylactic reaction to eggs. Immunization against measles, mumps and rubella is also not advised for people who have neomycin and kanamycin allergies (Elbahi et al.).

AUTISM

Autism is a developmental disorder that typically manifests as poor speech, aberrant conduct and lifetime dependency in its victims. Autism in children was stigmatized for decades as schizophrenia, mental retardation, or at best, strange conduct. Given that autism is a neurological disorder, a vaccine carrying thimerosal must be the cause of the condition (Davidson, 2017).

Vaccination Contradiction in Neurological Abnormalities

Following concerns about the link between the pertussis vaccine and persistent neurological deficiency, they were marked as contraindications. Since the beginning of time, myths concerning a variety of ailments have been widely accepted. Myths can affect society to varied degrees and are influenced by culture. Numerous beliefs regarding various infections (such as leprosy, tuberculosis and influenza) have been debunked over time using evidence-based methods (Begg and Nicoll, 1994).

Numerous myths have emerged as a result of the ambiguity surrounding the present COVID-19 outbreak. A few of these fallacies are causing social stigma to proliferate widely. These misconceptions may also cause people to become overconfident which increases their chance of contracting the virus. These misinformation campaigns are all greatly influencing public opinion and the spread of illness. Therefore, the proper authorities should act as soon as possible to dispel the falsehoods and take action to do so. Individuals should thoroughly assess anything before deeming it useful (Sahoo et al., 2020).

Allergies, Asthma and Vaccines

In developing nations, vaccinations have been linked to an increase in the prevalence of allergic diseases and asthma. Allergy-related disorders like asthma have a complex etiology, involving influences from the environment, heredity and lifestyle choices. Nonetheless, a number of carefully designed studies have demonstrated that vaccination both lowers and does not raise the incidence of SIDS (Fleming et al., 2001). On the other hand, it has been demonstrated beyond dispute that additional variables, including low birthweight, prone sleeping, smoking by the mother, soft bedding, infections and bottle feeding, are linked to SIDS (Nagler, 2002).

Connection between Autism and Mercury Poisoning

The inclusion of adjuvants containing mercury in some vaccinations is one of the key lies that perpetuates the myth of immunizations and autism. A substantial amount of research indicates that severe developmental abnormalities, such as autism spectrum disorders (ASD), can be brought on by substantial levels of neurotoxic drug absorption throughout early life (Fleming et al., 2001) Since natural mercury contact (mostly MeHg) can occur through diet (contaminated seafood, grains, etc.), medical treatment (syphilis treatment, teething powder, etc.), occupational settings (chronic exposure to mercury vapors, etc.), or other means, mercury has been well-established as a neurotoxic factor. It has also been linked to several malignancies (Van Reeth et al., 2009).

Vaccine and Sudden Infant Death Syndrome

A reasonable percentage of children who pass away from SIDS have recently received vaccinations, which has led to the unverified assumption that vaccines cause the disease. Since SIDS deaths happen in the age range when vaccination rates are highest, it makes sense that vaccination rates would naturally precede SIDS (Vennemann et al., 2007).

Vaccines Cause Autoimmune Diseases

Another commonly raised safety concern is links between vaccines and autoimmunity. These links have been extensively researched in relation to a variety of autoimmune (AI) disorders, such as multiple sclerosis (MS), diabetes mellitus type 1, Guillain-Barré Syndrome (GBS) and other demyelinating disorders. Current epidemiologic research has not discovered any links between the quantity of immunizations received and a higher chance of developing autoimmune diseases (Mailand and Frederiksen, 2017).

There is no correlation between receiving a vaccine and developing multiple sclerosis (MS). The relationship between various individual vaccines and central demyelinating disorders, including hepatitis B, human papilloma virus (HPV), influenza, MMR, varicella, tetanus, Bacillus Calmette Guérin (BCG), polio or diphtheria was examined. Systemic lupus erythematosus, rheumatoid arthritis, inflammatory myopathies, multiple sclerosis, Guillain-Barré syndrome and vasculitis are among the infrequently documented post-vaccination autoimmune disorders. Aluminum-containing vaccines, macrophagic myofasciitis and the latest evidence of autoimmunity after using the human papillomavirus vaccine (Mouchet et al., 2018).

Vaccines in Pregnancy

Vaccinations against influenza and pertussis are advised for pregnant women by both non-governmental advisory groups and federal agencies. There is a substantial body of evidence demonstrating the safety of the influenza vaccine because millions of expectant mothers have had it during their pregnancies. There is no correlation between the influenza vaccine and unfavorable pregnancy and newborn outcomes, such as birth defects, stillbirths, spontaneous abortions, low birth weights and poor Apgar scores, according to a number of well-designed cohort studies (Kharbanda et al., 2017).

Vaccination Myths in Human

Vaccine exposure does not seem to have any negative effects on the nervous system. There is no link between autism and the vaccinations against measles, mumps and rubella. Local side effects from vaccinations include erythema and discomfort. While other vaccines minimally raise the risk of syncope, the rotavirus vaccine slightly boosts the rate of intussusception.

Accumulating evidence suggests that many of the social and behavioral concerns associated with HPV vaccine that have sparked resistance among patients and providers (and have been the focus of media reports) have little or no basis in reality. One of the presumed concerns about HPV vaccine is the fear that adolescents will respond to vaccination with sexual risk compensation (also referred to as sexual disinhibition), initiating sexual activity at a younger age and/or reducing self-protective sexual behaviors (Zerbo et al., 2017).

There is no connection found between autism and the MMR vaccine or vaccinations containing mercury, like the DTaP (diphtheria, tetanus, acellular pertussis) shot. Further research revealed no correlation between type 1 diabetes and either the DTaP or MMR vaccines and, lastly, between Bell's palsy or asthma flare-ups and TIV. Recent research has revealed no connection between the human papilloma virus (HPV) vaccine and the emergence of a number of clinical issues, such as postural orthostatic tachycardia syndrome (POTS) and complex regional pain syndrome (CRPS) (Zimet et al., 2013). Due to these issues, vaccine coverage has significantly decreased as a result of concerns highlighted (Principi and Esposito, 2016).

Vaccination Myths in Animals

Mass vaccination campaigns against domestic animals and humans can indirectly promote the natural evolution of new pathogens or more virulent mutations. This is particularly true when the effects of these "public health" initiatives are not fully understood and addressed, as in the case of constantly rising population densities and concentrations. This implies that investors and producers of drugs and vaccines will always have business (Fox et al., 2020).

The vaccination campaigns against rinderpest and smallpox have been hailed as major achievements in the worldwide eradication of both diseases, which respectively affect human populations and those of domestic and wild ruminant animals.

It has become clear over the last ten years that opinions about vaccinations as a useful weapon for eradicating foot and mouth disease have changed. The previous unfavorable opinion stemmed from false beliefs, primarily from the notion that vaccinations conceal asymptomatic virus circulation and are not totally effective. The introduction of vaccination policies in the 1990's as part of a planned eradication effort in South America and during disease recurrence in disease-free areas helped to produce more consistent and obvious results from vaccination campaigns, opening the door to a shift in public opinion. The creation and use of cutting-edge sero-diagnostic techniques to evaluate silent virus circulation independent of vaccination was particularly important (Alwan, 2022).

Adverse reactions to canine vaccinations are reported. These included interstitial nephritis and ocular opacity after immunization with a particular strain of infectious canine hepatitis (Appel and others 1973), as well as encephalitis after co-administration of a canine distemper virus vaccine (Cornwell and others 1988).

Concerns about Safety

Certain parental concerns regarding the HPV vaccine, such as the belief that the immunization is too recent, are rooted in uncertainties regarding the safety of the vaccine (Fisher, 2012). These concerns may have been exacerbated by frightening news reports that have occasionally misreported data from the Vaccine Adverse Event Reporting System, falsely claiming that HPV vaccination frequently results in serious side effects, including death (Gulli, 2007). Many extensive studies on the safety of the HPV vaccine have been published and they largely indicate that there is little to no evidence of serious side effects from immunization (Lu et al., 2011).

Like other vaccination side effects, minor discomfort and bruises at the site of injection, drowsiness and syncope are the most commonly reported side effects (Naleway et al., 2012). These are temporary phenomena. It is imperative to emphasize that the occurrence of an adverse event following immunization does not inevitably indicate that the vaccine was the cause. Effectively conveying to parents the information that the HPV vaccine is safe is a significant barrier, though. the extremely high risks of not getting vaccinated in the context of late marriage, serial monogamy, generalized, relatively early sexual debut are the causes of gradual increase in risk of HPV infection (Zimet et al., 2013).

Vaccination and Public Concerns

People in Pakistan avoid vaccines because of the numerous myths and debates surrounding polio vaccination. As a result, polio cannot be eradicated. A significant portion of the public believes that children receive polio vaccines in order to manage the population (Ghinai et al., 2013).

According to one idea, the polio vaccination would cause children to die, while another believes that it will deprive us of our manhood and prevent us from having more than two children in the future. The anti-vaccination effort in the province afflicted by polio was the most damaging. Starting a constructive conversation with the public and educating them about the need to avoid unquestioningly believing conspiracy theories is the only method to methodically combat conspiracy theories and misconceptions (Kitta, 2012). Changing people's perceptions must be the ultimate goal of all these actions. This will eventually show to be a successful long-term plan that aids in the eradication of polio (Ahmad et al., 2022).

Refusal to Vaccination

The complicated causes behind vaccine refusal vary depending on the location and cultural background. Nonetheless, in the majority of situations, vaccine hesitancy remains a significant contributing factor to declining vaccination rates (Larson et al., 2014). Parental refusal of some or all vaccines is becoming more common. The American Academy of Family Physicians (AAFP) opposes vaccination exemption laws unless they are necessary due to an allergy or other medical condition. Parents who are thinking about refusing or delaying vaccinations should receive vaccine information statements from doctors, who should also point them toward reliable sources of information (Spencer et al., 2017).

Anti-Vaccination Movements

Many illnesses, including autism, developmental disorders and sudden infant death syndrome (SIDS), as well as new variants of Creutzfeldt-Jacob disease, diabetes, allergies, asthma, multiple sclerosis, cancer and even AIDS, have been incorrectly linked to vaccinations. Campaigns against vaccination are a serious threat to public health as it will trigger disease spectrum.

Justification for Mandates

The justifications for making vaccinations mandatory are far more consistent. Put simply, proponents of mandatory vaccinations contend that shots shield recipients from serious illnesses while also shielding society from the spread of infections that result from individual cases. The hazards associated with vaccination are acknowledged by proponents for each person who receives the shot. These hazards, they calculate, are considerably lesser than the risks associated with

the illnesses for which the vaccinations are administered (Lantos et al., 2010). Consequently, they conclude that becoming immunized is in each person's best interest. All things considered, the net benefit to society as well as the benefit to individuals justify the inherent interference with freedom of choice that characterizes every mandate (MacIntyre and Leask, 2003).

Conclusion

Myths are a significant barrier to vaccination. Many myths have been debunked, but others persist. As of right now, the fight is winning: vaccination coverage for all antigens now above 90% and the prevalence of diseases that can be avoided. In order to ensure that vaccination programs are as safe as possible, it is important to identify adverse events that follow vaccination and lower the possibility of low vaccination rates. Globally, vaccination misinformation threatens lives so it's critical that front-line primary care providers be aware of the facts and able to persuasively explain that vaccines are a safe and effective intervention. The greatest defenses against these myths are information and an optimistic view of immunization.

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Chapter 57

Liver Fluke Vaccination in Large Ruminants

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ABSTRACT

Globally, liver fluke infection causes significant economic losses for the livestock industry and has emerged as an important public health concern. The use of anthelmintic medications is the basis of disease management. However, anthelmintic resistance has arisen as a major concern in the treatment of liver fluke. To design viable vaccinations, a deeper understanding of host-parasite interactions is required. Despite substantial efforts over the last two decades, no vaccine candidate has demonstrated reliable and persistent protection in large ruminants. Even though there are not any obvious standout vaccine, we need to find important molecules in fluke biology (the tegument, secretory products or extracellular vesicles) and conduct trials, particularly in livestock by utilizing both current and developing vaccination technologies. Bioinformatics tools are bringing the development of a marketable vaccination against liver fluke closer. The current study provides updates on the various vaccine types and possible vaccination candidates for liver fluke infection in large ruminants.

KEYWORDS
Fasciola hepatica, Vaccine, Liver fluke, *Fasciola gigantica*, Monovalent vaccine, Combined vaccine

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INTRODUCTION

Species of the genus *Fasciola* is the cause of fasciolosis, a disease, primarily affecting livestock and occasionally infecting human beings. *Fasciola* (*F.*) *hepatica* and *Fasciola* (*F.*) *gigantica* commonly known as liver flukes are the significant flatworms in the world due to their largest latitudinal, longitudinal, and altitudinal dissemination (Fentie et al., 2013; Mas-Coma et al., 2005). It is estimated that approximately 180 million persons are at the risk of developing zoonotic infection, with 35-72 million of those cases being fasciolosis (Cwiklinski et al., 2016; Sabourin et al., 2018). The livestock sector suffers significant financial losses globally due to liver fluke infection. The losses exceed US$3 billion annually (Mas-Coma et al., 2019; Zafra et al., 2021). These expenses are brought on by using anthelmintic medications and production losses (milk, carcass composition, and delayed arrival at an acceptable slaughter weight) in the livestock sector. The infected animals exhibit reduced weight, anaemia, low fertility, decreased milk output, poor feed processing efficacy and decreased work capacity (Dar et al., 2005; Ur Rehman et al., 2023).

There are numerous variations in the life cycle of liver flukes, but they all generally involve a final vertebrate host for sexual reproduction and a primary molluscan host for larval multiplication (Roldán et al., 2021). Unembryonated eggs of the parasite passed in the stool of the final host hatches in water and develops into miracidia. Miracidia enters the intermediate host, where it develops into cercariae, rediae, and sporocysts (Rehbein et al., 2021). After emerging from intermediate host, cercariae encysts into metacercaria, which the ruminant host consumes. After excysting in the duodenum, metacercaria pass through the liver to enter the bile duct and the cycle continues (Flores-Velázquez et al., 2023).

Due to variables such as intensified farming practices, climate change, extensive resistance to drugs and possible hybridization of related parasites, there is increasing likelihood of animal infections and subsequent human disease (Mehmood et al., 2017). Currently, anthelmintic medications, mainly triclabendazole, are used for controlling flukes in both humans and animals. However, the ongoing growth of drug-resistant parasite populations makes the long-term use of chemical treatments to control parasitic infections unsustainable. Furthermore, consumers concerns regarding chemical residues in food and their negative effects on the environment are mounting (Cooper et al., 2012; Zhang et al., 2021).

The need for sustainable preventative measures, especially vaccines, is growing due to the environmental harm caused by intensive farming and chemical parasite control (Zerna et al., 2022). New adjuvant developments and vaccination technologies are significantly enhancing food safety by preventing residues from being left in food. Vaccines, when combined with better farm management and diagnostic techniques, would offer a multifaceted approach to control

fasciolosis. Vaccines are widely accepted among users and consumers (Afshan et al., 2013; Toet et al., 2014). Several efforts have been made to vaccinate the sheep, cattle and research animals with different liver fluke preparations, including excretory/secretory proteins and crude somatic proteins, somatic (36-55 kDa) and excretory-secretory antigens of *Fasciola* species not only advances diagnostic capabilities but also has significant implications for improving vaccination strategies in large ruminants (Afshan et al., 2022; Flores-Velázquez et al., 2023; Komal et al., 2021). The advancement of diagnostic techniques plays a crucial role in controlling parasitic diseases, both for initial detection and for monitoring the outcomes of vaccination programs (Afshan et al., 2013). The development of the Copro-ELISA exemplifies a sensitive tool that aids in the post-vaccination surveillance by efficiently detecting residual infections in cattle and buffaloes (Afshan et al., 2021; Kiran Afshan et al., 2017). Concurrently, the ELISA test for *F. hepatica* is particularly valuable for assessing immune responses to vaccinations. Moreover, understanding the genetic diversity of liver fluke species is essential for designing broad-spectrum vaccines (Mufti et al., 2014). Despite the enormous advancements made in vaccine development against bacteria and virus over the past 200 years, currently there is no commercially viable vaccines for animal or human helminth parasites except for the live attenuated huskvac vaccine against the lungworm (Molina-Hernández et al., 2015). This chapter's main goal is to go over more recent information on the development of specified vaccinations against fasciolosis in large ruminants, including approaches for creating novel subunit vaccines and irradiated attenuated vaccines. Prospects and the existing state of knowledge regarding several potential antigens are considered in this chapter.

Liver Fluke Vaccines: Types and Antigenic Candidates

A pathogen is composed of multiple types of antigens that can serve as an infection source so a vaccine that demonstrates immune responses to a range of antigens is thought to offer superior protection than other vaccinations (Golden et al., 2010; Tadesse et al., 2021). Highly specialized organs such as the digestive tract, teguments and secretory glands of the fluke have been discovered to release proteins and enzymes. Antigens have been characterized by cytological and biochemical methods according to their site of release in the parasite. The association between the cellular localization of antigens and their apparent function can be predicted through immunolocalization. This connection aids in the subsequent determination of the antigen location and its route of emission (Khan et al., 2017).

Numerous methods, such as: reviewing markers that exhibit cross-reactivity with sera taken from different trematodes, evaluating antigens that are closely related to already available vaccine candidates in different animal species and/or the rational selection of antigens that are considered essential in liver fluke biology, have been employed to find out potential antigens for liver fluke vaccine (Toet et al., 2014; Turner et al., 2016; McManus, 2020). About half a dozen pure, native, and recombinant antigens have been discovered to have immunoprophylactic potency against liver fluke infection (Toet et al., 2014).

Though there have been significant developments in the identification of possible vaccine molecules, it is reasonable to state that the degree of efficacy necessary for commercialization has not yet been attained for the control of fasciolosis in large ruminants (McManus, 2020).

Research has been carried out for enhancing humoral or cell-mediated immunity against fasciolosis in large ruminants through immunization with recombinant, attenuated, or a combination of these vaccines (McManus and Dalton, 2006; Ur Rehman et al., 2023). The most common methods of administering vaccines in research trials is intramuscular or subcutaneous (Table 1). A few studies using mucosal vaccination administration have shown encouraging outcomes (Ur Rehman et al., 2023). In addition to lowering fluke loads, vaccinations reduce eggs counts and liver damage subsequently improving animal health (Turner et al., 2016). Table 1 shows monovalent vaccine types used against liver fluke.

Attenuated Vaccines

Metacercaria

Experimental infection of cattle with irradiated metacercaria of *F. hepatica* can help to control the total number of worms, the progression of the infection, liver damage and the amounts of glutamate dehydrogenase and γ-glutamyl transferase (Lalor et al., 2021; Ur Rehman et al., 2023). Metacercaria irradiated with γ exposure at a level of 3 Krad provided considerable resistance in calves against fasciolosis by decreasing the number of worms and fecundity (Khan et al., 2017; Ur Rehman et al., 2023).

Excretory/secretory Product Vaccines (ES)

Majority of vaccine trials that have been conducted so far have focused on a limited number of parasite molecules specifically fatty acid binding proteins (FABP), glutathione-S-transferases (GSTs), Leucine aminopeptidase (LAP), and cathepsin L (CAT L) peptidases. These molecules attracted attention because the adult worms release them in large quantities under laboratory conditions. Additionally other proteins such as haemoglobin (Hb), *F. hepatica* kunitz-type molecule (FhKTM) and paramyosin have been studied as vaccine antigens and have shown significant results (Spithill et al., 2021; Toet et al., 2014; Ur Rehman et al., 2023).

Fatty Acid Binding Proteins

The first identified and refined antigen fractions to be evaluated as a liver fluke vaccine were FABPs. The soluble tegumental proteome of adult *Fasciola* contain a significant amount of FABPs (Morphew et al., 2013). Results of Hillyer's

tests led to the identification of this antigen as protective against liver fluke (Hajizadeh et al., 2021; Hillyer et al., 2008). According to different experimental studies, both native and recombinant FABPs significantly reduce animal infection with *F. hepatica* and provide cross-protection against *S. mansoni* and *S. Bovis* in various animal models. Various FABP isoforms have demonstrated minimal replicable efficacy in cattle (López-Abán et al., 2008; Nambi et al., 2005; Ur Rehman et al., 2023). Given the poor efficacy so far, *F. hepatica* FABP is not a good candidate for fasciolosis vaccination in cattle based on the data to date. Fatty acid binding protein Fh12 has been found to be critical to manipulate the host immune response (Ruiz-Jiménez et al., 2021). It is involved in not only suppression of macrophage function but also the reduction of initiation of primary immune response through inhibition of dendritic cells activity. Therefore, it can be the ideal target for vaccine development to not only disrupt the life cycle of the parasite but to boost the immune response against it.

Table 1: Monovalent vaccine types against liver fluke.

Antigen	Type	Fasciola spp.	Administration route	Adjuvant	Efficacy	Host	Reference
Metacercaria	Attenuated	*F. hepatica*	Oral	Nd	29%	Calves	(Nansen et al., 1975)
Fatty acid binding protein	Native	*F. gigantica*	Intramuscular	Montanide 70 M-VG	31%	Cattle	(Estunningsih et al., 1997)
	rFh15	*F. gigantica*	Subcutaneous	FCA/FIA	35%	Buffalo	(Nambi et al., 2005)
	rFABP	*F. gigantica*	Intramuscular	MontanideTMM-70VG	ns	Buffalo	(Kumar et al., 2012)
GST	Native	*F. hepatica*	Subcutaneous	FCA/FIA	33%	Cattle	(Zerna et al., 2022)
	rSb28GST	*F. hepatica*	Intramuscular	Al (OH)3,/Quil A,/PBS/FCA	ns	Cattle	(De Bont et al., 2003)
	rFgGST	*F. gigantica*	Intramuscular	MontanideTMM-70VG	ns	Buffalo	(Kumar et al., 2012)
	Native	*F. hepatica*	Subcutaneous	Quil Alsqualene Montanide®	49–69%	Cattle	(Morrison et al., 1996)
Cathepsin L1	Native CatL1	*F. hepatica*	Intramuscular	FCA/FIA	42-69%	Cattle	(Dalton et al., 1996; Toet et al., 2014)
	rCatL1	*F. hepatica*	Subcutaneous	MontanideTMISA70VG or 206VG	48%	Cattle	(Flores-Velázquez et al., 2023; Golden et al., 2010)
Cathepsin (CPFhW)	rCPFhW	*F. hepatica*	Intranasal	Not defined	54.2%	Cattle	(Wedrychowicz et al., 2007)
	rCPFhW	*F. hepatica*	Oral	None	56.20%	Cattle	(Wesołowska et al., 2018); (Flores-Velázquez et al., 2023)
Hemoglobin (Hb)	Native	*F. hepatica*	Intramuscular	None	43.80%	Cattle	(Dalton et al., 1996)
	rHbF2	*F. hepatica*	Not defined	Quil A/Aphigen/ cholesterol	ns	Cattle	(Dewilde et al., 2008)
Paramyosin	Native	*F. hepatica*	Subcutaneous	QA/SM	47%	Cattle	(Spithill et al., 1999)
Kunitz type molecule	Native	*F. hepatica*	Not defined	Quil A	ns	Cattle	(Spithill et al., 1999)
Thioredoxin	rFhTGR	*F. hepatica*	Subcutaneous	FIA	8.20%	Cattle	(Flores-Velázquez et al., 2023; Maggioli et al., 2016)
	rFhTGR	*F. hepatica*	Subcutaneous	Adyuvac50	3.80%	Cattle	(Flores-Velázquez et al., 2023; Maggioli et al., 2016)
	rFhTGR	*F. hepatica*	Subcutaneous	Alum	23%	Cattle	(Flores-Velázquez et al., 2023; Maggioli et al., 2016)
Tegument	Recombinant	*F. hepatica*	Subcutaneous	FCA/FIA	0%	Cattle	(Zerna et al., 2022)
Tetraspanin	rFhTSP2	*F. hepatica*	Subcutaneous	FCA/FIA	-1.6%	Cattle	(Flores-Velázquez et al., 2023; Zerna et al., 2021)
LeucineAminoPeptidase	rFgLAP	*F. gigantica*	Intramuscular	MontanideTMM-70VG	ns	Buffalo	(Raina et al., 2011)
Peroxiredoxin	rPrx	*F. gigantica*	Not defined	montanide M-70 VG	ns	Buffalo	(Raina et al., 2011)

r; recombinant, FCA; Freund complete adjuvant, FIA; Freund incomplete adjuvant, ns; not significant, Fh; *Fasciola hepatica,* Fg*; Fasciola gigantica*

Glutathione S Transferases

Glutathione S-transferases are present in nearly all animals and function as immune-evasion molecules in helminths. GSTs are found frequently in parasitic helminths and have been substantially conserved throughout evolution (Brophy et

al., 1990; Zerna et al., 2022). Apparently, the high concentration of GSTs in the fluke suggests that these enzymes are crucial to the parasite's metabolism and help prevent haematin from crystallizing into big particles that could obstruct the parasite digestive tract (Duan et al., 2024). These enzymes contribute to the cellular detoxification of drugs, provide defence against immune-induced impairment and aids in excretion of several xenobiotic compounds (Zerna et al., 2022). When the host immune's triggered free-radical attack on the parasite, GSTs lessen the impact of these free radicals (produced from degranulating cells on juveniles flukes) by detoxifying the secondary products of lipid peroxidation (Cervi et al., 1999; Piedrafita et al., 2001; Toet et al., 2014; Zerna et al., 2022). When properly prepared, GSTs can considerably protect cattle against liver fluke exposure. The mean efficacy demonstrated by the GSTs in cattle is 43% (Toet et al., 2014). The immunogenic GST protein from *F. gigantica* belonging to mu-class is utilized to develop the vaccine (Kalita et al., 2020). The resultant vaccine showed high docking with TLR2 receptor while molecular dynamics analysis revealed a stable interaction. However, immunogenic behavior and safety is still to be validated experimentally. Similarly, GST purified from *Gigantocotyle explanatum*, a foodborne liver trematode infecting Indian water buffalo, has shown high immunogenicity as polyclonal antibodies against it were raised in rabbits (Rehman et al., 2020). Furthermore, its antibodies against this antigen have not shown any cross-reactivity against *F. gigantica* which infects land buffaloes.

Cysteine (cathepsin) Proteases

The vast family of cysteine proteases includes cathepsins L and B, which have been investigated in terms of parasite immune evasion strategies, nutrition acquisition, and production of eggs and as candidate for potential vaccine. They are highly expressed in liver flukes because they facilitate the parasite's penetration into the host tissue (Cwiklinski et al., 2019; Maggioli et al., 2011)). Cathepsin L1 proteins are produced in cecal epithelial cells and released into ES products. Various CatL1 subtypes are involved in the migration and digesting of parasites and are expressed at different phases of the life cycle. In cattle, the effectiveness of two distinct secreted cathepsin L homologues, cathepsin L1 (CatL1) and cathepsin L2 (CatL2), has been evaluated independently and in combination with *Fasciola* haemoglobin (Mulcahy et al., 1998 ; Ur Rehman et al., 2023). The use of cathepsin L2 in combination with haemoglobin produced the greatest level of protection against infection (Table 2). Furthermore, it was discovered that cathepsin L1 reduced the fertility of parasite's eggs. In cattle, individual native CatL1 has demonstrated noteworthy efficacy of 42–69% (Dalton et al., 1996). According to the available data, CatL1's effectiveness in cattle may be sufficient for commercial production if an appropriate adjuvant is added to increase efficacy. *F. hepatica* is known to secrete both cathepsin peptidases and wide range of peptidase inhibitors to maintain the balance. This ensures the sustained survival of the parasites within their hosts. In this regard, Cwiklinski and and Dalton (2022) have designed an interesting strategy to disrupt this balance by targeting different inhibitors. This approach has helped to increase the immune response significantly, although any of the trials has shown reduction in fluke burden. Table 2 shows the combination of vaccines used against liver fluke infection.

Table 2: Combination vaccines against liver fluke infection

Antigens	Types	*Fasciola* spp	Administration route	Adjuvant	Efficacy	Host	Reference
CatL1 + Hb	Native	*F. hepatica*	intramuscular	FCA/FIA	51.90%	Cattle	(Dalton et al., 1996)
CatL2 + Hb	Native	*F. hepatica*	intramuscular	FCA/FIA	72.40%	Cattle	(Dalton et al., 1996)
CatL2 + Hb	Native	*F. hepatica*	intramuscular	FCA/FIA	72.40%	Cattle	(Mulcahy et al., 1998)
CatL2 + Hb	Native	*F. hepatica*	subcutaneous	FIA	11.00%	Cattle	(Mulcahy et al., 1998)
CatL2 + Hb	Native	*F. hepatica*	intramuscular	FCA/FIA	29.00%	Cattle	(Mulcahy et al., 1999)
CatL1 + CatL2	Native	*F. hepatica*	intramuscular	FCA/FIA	55.00%	Cattle	(Mulcahy et al., 1999)
rmFhCL1+ rmFhCL3	Recombinant	*F. hepatica*	subcutaneous	ZA1	37.60%	Cattle	(Flores-Velázquez et al., 2023; Garza-Cuartero et al., 2018)
FhTeg1+ FhTeg5	Recombinant	*F. hepatica*	not defined	FCA/FIA	0%	Cattle	(Flores-Velázquez et al., 2023; McCusker et al., 2020)
LTB-FhTSP2	Recombinant	*F. hepatica*	Intranasal	None	ns	Cattle	(Flores-Velázquez et al., 2023; Zerna et al., 2021)
FhGST+FhTeg	Recombinant	*F. hepatica*	subcutaneous	FCA/FIA	33%	Cattle	(Zerna et al., 2022)
rFABP+GST	Recombinant	*F. gigantica*	not defined	Montanide 70 M-VG	35.8	Buffalo	(Kumar et al., 2012; Lalrinkima et al., 2021)

***F. hepatica* Kunitz-type Molecule**

FhKTM are used as antigens, consists of an only 58-amino acid long polypeptide. It is widely distributed in the adult *F. hepatica's* tegument, parenchymal tissue, and gut (Mulcahy et al., 1998; Silvane et al., 2020). FhKTM's abundance indicates that it would be a good option for a vaccine, since gut-associated molecules are effectively employed in vaccinations against other parasites including *Boophilus microplus* and *Haemonchus contorts* (Sasaki et al., 2006; Di Maggio et al., 2016). Aside from its abundance, this molecule is an effective protease antagonist (Silvane et al., 2020). The formulation of a 6-O-ascorbyl palmitate ester (Coa-ASC16) and a synthetic oligodeoxynucleotide with unmethylated cytosine-guanine motifs (CpG-ODN) is done utilizing liquid crystal nanostructure to produce a vaccine against *F. hepatica* (Silvane et al., 2020).

These compounds strengthen the humoral immune reaction directed against *F. hepatica* (Cervi et al., 2004; Fracasso et al., 2017; Toet et al., 2014). Research has indicated that the addition of FhKTM/CpG-ODN/Coa-ASC16 boosts interleukin (IL)-17A and interferon-gamma (IFNγ) production, both of which are useful in the management of fasciolosis infection (Falcón et al., 2012). Likewise, additional research has demonstrated that IFNγ and IL-17A cooperate by prompting macrophages to produce nitric oxide, which protects against *Fasciola* infection (Gao et al., 2016; Kumar et al., 2012; Nascimento et al., 2015; Ur Rehman et al., 2023). It has also been noted that FhKTM enhances host survivability and avoids damage to the liver (Silvane et al., 2020).

Hemoglobin

Though not much is known about helminth hemoglobins, they are thought to serve as oxygen reserves like myoglobin in addition to being engaged in the transport of oxygen across tissues (Hotez et al., 2008; Hotez et al., 2016). The energy consumption of migrating liver flukes is primarily aerobic, and their Krebs cycle is mainly active in the tegument (Williamson et al., 2003). Anaerobic respiration is triggered by the bile duct's anaerobic conditions as well as the parasite's growth, which restricts oxygen transport to the cells. Therefore, it's likely that the Hb vaccination caused an immune response that could disrupt the parasite's ability to metabolize oxygen in its tegument during migration (Williamson et al., 2003; Knox, 2011). In addition to producing immunoprotection and reducing the pathology linked to liver fluke infection, this antigen vaccinations have a deleterious effect on the growth and viability of the fluke eggs. In cattle, liver fluke Hb can also induce very high levels of protection (Toet et al., 2014; Spithill et al., 2021).

Paramyosin

Paramyosin, a sub-tegumental protein of *Schistosoma*, has been used as a mouse vaccine, in several studies (Jiz et al., 2008; 2015; Zhang et al., 2006). The potential of paramyosin isolated from *F. hepatica* in two vaccination experiments in ruminants has been investigated considering the capacity of antigens from *Fasciola* and *Schistosoma* to cross-protect. Some studies have tested the potential of paramyosin as vaccine target against *Clonorchis sinensis*, the Chinese liver fluke which infects fish-eating mammals including humans (Park et al., 2009; Kang et al., 2022). For instance, Wang et al., have used paramyosin from *C. sinensis* in mice and observed significantly higher production of IgG1 and IgG2a (Wang et al., 2012). Furthermore, it resulted in activated of immune type 1 and immune type 2 leading to 54.3% reduced fluke burden in mice. Similar promising results were observed in another study where recombinant paramyosin was used to immunize the mice (Sun et al., 2020).

Saponin Like Protein

A class of proteins known as SAPs interacts with different types of lipids. They can cause host cells to lyse, which makes it easier for parasites to be processed and absorbed. SAP-1 and SAP-2 are the two types of SAPs found in liver fluke (Kueakhai et al., 2017). SAP-2 alone and in combination with leucine aminopeptidase has been tested as potential vaccine candidate against *F. gigantica* (Changklungmoa et al., 2023). The results have revealed that both individual proteins and in combination are able to significantly increase the serum IgG1 and IgG2a in vaccinated mice. Moreover, reduced liver damage was observed. Overall, study has shown that the combination of these two immunogenic proteins is more effective as vaccines as compared to the single proteins. Recombinant SAP vaccinations are not currently available for ruminants.

Recombinant Vaccine

Even though native proteins provided large ruminants with good protection in trials. However, it is not feasible to use proteins in native form for development of a commercial vaccine. For this reason, most subsequent vaccine trials have used recombinant proteins from different stages of the parasite (Spithill et al., 2021). Recombinant protein vaccination studies have shown significant protection of up to 89% (fluke reduction) (Tables 1, 2), S but under various laboratories and environmental settings, this high level of protection has not proven consistent. It's interesting to note that majority of the vaccine candidates were initially isolated as native proteins typically from secretome of adult worm as antigen preparation was simple and not overly complex. Later, prokaryotic bacterial and/or eukaryotic yeast expression systems were used to reassemble these proteins into recombinant subunit vaccines (Spithill et al., 2021). One recent cocktail recombinant vaccine against *F. hepatica* in sheep has utilized protease inhibitors and antioxidants (Cwiklinski et al., 2023). Although, different vaccine combinations from either category are unable to confer the protection, but sustained immune responses are observed. Therefore, further optimization of protocols is expected to yield promising results in future. It is crucial to understand that the purpose of vaccination is not limited to the reduction of fluke burden, but also to minimize the damage to the host animals. In this regard, one study based on a partially protective vaccine of four different recombinant proteins from *F. hepatica* in combination with adjuvant Montanide 61 VG showed both desired effects (Molina-Hernández et al., 2021). The vaccine was able to reduce the fluke burden along with reduced liver damage of the host sheep as compared to non-protective vaccine, based on the same antigens in different adjuvant, and infected control group. This study has taken another important step not only towards animal welfare but to maximize the economic benefits as well.

Nucleic Acid Vaccine

The application of nucleic-acid vaccines against liver fluke infection has overcome the difficulties associated with recombinant vaccines and offers an enhanced way of vaccine manufacture (Carmona et al., 1993; Smitha et al., 2010).

Using naked *F. gigantica* FABP DNA with non-sylated polyethylenimine *F. gigantica* FABP causes mice to produce a considerable amount of Th1 cytokines (tumour necrosis factor and IFN-γ), which shield the host from parasite infection (Kofta et al., 2000). It has been discovered that supplementing DNA vaccine with cysteine proteases completely protects male mice against liver fluke infection (Kofta and Wedrychowicz, 2001). However, trail with the large ruminants as model animals are required (Ur Rehman et al., 2023). It is vital to utilize the latest molecular identification techniques aids in developing strain specific regionally tailored vaccines (Komal et al., 2024). Rinaldi et al. (2020) have provided a refined protocol to utilize RNA interference technology for gene silencing in liver flukes (Rinaldi et al., 2020). This optimized protocol is not only important to determine novel targets for drug discovery but also for the vaccine development. Using a similar approach, *Ov-grn-1* has been found to be an important factor for malignant transformation by food-borne liver fluke *Opisthorchis viverrini* (Chaiyadet et al., 2022). Their RNA-guided knock-out study resulted in reduced fibrosis in hepatobiliary tract, specifically periductal fibrosis. Therefore, this target can be utilized for future vaccination purposes to reduce chronic fibrosis. Another approach to develop nucleic acid vaccines against liver flukes can be based on understanding of host-parasite molecular interactions. For instance, the proteomics approaches have been utilized to determine the proteins related to signaling pathways, fibrosis, oxidative stress, metabolism of fatty acids and proteins (Šimonji et al., 2022). Therefore, the DNA and RNA sequences which encode the proteins interacting with hosts can be the ideal targets for vaccine development.

Combination Vaccines

It is unclear if multivalent immunizations are required for the optimum commercial-level efficacy against liver fluke as single antigen vaccines differ in their performance and the results are non-reproducible under different laboratories conditions. The proper combination of multivalent vaccines may result in increased performance, but the incorrect composition may cause immunological conflict and consequent decrease in efficacy (Li et al., 2006; Zhu et al., 2011). Creating a mixed multiepitope vaccine has a positive immunogenic impact against fasciolosis infection (Caffrey et al., 2018). In tests conducted on cattle, the bivalent combination of native CatL1 and CatL2 demonstrated 55% protection (Table 2). Leucine aminopeptidase of *F. hepatica*, haemoglobin, and cathepsin (L1 and L2) have all been reported to be utilized in combination to suppress fasciolosis (Dar et al., 2005). A study using *F. hepatica* haemoglobin and CL2 showed a 98% anti-embryonated impact on eggs (Haçarız et al., 2012). The cocktail of two different types of antigens of *F. hepatica* in sheep showed improvements of different parameters including hepatic lesions, fecal egg count and fluke burden (Zafra et al., 2021). Furthermore, the serological study revealed that there is a significant correlation between anti-*Fasciola* IgG levels and liver enzyme activities in patients with fascioliasis, suggesting these as potential biomarkers of disease pathogenicity (Afshan et al., 2020).

Conclusion and Future Directions

Compared to viruses, parasites especially large multicellular helminths are far more complex. They require numerous hosts to complete their life cycle, in which they undergo dramatic changes in growth and development and express hundreds of thousands of proteins as *Fasciola* spp., transcribes over 18,000 genes while developing in a mammalian host (Cwiklinski et al., 2015; Cwiklinski et al., 2018; Cwiklinski et al., 2021). Thus, a deliberate effort is required to analyse and reassess the gathered data to identify undiscovered factors in the liver fluke biology that will be crucial for future vaccine development. Currently, not enough is known about immunogenic proteins to develop vaccines that combat the *Fasciola* spp (Driguez et al., 2010; Eyayu et al., 2020). Additional research is necessary to understand how immunization alters the anatomy of liver fluke reproductive organs.

Even though some trials showed encouraging findings (up to 89% protection against a single challenge infection) (Spithill et al., 2021; Talaie et al., 2004), these studies ultimately failed to find a vaccine that consistently produced protection at levels that would have encouraged more research and development. Using scientific approach, we must screen as many antigens as feasible. Furthermore, there are significant differences in the research parameters such as the host selection, infection evaluation, adjuvant or vector selection, infection period and outcome analysis. Specifically, techniques used for rat experiments might not be appropriate for ruminants. Even though conducting medium-throughput vaccine screens on large animals (cattle, in particular) is impossible due to logistical, statistical, and financial difficulties associated with such trials. These studies necessitate close partnerships with government agencies and agricultural research organizations that have access to large animal research facilities. It is essential that funding and research agendas are realigned to ensure both immediate and long-term strategies are explored to combat complex parasitosis. A more practical and cost-effective approach that can help lessen the use of expensive animal model is to mimic vaccine manufacturing and forecast reactions without a host using bioinformatics. Massive genomic data sets are now being created for several worms as a result of the discovery and enhancement of next-generation sequencing technology (Forrester and Hall, 2014). Innovations in bioinformatics and genomics provide a promising avenue to bypass some of the traditional barriers faced in vaccine development, instead of concentrating on a single gene or protein, using all the "omics" data that is available for a certain parasite offers an objective method of understanding parasite biology. This will provide a full understanding of the genes, proteins and novel immunogenic targets that are significant at each embryonic stage, particularly those working at the host-parasite interface, which may be critical for invading and infections (Molina-Hernández et al., 2015).

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Chapter 58

The Evolving Landscape of Vaccines

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ABSTRACT

A vaccine is a biological agent that is utilized to boost the immune system when exposed to any pathogen. The evolvement of genetic engineering in the vaccines discovery has promoted the chance to target new diseases. The creation of vaccines is being advanced via genetic engineering, which makes it possible to precisely alter pathogen antigens to increase their immunogenicity and effectiveness. New vaccines may have a significant impact on costs, so individuals enrolled in individual health plans and small employers may be less inclined to pay for them. Different traditional approaches were used to develop including toxoid vaccine, subunit vaccines, inactivated vaccines, live attenuated vaccines. Nevertheless, modernity has brought about new customs and enhanced the development processes by introducing novel vaccines, such as mRNA, DNA, and viral vector vaccines, recombinant protein vaccines, virus-like protein vaccines, and nanoparticle vaccines. Currently, a number of infectious diseases that lack symptoms have been reported on the platform. In order to stop the spread of diseases, it is now essential to develop various vaccines against contemporary infectious diseases. The design of vaccines is one particularly fortunate and significant application. The use of artificial intelligence and machine learning in the creation of vaccines represents a dramatic change in the way we fight infectious diseases.

KEYWORDS
Vaccine, Pathogen, Infectious diseases, Pandemic, Genetic engineering, Machine learning

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INTRODUCTION

A vaccine is a biological agent that is utilized to boost the immune system when exposed to any pathogen. Recently, the word "vaccine" was created, derived from the Latin word vacca, which means cow. A vaccine usually contains an agent that looks like a disease-causing germ (microorganism) and is manufactured from weakened or destroyed microbes, one of their surface proteins, or their toxins. The agent induces the body's immune system to detect the agent as a threat as well as any microbes connected with it (Melief et al., 2015). Immunization is still one of the best ways to avoid infectious diseases and has a significant impact on human health (Sallusto et al., 2010). The vaccine trial process consists of various steps that must be followed quantitatively and systematically. The length of this process is proportional to the vaccine's purpose and nature, which is to protect healthy people from pathogen infection (Deb and Goel, 2020).

Brief Overview of the Historical Significance of Vaccines

Pandemic-prone infectious illnesses (including the plague, cholera, influenza, and several corona viruses) have frequently arisen and spread throughout history (Piret and Boivin, 2021). The development of bacteriology over time led to the quick development of vaccinations against typhoid, tuberculosis, anthrax, cholera, plague, and cholera (Aida et al., 2021). The first vaccine for smallpox disease treatment was introduced in the 15th century. The man who successfully invented the vaccine to treat cowpox was Edward Jenner. Additionally, ailing people occasionally started new smallpox outbreaks. At the close of the 1700s, already cowpox infected milkmaid did not develop smallpox, according to Edward Jenner, who had variolation when he was just 8 years old (Nabel, 2013; plotkin, 2014). In the 18th century, Louis Pasteur was the first scientist to discover a vaccine in the 18th century to treat both rabies and fowl cholera in chickens. The initial vaccination against the influenza virus was released in 1917–1918. Jonas Salk develops the first immunization against polio.

Importance of Staying Updated with Advances in Vaccine Development

There is little question that advancement in science and these expanded applications will lead to better health-related outcomes. The evolvement of genetic engineering in the vaccines discovery has promoted the chance to target new diseases. The best method to boost the value of upcoming vaccinations would be to calculate the potential short- and long-term cost related to the product's availability (Aleem et al., 2024). Because new vaccines may have a large influence on rates, small employers and individual health plan members may be less likely to pay for them. Since the introduction of more recent vaccinations, more atypical vaccinators, pharmacies and other businesses that were formerly beyond the healthcare system—have become aware of the financial consequences of vaccination, which has been a dilemma for physicians.

Traditional Vaccine Development Approaches

Live Attenuated Vaccines

Antimicrobial drugs, including neomycin, streptomycin, and polymyxin B, may be included in trace levels in live or inactivated virus vaccinations. Live vaccines come from bacteria or viruses that are found in the wild. These natural viruses or bacteria are repeatedly cultured in a lab to attenuate (weaken) them. In 1954, a youngster who had the measles sickness was the source of the measles virus utilized in modern vaccinations. Attenuated live vaccines need to proliferate in the recipient in order to elicit an immune response. The unchecked proliferation of the vaccination virus or bacteria following a live, attenuated injection can result in serious or even deadly illnesses (Assenmacher et al., 2005)

Inactivated Vaccines

Vaccinations that have been inactivated are not living and cannot spread. No one with impaired immunity can get a disease from these vaccinations. The immunity produced by live, attenuated vaccinations lasts longer than the immunity produced by inactivated vaccinations. After the second or third dose, the immune system develops a defensive reaction(Hall et al., 2021). When an inactivated vaccination is administered, the immune system primarily produces antibodies, as opposed to live vaccines, which cause an immunological response that closely mimics a genuine infection.

Subunit Vaccines

Vaccines called subunits comprise a fraction of the pathogen. In subunit vaccinations, the antigens may be polysaccharides, proteins, or a mix of polysaccharides and proteins. In other words, pure polysaccharide vaccines can excite B-cells without the involvement of T-helper cells, and they usually elicit an immunological response independent of T-cells. Conjugation is the chemical process that joins a polysaccharide from a bacterial surface to a protein molecule to create conjugate subunit vaccines. Immune response-stimulating vaccines have advanced from using pathogens that were inactivated or attenuated to using subunits that included pathogen components (Gote et al., 2023).

Toxoid Vaccine

Anti-exotoxin vaccines have shown to be extremely beneficial for public health, as a number of human pathogens produce them (Plotkin, 2014). Since their first administration in the 1920s, toxoid vaccines have been used and are known to produce a potent and defensive humoral immune response (Liang, 2018). The basis for the tetanus-toxoid vaccine is full-length tetanus toxin that has been formalin-inactivated. Despite their effectiveness, these vaccines come with risks related to formaldehyde use during production, complicated manufacturing processes, and pollution (Behrensdorf-Nicol et al., 2018).

Modern Vaccine Technologies

"Platform vaccine" describes vaccination strategies in which a nearly identical mechanism, gadget, delivery vector, or cell line can be readily modified and used to target new infections based only on their genetic makeup (Adalja et al., 2019).

mRNA Vaccines

The discovery of next generation vaccines includingly recombinant viral vector vaccines, virus-like particles, protein-based, and toxoid vaccines are significant turning points in the history of vaccine research. The discovery of mRNA vaccine was indicative attention towards intracellular effectiveness, quick development and authenticity specifically to COVID-19 (Gote et al., 2023). These improvements have been made through the introduction of modified nucleotides, lipid nanoparticles, capping, tailing, and effective purification techniques, as well as improved mRNA delivery and decreased immunogenicity. Furthermore, mRNA vaccines can encode for several antigens, enhancing the immune system's defense against certain hardy infections (Freyn et al., 2020). The first-generation mRNA vaccines were formerly considered lowly, but their safety, efficacy, handling, storage, and storage were all improved upon before the development of the second-generation vaccinations (Gote et al., 2023).

Viral Vector Vaccines

In the family Rhabdoviridae, VSV (vesicular stomatitis virus) and RABV (rabies virus) are enveloped NNSVs (non-segmented, negative strand RNA viruses). Five structural proteins make up Rhabdoviridae: RNA-dependent RNA

polymerase, matrix protein (M), glycoprotein (G), phosphoprotein (P), and nuclear protein (N) (Gomme et al., 2012). An RNA virus that is segmented and encapsulated, IFV is a unit of the Orthomyxoviridae that mobilized for the manufacturing of viral vector based vaccine (Ritchey, 1976). The viral NS1 protein is a virulence factor that has the ability to suppress the synthesis of interferon and allow the virus to evade the body's first defense (García et al., 1998). The creation of an IFV-vectored vaccine elaborated that removing of the NS1 gene considerably reduced viral pathogenicity (Wang et al., 2019). Adenoviridae family of viruses includes non-enveloped dsDNA viruses. AdVs categorized into multiple serotypes have a broad host range. They can manipulate the size of their genome, which spans from 26 to 45 kb (Norrby et al., 1976; Davison et al., 2003) As demonstrated by the latest rVSV-ZEBOV vaccine against Ebola, for instance, viral vector vaccinations have proven to be therapeutically effective(Goldstein et al., 2020).

Technological Advancement in Nucleic Acid Vaccines

In 1990, Wolff et al. unexpectedly found genes on the DNA recombinant expression vector, that explicit in confined muscle cells, resulting in genesis of antibodies, after injecting the vector into the mice skeletal muscle. DNA vaccines were first developed as a result of this important discovery (Nagy et al., 2021). DNA vaccines are superior to conventional vaccinations in a number of ways. DNA vaccines exhibit remarkable versatility in that they can encode several gene types, such as immunological and biological proteins, as well as viral or bacterial antigens. Furthermore, DNA vaccines may be generated in large quantities and are stable, portable, and easy to store (Niño et al., 2022). Since mRNA vaccines are more effective than standard vaccines and there is interest in nucleic acid vaccines, they have taken center stage in investigation and evolvement of the vaccine (Khan et al., 2021). Since mRNA vaccines are manufactured without using cells or eggs, they are a viable option that could close interruption among communicable diseases, growing epidemics, and pressing demand for strong vaccinations (Jin et al., 2022).

Vaccine Platforms of the Future Generation

DNA Vaccines

Developed in the 1990s, DNA vaccines can be modified easily, versatile, stable, and suitable for hoarding (Cui, 2005; Jazayeri et al., 2019). DNA plasmids, which are DNA antigens, are delivered to cells using DNA vaccines. Since mRNA and DNA are charged, hydrophilic, and polar molecules, they cannot freely traverse the cell membrane, which is a barrier for vaccines containing both of these materials must overcome (Chavda et al., 2021). The worldwide requirement for a durable and scalable vaccination against SARS-2-CoV in 2020 prompted the licensing of DNA vaccines (Sheridan et al., 2021). DNA vaccines were successfully studied using the biolistic approach, which involves introducing DNA into the cell. This technique is still being researched. Both sound and electricity have the ability to damage the plasma membrane. Particularly to aid in mucous membrane administration, certain vectors now contain cell-population-targeting motifs (Kozak and Hu, 2024).

Virus Like Protein (VLP)

The extremely repeated presentation of antigenic epitopes in VLP vaccines makes them highly immunogenic, capable of inducing strong humoral and cellular immune responses (Grgacic and Anderson, 2006; Ahmad et al., 2024). VLPs are very attractive platforms for vaccine design because they have many features that set them apart from conventional vaccines. They are the same size (20–200 nm) as the viruses that the VLPs are derived from (Bachmann, 2010). Human papillomavirus protection has been granted worldwide approval for three VLP-based vaccines: Cervarix, Gardasil-9, and Gardasil-4 (Zhai, and Tumban, 2016; Joura et al., 2015).

Recombinant Protein Vaccines

The last few decades have seen the development of recombinant protein technology, which makes it possible to produce synthetic proteins in microbe and various other expression host organisms at a reasonable cost (Puetz and Wurm, 2019). Worldwide, clinically, numerous recombinant protein vaccines are now being used (Vetter et al., 2018). There are presently several expression platforms accessible for the synthesis of recombinant proteins, inclusively microbial systems like *Escherichia coli* and other yeasts, as well as insect cells, mammalian cells, and even plants (Nigrovic and Thompson, 2007). Insect cells and yeasts often have production costs that are in the middle of those of mammalian cells, which are the most expensive, and *E. Coli*, inexpensive alternative, for protein producing (Pollet et al., 2021). Definitely, recombinant protein vaccines have advantages over mRNA and viral-vector vaccines, even though they need longer time for production and development (King, 2020).

Nanoparticle Vaccines

Modern vaccination research has shifted to emphasis on innovative adjuvants, and the application of nanoparticulated delivery systems to provide antigens typically results in vaccines that are more effective (Yasamineh et al., 2022). Nanovaccines were created through decades of research in nanotechnology. These formulations allowed for alternate routes of administration, facilitated uptake by the targeted cells, and even modulation of the immune response (Zhang et al., 2022). It's important to be careful when selecting excipients like buffers, tonicity-adjusting agents, surfactants, and other stabilizers, is necessary when formulating vaccines containing aluminum salts (Krajišnik et al., 2019). It has been

demonstrated that using MSN (mesoporous silica nanoparticles) as an immunological adjuvant in animal models can successfully improve humoral and cellular immunity (Mody et al., 2013). Natural nanocarriers that can evade the host immune response and infiltrate the host cell to transfer targeted genetic material have become an important tool in the vaccination industry (Ojha et al., 2022).

Challenges Posed by Emerging Infectious Diseases

The development of new viruses and the spread of old ones in recent decades have been attributed to a number of factors, including increased international trade and travel, population growth, anthropogenic environmental change, and interactions between people, wildlife, and domestic animals (Hassell et al., 2017). During the COVID-19, H1N1, and Ebola outbreaks that involved voluntary and enforced social separation among humans, it has been observed how individuals in social groups modify their behavior in response to EIDs (Block et al., 2020; Desclaux et al., 2017). Since parasite prevalence and intensity vary among social species, living in groups has long been believed to have an automatic and universal negative impact on the spread of parasites (Alexander, 1974; Patterson and Ruckstuhl., 2013). Pathogens may enhance adaptability in humans by strengthening the divide between in-group and out-group. Humans have a hypervigilant, especially prone to mistake pathogen-avoidance psychology in the existence of indicators signaling high disease-causing agent strain, whereby even little behavioral and physical variations from anticipated phenotype might be be interpreted as possible infection sign (Petersen., 2017; Van Leeuwen and Petersen., 2018).

Adult Immunization Challenges

A substantial portion of morbidity, disability, and mortality are caused by infectious diseases (Verma et al., 2015). Every year, a considerable segment of the adult population is admitted to hospitals as a result of VPDs. Adults who have comorbidities and risk factors like diabetes, cancer, cardiovascular and respiratory disorders, or both increase their risk of developing VPDs. This makes managing VPDs within our current healthcare system extremely difficult (de et al., 2018). Adults may only need one booster shot, but because their immune systems are deteriorating with age, older people frequently need stronger vaccinations (Rappuoli et al., 2014). Immunization reduces the intensity of disorder, lessens complications and comorbidities in elderly. According to a study, vaccination against pneumococcal disease and influenza can reduce myocardial infarction by up to 50% (Lamontagne et al., 2008). The Advisory Committee on Immunization Practices (ACIP) recommends vaccinations based on risk in addition to vaccination for healthy adults (Centers for Disease Control and Prevention, 2017; Kim et al., 2019). Physicians' ability to make clinical decisions regarding adult vaccinations will be aided by the recently revised ACIP guidelines. Additionally, doctors can take a proactive approach to increasing adult vaccination rates (Kempe et al., 2021).

Case Studies of Vaccines Developed in Response to Recent Outbreaks (e.g., Zika, Ebola)

The emergence of COVID-19 and other infectious diseases has made society more prone to unexpected public health threats (Bloom and Cadarette, 2019). Vaccines against large number pathogens that dispose a serious jeopardise to community are not available, despite their many advantages e.g. G. Zika, Marburg, Lassa (Excler et al,. 2021). A comprehensive grasp of the intricate web of interdependent stakeholders, procedures, and decision-making points involved is necessary to address these issues. Silos among stakeholders can result in fragmentation and inadequate coordination because they frequently have different objectives and resources (Bloom and Cadarette, 2019). Globally, the number of cases of the Zika virus sickness decreased starting in 2017, however the virus is still spreading at low levels in a number of American countries as well as other endemic areas. Furthermore, Zika virus epidemic activity was discovered in India in 2021, and the first instances of the disease spread locally by mosquitoes were documented in Europe in 2019. Although there has been evidence of Zika virus infection spread by mosquitoes in 89 nations and territories thus far, there is still a lack of worldwide surveillance (WHO, 2022). The Ebola (Sudan virus) epidemic in Mubende District was confirmed by Uganda's Ministry of Health in September 2022. The Sudan ebolavirus was responsible for Uganda's sixth Ebola outbreak. There were 55 fatalities and 142 confirmed cases by January 2023 (CDC, 2024).

EBOV (Ebola virus) Case Study

First reports of Ebola virus disease (EVD) were made in the Democratic Republic of the Congo (DRC) in 1976. Ebola is a highly fatal and severe illness caused by infection with viruses belonging to the Filoviridae family (Jacob et al., 2020). The virus is believed to primarily reside in fruit bats, but other animals, such as non-human primates, serve as intermediate hosts by secreting the virus and possibly contaminating humans who come into contact with them—for example, through hunting (Hussein, 2023). In addition to the many vaccines being developed against other viruses in the Filoviridae family, such as Marburg (MARV) and the Sudan species of Ebola (SUDV), there are currently two EBOV vaccines that have accomplished WHO pre-qualification (Parish et al., 2023). According to report of WHO, Globally, the number of cases of the Zika virus sickness decreased starting in 2017, however the virus is still spreading at low levels in a number of American countries as well as other endemic areas. Furthermore, Zika virus epidemic activity was discovered in India in 2021, and the first instances of the disease spread locally by mosquitoes were documented in Europe in 2019. Although there has been evidence of Zika virus infection spread by mosquitoes in 89 nations and territories thus far, there is still a lack of worldwide surveillance.

Role of Advanced Vaccine Technologies in Addressing Emerging Threats

Unfortunately, a lot of people are still at risk of dying from most infectious diseases that may be prevented with vaccinations, particularly in emerging and impoverished nations (Shaker et al., 2018). Prophylactic vaccinations typically contain one or more antigens that trigger the immune system's highly evolved reaction, allowing the body to recognize and remember a particular infection (Vetter et al., 2018). Although vaccinations based on DNA and RNA might not have the same safety issues as vaccines based on microorganisms, they nevertheless have some of their own. Although an initial investigation revealed that random chromosomal integration could happen occasionally as a result of DNA vaccination, it was found that this happened far less frequently than random genetic alterations (Wang et al., 2004).

Access to Vaccines: Global Disparities, Vaccine Nationalism, and Supply Chain Issues

When the COVID-19 pandemic hit, LDCs saw a greater percentage of severe cases than developed nations (Altindis, 2022). In order to address this issue, the WHO launched the COVID-19 Vaccines Global Access (COVAX) initiative. It is a vaccination partnership that aims to provide six billion COVID-19 vaccines to poor nations (Louden, 2022). Instead of encouraging international cooperation, the geopolitical environment has driven nations into competition with one another due to "vaccine nationalism" (Myre, 2020). This inability to unite in the face of the epidemic highlights a convoluted web of political economy issues firmly entrenched in "intellectual monopoly capitalism," presenting formidable obstacles to the realization of vaccination equity on a worldwide grand scale (Sell, 2020). We have proposed the continuous cell lines as a way to get around these challenges and come up with workable solutions that prioritize vaccination equality in the post-pandemic age without compromising IP rights (Park et al., 2023). The international community will still have difficulty reaching a consensus on vaccine fairness in multilateral trade discussions, especially in the post-COVID-19 environment. COVID-19 has highlighted the shortcomings of the WHO and has accelerated efforts to eradicate "Vaccine Nationalism" (Park et al. in 2023).

The Use of Artificial Intelligence (AI) and Machine Learning (ML) in Vaccinations

The merging of artificial intelligence (AI) and machine learning (ML) has transformed into a disruptive power in many scientific fields in current years. A notably auspicious and consequential application lies in the field of vaccine design. The combination of machine learning and artificial intelligence in vaccine design marks a significant shift in how we combat infectious diseases. These technologies enable faster identification of targets, accurate predictive modeling, efficient manufacturing processes, and personalized medicine. The influence of AI and ML can be felt in every stage of vaccine development (Fienke, 2023). The production of large immunopeptidomic datasets and an extensive immune repertoire has propelled advances in machine learning methods for immunology, which have been systematically curated and annotated in specialized databases (Vita et al., 2018). The antigen-antibody interaction surface can generally be optimized by targeted protein structural characterization efforts, which have already been made through protein-protein docking and comparative protein structure modeling (Atanasova, 2023; Muhammed., 2019). Vaccination recommendations can be made based on a patient's profile using artificial intelligence integrated into hospital information systems (Ghia et al., 2021).

Conclusion

A vaccine is typically made from weakened or destroyed microbes, one of their surface proteins, or their toxins, and it contains an agent that resembles a disease-causing germ (microorganism). Targeting new diseases has become more likely as genetic engineering has advanced since vaccines were discovered. Increasing the value of immunizations that are about to be administered can best be accomplished by estimating the potential short- and long-term costs associated with the product's availability. Many factors, such as increased international trade and travel, population growth, anthropogenic environmental change, and interactions between people, wildlife, and domestic animals, have been linked to the development of new viruses and the spread of old ones in recent decades. In recent years, the combination of machine learning (ML) and artificial intelligence (AI) has become a disruptive force in many scientific fields. Future vaccine research prospects include developments in mRNA technology, which will allow for more quick and adaptable vaccine production against new infections. Furthermore, individualized vaccines matched to individual genetic profiles may improve efficacy while reducing side effects. Improved distribution networks and global cooperation are also critical for guaranteeing fair access to vaccines, reducing health inequities, and improving pandemic preparedness.

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Chapter 59

Vaccine: The Savior in Cats

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ABSTRACT

Cats are one of the most common pets but prone to several diseases and parasites. There are solutions to keep the cat safe from these illnesses like vaccines and role of veterinarian in management of diseases. This chapter emphasizes the role of vaccine to prevent these diseases in cats. It describes the different types of vaccines like live vaccine, attenuated vaccine, and subunit ones. It discusses vaccine schedule and core and non-core vaccines for cats. The manufacturing and the material used in manufacturing process is also presented. The efficacy of different types of vaccines, duration of immunity they provide and recommendation regarding their use is also discussed.

KEYWORDS
Felis Catus, Cat diseases, Vaccines and types, Duration of immunity, Vaccines efficacy

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INTRODUCTION

The domestic cat (*Felis catus*), also called the housecat, and is a little carnivorous animal that people cherished for its hunting ability and company. Cats are the most popular pets worldwide and have been associated with humans for about 9,500 years. Their close association with humans has led to their presence almost everywhere on Earth, causing concerns as an invasive species due to their impact on native animals (Brown, 2020). Feral cat populations, reaching up to 60 million in the US alone, contribute to these issues. Cats share similarities in anatomy and size with further felids, equipped through agile figures and specialized teeth for hunting. They are skilled predators, relying on keen senses for hunting. Cats are gregarious animals even though they are solitary hunters, using various vocalizations, scents, and gestures that convey information, such purring, hissing, and meowing. Some are bred and exhibited as pedigreed pets, a hobby known as cat fancy (Robbins, 2012).

Cats can withstand high temperatures better than humans. Their discomfort typically sets in when their skin reaches about 52 °C (126 °F), but with access to water, they can endure temperatures up to 56 °C (133 °F) without significant discomfort. They stay warm by reducing blood flow to their skin and cool down by panting and vaporization through their mouth. Cats don't perspire and rarely gasp except in extreme heat. Unlike humans, their body temperature remains constant throughout the day due to their lack of circadian rhythms. Their dry feces and concentrated urine help them retain fluids, and their efficient kidneys allow them to survive on a meat-only diet and even drink seawater to replenish (Eldredge et al., 2008).

Cats can get sick with lots of different problems, like infections, bugs, injuries, and long-lasting sickness. In modern preventive veterinary medicine, vaccination is vital for controlling common infectious diseases in cats. Passive immunization involves administering preformed antibodies to animals, serving as an alternative to maternal immunity. This method is particularly useful for colostrum-deprived neonates, unvaccinated high-risk puppies and kittens, and immunocompromised animals. Active immunization, achieved through various vaccine types like modified live, inactivated, and subunit vaccines, is also crucial. Understanding the unique features of these vaccines and potential causes of vaccination failures is essential for implementing successful vaccination schedules in small animal practice (Para et al., 2022).

Vaccination is a well-established idea in preventative medicine and a significant revenue stream for the majority of veterinary clinics. Vaccines administration is a daily task for the majority of veterinary surgeons, but it has become so automatic that few ever pause to think about the science underlying this area of veterinary medicine. Both the medical and veterinary communities highlighted potential side effects of vaccination in the late 1990s.

According to international vaccination guidelines from organizations like World Small Animal Veterinary Association, Advisory Board on Cat Diseases - ABCD, The American Association of Feline Practitioners – AAFP, every feline should receive immunization against specific prevalent illnesses such as *Feline parvovirus* (FPV), *Feline calicivirus* (FCV), and *Herpesvirus-1* (FHV-1), no matter where they live (Larson Rabies vaccination is also important, especially in areas where

rabies is common (Larson and Schuitz, 2021). Additional vaccines may be recommended based on a cat's lifestyle and where they live. In Italy, core vaccinations include those against *Parvovirus*, *Feline calicivirus*, and *Herpesvirus-1*, with rabies vaccination required only for travel outside the country. Inoculation against *Feline Leukemia* (FeLV) is also advised for kittens and adult cats at heightened risk. The vaccination for feline infectious peritonitis is typically not endorsed.

Vaccinating dogs and cats has raised concerns among vets and pet owners due to worries about safety and how long the immunity lasts. New evidence suggests, we might be vaccinating too often for some diseases, and there can be rare but serious problems linked to vaccination. There are also questions about how well vaccines work and if it's necessary to use all available vaccines on every animal. Various individuals and groups are suggesting changes to current vaccination practices. The Canadian Veterinary Medical Association has recently released a strategy to address vaccine procedures, including a Public Announcement regarding Vaccinations (Carpenter et al., 2022).

Vaccine Design

Viruses, bacteria, fungus, protozoa, or helminths can all be used to make vaccines. Manufacturers produce vaccines through either attenuating ("modified live virus (MLV)") by serial passage over culture or inactivating ("killed") methods. Animals infected with attenuated products have restricted replication and humoral, cell-mediated, and mucosal immunity. Such vaccines typically penetrate maternally derived immunity at a younger age than inactivated products and prompt immunity more swiftly than the latter. Moreover, they elicit protective immunity after just one dose (provided maternal immunity has diminished). Due to being living organisms, attenuated products may become deactivated due to incorrect storage or administration. A less common yet more significant concern is the possibility that these vaccines may not be sufficiently weakened, potentially resulting in disease onset (Alamgir and Alamgir, 2018).

There are only inactivated treatments available for some diseases, such as the *Feline leukaemia virus* (FeLV), *Leptospira spp*., *Borrelia spp*., and rabies virus. However, only weakened products are available for diseases such as the measles virus, canine adenoviruses 1 and 2, feline coronavirus, canine distemper virus, and parainfluenza virus. Certain diseases, including *Feline calicivirus*, *Canine coronavirus*, *Canine parvovirus*, *Bordetella species*, *Herpesvirus*, and *Chlamydia psittaci*, can be treated with any of the two product variants (Baneth, 2020). Numerous vaccines incorporate antigens from various pathogens for convenience, yet regulatory agencies overseeing product licensing mandate that these multi-antigen products demonstrate equivalent efficacy to vaccines containing singular antigens. Nonetheless, there is evidence indicating that in certain instances, multiple antigens might disrupt the immune response to the vaccine. Conversely, some argue that using combination vaccines in cats may reduce the risk of post-vaccinal sarcomas by administering less adjuvant. These problems are yet unresolved.

Diluents include water for suspension, indicator colors, buffers and preservatives are used in vaccines. These diluents are specifically intended for each vaccine product, thus they should not be mixed with diluents from different vaccine brands. Emerging vaccine technologies strive to improve safety, incorporating "isolated" Bactrians (Without bacterial endotoxin and made up only of bacterial surface antigens) and "subunit" products, for instance a FeLV vaccine having disassembled FeLV virus derived from transformed cell lines. Recombinant DNA technology is also used, where genes encoding protective antigens are cloned and expressed in Escherichia coli to produce non-pathogenic, high antigen concentration products (Hansson et al., 2000). Yet, it remains uncertain whether exposing isolated antigens to the immune system is equally efficient in triggering protective immunity as presenting antigens within the framework of the infectious agent, a factor that may differ depending on the pathogen. Another method using recombinant DNA entails removing virulence genes from the pathogen while preserving protective antigens. Although not yet available for dogs and cats, human hepatitis B virus, swine pseudorabies, and viral vaccines have been authorized using this method. Another strategy is the "vectored" vaccination, where genes encoding protective antigens are inserted into an unrelated virus serving as a delivery system (Trovato and De Berardinis, 2015). For instance, Canada will soon have access to a canine distemper virus vaccination vectored by canary pox. Despite the fact that recombinant vaccinations are probably safe and may not require adjuvants, their effectiveness compared to traditional vaccines remains to be demonstrated (Kruth and Ellis, 1998).

The Efficacy of Vaccination

Challenge experiments have yielded minimal evidence, but vaccinations against canine distemper and pan leukopenia seem to protect most animals for an extended period of time. Nevertheless, certain weakened inoculations used previously underperformed as a result of the vaccination strains' extreme weakness. Such as, it was found that numerous canine parvovirus vaccines didn't trigger protective immunity due to excessive weakening and insufficient antigen content. This issue has been addressed with the development of parvovirus vaccines with lower passage (less weakened) and higher antigen content. Pathogens linked with chronic or latent infections are typically harder to vaccinate against compared to those causing severe infections. The nature of feline herpesvirus, feline calicivirus, and FeLV infections makes vaccination less effective compared to diseases like canine distemper and pan leukopenia. Relying solely on vaccination might not be sufficient to control these types of pathogens, and it's essential to emphasize proper animal care for those at high possibility of such infections (Larson and Schultz, 2021).

Duration of Immunity

The length of time that vaccines protect animals can vary, both depending on the disease and among individual animals vaccinated against the same disease. Sometimes, vaccine protection doesn't last for the entire life of the animal.

Because of this, manufacturers recommend getting revaccinated. Since we don't know how long most vaccines protect for, it's typically recommended to get revaccinated every year, assuming that vaccines are safe. However, there's a concern that vaccination might not always be safe for certain animals. This means that the chances of vaccine-related problems might be lower if cats were vaccinated less often. The yearly schedule for revaccination is a convenient suggestion, but it's not based on studies determining the longest time vaccines protect animals from disease. Some studies have been done to find out the shortest time a vaccine provides immunity, showing that certain vaccines protect for a minimum of a year. Veterinarians understand that the plain way dogs' and cats' immune systems work is probably not very different from humans', who don't need yearly booster shots for many diseases (Gotuzzo et al., 2013). However, the assistances of getting every year check-up along with vaccination have been thought to partly explain this practice in pets. There's now proof that vaccination can sometimes be harmful to certain animals. For instance, some cats develop leiomyosarcoma at vaccination sites, and there's a link between recent vaccination and immune-mediated anemia in dogs. Interestingly, there is now information emerging about how long some vaccines provide protection, especially rabies vaccines. Studies have shown that rabies vaccines can protect for 3 years. One way to measure immunity is by checking the levels of virus-neutralizing antibodies in the blood. For some diseases like canine distemper and pan leukopenia, these antibody levels closely match the level of protection. However, for other diseases like *Feline Herpesvirus* and *Feline Calicivirus*, where protection relies on different parts of the immune system, antibody levels may not accurately show how protected the animal is. Recently, the duration of antibody levels in the blood was studied for *Feline Parvovirus*, *Herpesvirus,* and *Feline Calicivirus*. This study recorded the continuation of antibody levels deemed protective for at least 3 years in all tested cats (Bergmann et al., 2020). Using this information, the American Association of Feline Practitioners and Academy of Feline Medicine have released guidelines suggesting that cats receive initial immunization for feline calicivirus, herpesvirus, and parvovirus, and rabies virus as directed by the manufacturer. Consequently, a booster shot should be administered after 1 year, followed by additional vaccinations every 3 years. The recommendation also says that depending on how they evaluate the risks to their patients, vets may decide to vaccinate more often. Herpesvirus, feline calicivirus, parvovirus, and rabies virus are deemed essential vaccines for entirely felines, considering the widespread prevalence and severity of these infections, or their potential to spread to humans, given the safety and effectiveness of the vaccines. While acknowledging that these recommendations align with available evidence, it's crucial to recognize that they are mostly based on a single trial with a limited number of cats (rabies vaccinations excluded). Only one product was used to vaccinate these cats, thus it might not be representative of other products on the market. Moreover, an experiment publicity test was not conducted. Undoubtedly, this data alone would not be used to approve a new product. "Non-core vaccines" (i.e., only advised for cats with established risk) include *Chlamydia psittaci*, FeLV, and feline coronavirus (Stone et al., 2020). The longest period of protection provided by these vaccines has not been disclosed, and the current suggestion from the American Association of Feline Practitioners is to administer annual booster shots with these products, if the cat is deemed to be at risk of contracting these infections. Similar recommendations have been made for dog vaccinations, wherein the canine distemper virus, adenovirus-2, parvovirus, and rabies virus are considered "essential" vaccinations. These recommendations state that the first vaccination should be administered in accordance with the manufacturer's instructions. The dog must receive a booster shot after 1 year, followed by subsequent vaccinations every 3 years. While this schedule seems biologically reasonable based on serologic evidence, there is limited published data from challenge studies to endorse this timing, except for rabies vaccinations. Parenteral parainfluenza virus vaccines have not been shown to be useful for "non-core" vaccinations (Mitchell and Brownline, 2015). Administering intranasal vaccines containing attenuated *Bordetella bronchiseptica* and parainfluenza virus is recommended for at-risk dogs. However, the duration of immunity provided by these products remains unknown and may last only for months. The *Leptospira* vaccines provide immunity for a short time (months) and don't offer protection against certain serovars like *pomona* and *grippotyphosa*, which are becoming more common. The usefulness of *Borrelia sp*., vaccines is still under debate. It's hard to confirm if canine coronavirus is a significant threat, so the need for vaccination is uncertain. Why not just check serum virus-neutralizing antibody levels and vaccinate when obligatory? As stated earlier, whereas serum levels are good indicators of protection for some diseases, they might not be accurate for others where cell-mediated immunity and mucosal immunity play vital roles. Plus, measuring titers isn't consistent across labs, so results can vary widely. To make titers useful for deciding when to vaccinate, it's important to standardize the tests or have them done by a central lab (Cross et al., 2007).

Below is a quick summary of common feline illnesses and the vaccinations that protect against them:

Feline Pan Leukopenia (FPV)

The feline pan leukopenia (FPV) is a serious, contagious disease that affects a cat's digestive system and immune system. The virus responsible, FPV, belongs to the *Parvovirus*. It spreads through contact with infected feces, including indirect contact through objects like shoes or clothing. Even indoor cats can catch it this way. The kittens are especially vulnerable. The FPV is tough and can endure a long period in the environment, so it's common in many cat populations. In one study, it was found to be the cause of death in 25% of examined kittens. It mainly targets fast-growing cells. In newborn kittens, it can be deadly, causing sudden death or neurological issues like trouble walking or seeing. Older kittens may get sick with symptoms like low white blood cell count and diarrhea. The disease is most often seen in kittens between 2 and 5 months old, while older cats usually have milder symptoms or none at all (Jakel et al., 2012).

Diagnosis

Commercially available test kits detect the presence of feline pan leukopenia virus antigen in feces. Special labs perform PCR tests on either blood or feces. The serological tests are not advised because they can't tell the difference between infection and vaccination.

Disease Management

The supportive care and attentive nursing greatly reduce mortality rates. For enteritis cases, it's advised to administer broad-spectrum antibiotics intravenously. The effective disinfectants include bleach (sodium hypochlorite), per acetic acid, formaldehyde, or sodium hydroxide.

Vaccination Recommendations

All cats, even those that stay indoors, should receive vaccinations. It's typically advised to administer two shots when they're 8-9 weeks old, followed by another shot 3-4 weeks later, and a booster one year later. For kittens from places with a lot of infections, like shelters, or born to vaccinated mothers, another shot at 16-20 weeks old is suggested. After that, boosters ought to be administered at least every three years. The pregnant cats or kittens under 4 weeks old shouldn't get vaccines with live viruses. Vaccines for FPV are typically included in standard vaccinations for cats. They work by triggering the cat's immune system to produce protective antibodies against the virus. These antibodies can be checked to see if the cat is protected (Truyen et al., 2009).

Feline Herpesvirus-1 (FHV-1)

In cats and kittens, feline herpesvirus type 1 (FHV-1) is frequently responsible for ophthalmic and upper respiratory conditions. It may also be a factor in eosinophilic dermatitis.

Hypothesis

Famciclovir (Famvir; Novartis), a systemic anti-herpes medication, is useful in the therapeutic treatment of conditions linked to FHV-1, such as keratitis, conjunctivitis, feline corneal sequestrum, rhinosinusitis, and dermatitis associated with FHV-1.

Clinical Outcome

Vaccines targeting FHV-1 are included in core vaccines for cats. Ten cats with signs of FHV-1, such as eye problems, nose and sinus issues, and skin inflammation, were treated with oral famciclovir. Cats in Australia and Europe took 62.5 mg once or twice a day, while those in the USA were given 125 mg three times a day. The treatment was well tolerated and had a positive impact on all cats. Lesions improved significantly compared to previous treatments, and one cat with severe rhinosinusitis showed marked improvement after a 4-month course. Corneal issues improved in most cats, and oral famciclovir was preferred over topical therapy for convenience. Cats with FHV-1 dermatitis also saw substantial improvement, although some experienced relapse. Overall, famciclovir proved effective in treating FHV-1 symptoms in these cases (Truyen et al., 2009).

Conclusions

Famciclovir seems like a hopeful systemic medication for addressing illnesses linked with FHV-I infection. Further thorough clinical trials are needed to refine the dosage plan for safe and efficient treatment of herpes in feline clinical practice (Malik et al., 2009).

Feline Calicivirus (FCV)

Similar to FHV-1, FCV also frequently causes respiratory infections in cats and causes symptoms including sneezing, nasal discharge, and mouth ulcers. One of the main vaccinations for cats includes an FCV vaccination as well. FCV, or feline calicivirus, is a very unpredictable virus. Recently, more severe, systemic FCV infections have been reported.

Infection

Cats, those with acute infections, or carriers release FCV in secretions from the mouth, nose, and eyes. Infection primarily happens through direct contact.

Disease Signs

The primary symptoms include mouth sores, respiratory issues, and a high body temperature. Feline calicivirus can be found in almost all cats with persistent mouth inflammation or gum inflammation. Cats with 'virulent systemic FCV disease' may exhibit varying signs such as fever, swelling of the skin, sores on the head and legs, and yellowing of the skin. The likelihood of death is high, and the condition is more serious in older cats (Radford et al., 2009).

Diagnosis

Detecting FCV can be done through virus isolation or reverse-transcriptase PCR. Viral RNA can be found in swabs from the mouth and eyes, blood, skin samples, or lung tissue using PCR. Positive PCR results should be approached carefully, as

they might indicate low-level the shedding by cats persistently infected. Diagnosing virulent systemic FCV disease depends on clinical symptoms and identifying the same strain in the blood of multiple sick cats.

Disease Management

It is crucial to provide supportive care (including fluid therapy) and excellent nursing support. Cats that refuse to eat should be given highly appetizing, pureed, or warmed food. Mucolytic medications (such as bromex) or nebulization with saline might provide relief. Administering broad-spectrum antibiotics may help prevent additional bacterial infections. *Feline Calicivirus* can endure in the surroundings for approximately 1 month and is resilient to numerous typical disinfectants.

Vaccination Recommendations

The recommendation is to administer two injections, at 9 and 12 weeks of age, followed by a first booster 1 year later. In situations where the risk is high, it is advised to administer a third vaccination at 16 weeks. Boosters should be administered every 3 years. However, cats facing high risks should receive annual vaccinations. Cats that have recuperated from caliciviral illness likely do not have lifelong protection, especially if infected with varying strains. It is still advisable to vaccinate these cats (Radford et al., 2009).

Feline Leukemia Virus (FeLV)

FeLV is a viral disease that can suppress a cat's immune system, leading to various health problems such as anemia, lymphoma, and secondary infections. Vaccination against FeLV is recommended for cats at risk of exposure, especially those who go outdoors or live in multi-cat households where FeLV-positive cats are present.

Feline Leukemia Virus (FeLV) is a common virus found in domestic cats worldwide. It's grouped into three main subgroups: FeLV-A, -B, and -C, based on their interference and neutralization patterns. FeLV-A is the most widespread, though less harmful. It's easily transmitted but not highly pathogenic. Cats infected with FeLV-A may not show symptoms for a long time, but could eventually develop diseases like lymphoma. FeLV-B and FeLV-C are less common. They develop from FeLV-A within infected cats and can lead to lymphoid malignancies or aplastic anemia, respectively. A newer subgroup, FeLV-T, has been identified, causing immune system issues in infected cats (Hofmann-Lehmann et al., 2007).

FeLV vaccines have been widely used in veterinary practices for years. While some are made from inactivated FeLV proteins, others contain recombinant FeLV surface proteins. Most vaccines include adjuvants to enhance effectiveness. A newer live virus vaccine uses a canary pox vector expressing FeLV genes. Previous studies on FeLV pathogenesis and vaccines were done before sensitive molecular diagnostic tests were available. Recent research using these tests showed that detecting FeLV DNA is more sensitive than antigen detection or virus isolation for assessing FeLV exposure. This led to identifying four categories of FeLV-host relationships after exposure. However, little was known about plasma viral RNA levels in these categories. Recent studies have begun to quantify provirus and viral RNA loads during FeLV vaccination, shedding light on the host-virus interaction. This study aims to provide insights into this interaction using sensitive molecular assays (Hofmann-Lehmann et al., 2007).

Feline Leukaemia Virus (FeLV)

Feline leukaemia virus (FeLV) is a retrovirus that can cause immune system suppression, anemia, and/or lymphoma. In the last 25 years, the prevalence of FeLV infection has been significantly decreased attributed to dependable tests for identifying carriers with the virus and to efficient vaccines (Hofmann-Lehmann and Hartmann, 2020).

Infection

Transmission among cats primarily happens through close interactions, including friendly contact and biting. In extensive populations of cats not vaccinated, approximately 30–40% will develop continuous viraemia, 30–40% experience temporary viremia, and 20–30% undergoes seroconversion. Young kittens are particularly vulnerable to FeLV infection.

Disease Signs

The primary symptoms of persistent FeLV viremia include immune suppression, anemia, and lymphoma. Less frequent symptoms include immune-related ailments, long-term inflammation of the intestines, reproductive problems, and peripheral neuropathies. The majority of cats with continuous viremia typically pass away within 2–3 years.

Diagnosis

In regions with low prevalence, there might be a chance of incorrect positive outcomes; hence, a potentially positive test outcome in a healthy cat should be verified, ideally through PCR for provirus. Asymptomatic cat’s positive for FeLV should undergo retesting.

Disease Management

Cats with FeLV need to be kept indoors and receive supportive care. Prompt treatment is needed for any secondary infections. It's important to continue vaccinating against common diseases using inactivated vaccines. The virus cannot last lengthy periods of time outside the body.

Vaccination Recommendations

All cats whose FeLV status is unknown should be tested prior to vaccination. Healthy cats which might be exposed to FeLV should be vaccinated against it. The first vaccination should be given to kittens between 8 and 9 weeks, the second at 12 weeks, and the booster one year later. Because they are less susceptible, cats older than 3–4 years old should have a booster every 2-3 years (Lutz et al., 2009).

Feline Immunodeficiency Virus (FIV)

Feline Immunodeficiency Virus (FIV) is another viral disease that weakens a cat's immune system, similar to HIV in humans. Vaccines for FIV exist, but their efficacy and necessity are still debated. FIV vaccines are not considered core vaccines and are typically only recommended in certain situations, such as for cats at high risk of exposure to the virus.

Feline the immunodeficiency virus (FIV) is a retrovirus closely linked to the human the immunodeficiency virus. While most felines can contract FIV, humans cannot. Feline the immunodeficiency virus is widespread in domestic cat populations globally. The virus rapidly loses its ability to infect outside the host and is vulnerable to all disinfectants (Hofmann-Lehmann et al., 2007).

Infection

Transmission of feline immunodeficiency virus occurs through bites. The likelihood of transmission is minimal in households with socially well-adjusted cats. Transmission from mother to kittens is possible, particularly if the queen is experiencing an acute infection. Cats with FIV remain infected persistently despite their capability to produce antibodies and activate cell-mediated immune responses.

Disease Signs

Affected cats typically show no clinical symptoms for several years, and in some cases, they never display any signs of illness, depending on the strain of the virus. The majority of clinical symptoms result from immunodeficiency and subsequent infections. Common manifestations include chronic gum inflammation, persistent nasal inflammation, swollen lymph nodes, weight loss, and immune-mediated kidney inflammation.

Diagnosis

Positive ELISA results acquired in a low-prevalence or low-risk group should consistently be verified by a laboratory. Western blot serves as the definitive laboratory test for FIV serology. PCR-based tests differ in their performance.

Disease Management

Cats should not be euthanized only because they test positive for FIV. Cats with FIV may have a similar lifespan to uninfected cats if they are properly cared for. Asymptomatic cats with FIV should be spayed or neutered to prevent fighting and the spread of the virus. Infected cats should undergo routine veterinary check-ups. They can share the same ward with other patients, but they should be kept in separate cages (Osorio et al., 1999).

Vaccination Recommendations

Currently, there is no commercially available FIV vaccine in Europe. The potential advantages and drawbacks of vaccinating FIV-infected cats should be evaluated on a case-by-case basis. Needles and surgical tools utilized on cats with FIV may spread the virus to other cats, thus stringent cleanliness is crucial (Hosie et al., 2009).

Rabies

Humans can get the viral illness rabies, which damages the central nervous system. Vaccination against rabies is essential for all cats, as it not only protects the cat but also helps prevent the spread of rabies to humans and other animals. Many regions often mandate rabies vaccination by law.

Since the late 1800s, scientists have been working to create better vaccines for rabies, a deadly disease caused by the rabies virus. Although many developed countries have controlled the disease in pets, it still poses a threat in other parts of the world (Aubert, 1992).

Three proteins in RV named N, P and L along with single stranded RNA forms Ribonucleoprotein (RNP). This RNP is covered with spike Glycoprotein and Matrix protein(M). The DNA plasmids vectors in DNA vaccines trigger the immune response due to which antibodies are produced and Cytotoxic T-Lymphocytes are activated (the same response that is produced due to live vaccines).

DNA vaccines' safety and efficacy have primarily undergone examination in mice. However, research has indicated their capability to elicit robust reactions in larger creatures such as ferrets, pigs, cattle, and non-human primates. Previous studies with DNA vaccines containing the rabies glycoprotein G proved to protect mice. In this study, we looked at how a rabies virus DNA vaccine given to dogs and cats through injections into the muscle and skin produced antibody responses. We used various delivery methods and doses of DNA to assess the results (Osorio et al., 1999).

Modern veterinary vaccines are stronger than older versions. However, because no vaccine works perfectly, and some may not work well against certain strains, vaccinated animals still need to be watched closely if they're involved in a biting

incident. This observation period is required for dogs, cats, and sometimes ferrets in some countries. Also, even if a pet is vaccinated, if there's a suspicion of rabies, the animal may still need to be euthanized, regardless of how good the vaccine is (Adedeji et al., 2010).

Bordetella Bronchiseptica

This bacterium is frequently linked with respiratory ailments in cats. Vaccination against Bordetella is generally advised for cats in settings where they face increased exposure risks, such as multi-cat households or boarding facilities. A major pathogen in domestic cats, *Bordetella bronchiseptica* is a Gram-negative bacteria that colonizes the respiratory system of animals. It is prudent to view *B. bronchiseptica* as an infrequent source of zoonotic infections. The bacterium is vulnerable to communal antiseptics.

Infection

The bacterium is released in the nasal and oral discharges of cats that are infected. Dogs suffering from respiratory illness pose a risk of infection for cats. The microorganism forms colonies on the ciliated epithelium of the host's respiratory tract, leading to chronic infections.

Disease Signs

B. bronchiseptica infection has been linked to a broad spectrum of respiratory symptoms, ranging from mild symptoms like sneezing, fever, eye discharge, coughing, and swollen lymph nodes to pneumonia with difficulty breathing, bluish discoloration, and fatalities.

Diagnosis

Bacterial culture and PCR demonstrate limited sensitivity. Samples for segregation can be collected from the oropharynx (using swabs) or through Tran's tracheal wash/bronchoalveolar lavage.

Disease Management

Even if symptoms are mild, it's important to treat bacterial infections. When we don't have sensitivity data, tetracyclines like doxycycline are recommended. Cats with severe infections need intensive care. Nasal vaccination with a modified live vaccine provides protection within 72 hours and immunity for at least a year against *B. bronchiseptica* infection. Widely using this vaccine, especially in at-risk animals, can aid in lowering *B. bronchiseptica*-induced feline upper respiratory tract illness (Williams et al., 2002).

It's important to note that vaccination recommendations may vary based on factors such as a cat's age, health status, lifestyle (indoor vs. outside), and geographic location. Therefore, it's best to consult with a veterinarian to determine the most appropriate vaccination protocol for your cat.

Conclusion

A fundamental comprehension of vaccine components and enhancers, along with the advantages and limitations of weakened and incapacitated formulations, is essential for informed vaccine decision-making. The commencement of an early protective reaction serves as guidance for the development of primary immunization regimens, and the duration of protection (based on challenge studies) remains uncertain for each pathogen or product. Finding that protection to some viruses may continue over decades but immunity to others would only survive for months (with existing vaccination technology) would not be surprising. The likelihood that a patient may have a serious disease in comparison to the possible hazards of vaccine-related consequences should be taken into consideration while making the choice to vaccinate (Kruth and Ellis, 1998).

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Chapter 60

Potential of Antioxidants-unleashing Natural Defense against Oxidative Stress in Fish

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ABSTRACT

Oxidative stress significantly impacts fish production, influencing growth, health, and meat quality. Antioxidants are vital in maintaining cellular homeostasis and protecting fish from oxidative stress damages. This literature highlights the potential of antioxidants in enhancing natural defense mechanisms against oxidative stress in fish. It examines various sources of oxidative stress, including environmental, nutritional, and physiological factors, and underscores the importance of endogenous antioxidant defense systems, consisting of enzymatic and non-enzymatic antioxidants. The potential of exogenous antioxidants from plants, algae, and microorganisms in fish nutrition is explored, along with their effects on growth, immune response, stress resistance, and flesh quality. Challenges and future perspectives related to antioxidant supplementation in fish nutrition are discussed, emphasizing the need for optimizing supplementation strategies, understanding interactions with other dietary components, monitoring potential adverse effects, and developing novel delivery systems. Future research directions include elucidating the mechanisms of action of different antioxidants, exploring synergistic or additive effects, and advancing sustainable strategies for promoting fish health and production through strategic antioxidant use in fish diets. Hence, this chapter provides valuable insights for researchers, nutritionists, and aquaculture practitioners aiming to improve fish welfare and optimize production through effective antioxidant use in fish nutrition.

KEYWORDS
Antioxidants, Natural Defense, Oxidative Stress, Fish

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INTRODUCTION

Oxidative Stress in Fish

Oxidative stress is a phenomenon that occurs when the balance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanisms is disrupted, leading to potential cellular damage (Sachdev et al., 2021). In fish, oxidative stress can be induced by various environmental, nutritional, and physiological factors, which can negatively impact their health, growth, and overall performance (Gopalraaj et al., 2024). Fish are particularly susceptible to oxidative stress due to their aquatic habitat, which exposes them to numerous stressors such as temperature fluctuations, hypoxia, and pollution (Abdikalikova et al., 2024).

ROS are highly reactive molecules that can cause oxidative damage to cellular components, including lipids, proteins, and DNA (Jomova et al., 2024). In fish, ROS are generated as by-products of normal metabolic processes, such as cellular respiration and immune responses (Hong et al., 2024). However, when ROS production exceeds the capacity of the antioxidant defense systems, oxidative stress ensues (Yang et al., 2024). This imbalance can lead to various pathological conditions, such as immunosuppression, growth retardation, and increased susceptibility to diseases (Cai et al., 2024). Studies have shown that oxidative stress in fish can be induced by a wide range of environmental factors, including temperature extremes (Islam et al., 2022), hypoxia and exposure to pollutants such as heavy metals (Turan et al., 2020) and pesticides (Yang et al., 2020).

Nutritional factors can also contribute to oxidative stress in fish. Diets deficient in essential nutrients, such as vitamins C and E, can impair the antioxidant defense systems and increase the susceptibility to oxidative damage (Xiao et al., 2024).

On the other hand, diets containing high levels of polyunsaturated fatty acids (PUFAs) can increase the risk of lipid peroxidation, as PUFAs are highly susceptible to oxidation (Islam et al., 2023). Sea bream (*Sparus aurata*) fed a diet high in PUFAs exhibited increased levels of lipid peroxidation and reduced activity of antioxidant enzymes compared to fish fed a diet with lower PUFA content (Bouraoui et al., 2023).

Physiological factors, such as growth, reproduction, and immune responses, can also contribute to oxidative stress in fish. During periods of rapid growth or reproduction, the metabolic demands increase, leading to higher ROS production. Additionally, the activation of the immune system in response to pathogens or stressors can generate ROS as part of the defense mechanism. However, if the ROS production is not adequately regulated, it can result in oxidative damage to the host tissues (Abdel-Tawwab et al., 2019).

Antioxidants are substances that can prevent or delay oxidative damage caused by ROS (Gulcin 2020). In fish, antioxidants play a crucial role in maintaining the balance between ROS production and elimination, thereby protecting the cells and tissues from oxidative stress Li et al., 2023). Antioxidants can be classified into two main categories i.e. enzymatic and non-enzymatic antioxidants (Naz et al., 2023).

The antioxidant defense systems in fish are influenced by various factors, such as species, age, tissue type, and environmental conditions. For example, marine fish generally have higher antioxidant enzyme activities compared to freshwater fish, possibly due to the higher oxygen solubility in seawater. Additionally, the antioxidant defense systems can be modulated by dietary factors, such as the intake of antioxidant nutrients (Tocher, 2003). Dietary supplementation with vitamins C and E, carotenoids, and other antioxidant compounds can enhance the antioxidant status and protect fish against oxidative stress (Gopalraaj et al., 2024).

Sources of Oxidative Stress in Fish

Oxidative stress in fish can be induced by various factors, both internal and external. These factors can disrupt the balance between ROS production and antioxidant defenses, leading to potential cellular damage and negative impacts on fish health and performance (Birnie-Gauvin et al., 2017).

Environmental Factors

Environmental factors play a significant role in inducing oxidative stress in fish. Fish are exposed to a wide range of environmental stressors in their aquatic habitats, which can influence their oxidative status. One of the major environmental factors that can cause oxidative stress in fish is temperature. Fish are ectothermic animals, meaning their body temperature is regulated by the surrounding environment. When exposed to temperature extremes, either too high or too low, fish can experience increased ROS production and oxidative damage (Menon et al., 2023).

Hypoxia, or low dissolved oxygen levels, is another environmental stressor that can induce oxidative stress in fish. During hypoxic conditions, fish may experience increased ROS production due to the disruption of mitochondrial electron transport chain and the activation of xanthine oxidase. This can lead to oxidative damage in various tissues, such as the brain, liver, and gills (Nitz et al., 2023). Exposure to hypoxia resulted in increased lipid peroxidation and altered antioxidant enzyme activities in the brain of common carp (Cyprinus carpio) (Jia et al., 2021).

Exposure to pollutants, such as heavy metals and pesticides, can also induce oxidative stress in fish. These contaminants can enter the aquatic environment through various sources, including industrial effluents, agricultural runoff, and sewage discharge. Heavy metals, such as cadmium, copper, and mercury, can accumulate in fish tissues and generate ROS through Fenton reactions or by depleting antioxidant defenses. Pesticides, such as organochlorines and organophosphates, can also induce oxidative stress in fish by disrupting the antioxidant system and increasing ROS production (Rani et al., 2022).

Nutritional Factors

Nutritional factors can significantly influence the oxidative status of fish. Imbalanced or deficient diets can lead to oxidative stress by affecting the antioxidant defense system and increasing the susceptibility to oxidative damage (Yu et al., 2022). One of the essential nutrients that play a crucial role in maintaining the oxidative balance in fish is vitamin C (ascorbic acid). Vitamin C is a potent antioxidant that can directly scavenge ROS and regenerate other antioxidants, such as vitamin E (Hamre, 2011). Fish cannot synthesize vitamin C endogenously and must obtain it from their diet. Studies have shown that dietary vitamin C deficiency can lead to increased oxidative stress and reduced growth and survival in various fish species (Kükürt and Gelen, 2024).

Vitamin E (tocopherols and tocotrienols) is another important nutrient that helps protect fish against oxidative stress. As a lipid-soluble antioxidant, vitamin E plays a crucial role in maintaining the integrity of cell membranes by preventing lipid peroxidation. Dietary vitamin E deficiency has been associated with increased oxidative damage, reduced growth, and impaired immune function in fish (Sivaramakrishnan et al., 2024).

The type and quality of dietary lipids can also influence the oxidative status of fish. Fish require essential fatty acids, particularly long-chain polyunsaturated fatty acids (LC-PUFAs), for optimal growth and health. However, LC-PUFAs are highly susceptible to oxidation due to their multiple double bonds. Dietary oxidized lipids can increase the oxidative load in fish and lead to oxidative stress, affecting growth, immune function, and flesh quality (Yan et al., 2024. Rainbow trout (*Oncorhynchus mykiss*) fed a diet containing oxidized soybean oil exhibited reduced growth, altered antioxidant enzyme activities, and increased lipid peroxidation in the liver and intestine Jiang et al. (2024).

Physiological Factors

Physiological factors, such as growth, reproduction, and immune responses, can also contribute to oxidative stress in fish. During periods of rapid growth or reproduction, fish may experience increased metabolic rates and oxygen consumption, which can lead to higher ROS production. For example, the activity of antioxidant enzymes (SOD and CAT) was found to increase in the liver of rainbow trout during the period of rapid growth, suggesting an adaptive response to increased oxidative stress (Huang et al., 2021).

Reproductive processes, such as gonadal maturation and spawning, can also influence the oxidative status of fish. During these periods, fish may experience increased oxidative stress due to the high energy demands and the production of ROS associated with steroidogenesis and gamete development (Nartea et al., 2023). The fish immune system also plays a role in inducing oxidative stress. During an immune response to pathogens or inflammatory stimuli, fish phagocytic cells (e.g., neutrophils and macrophages) produce ROS as part of their bactericidal activity. While this is an essential mechanism for defending against pathogens, excessive or prolonged ROS production can lead to oxidative damage in the surrounding tissues. The expression of antioxidant genes (SOD, CAT, and GPx) was found to increase in the head kidney of gilthead sea bream (*Sparus aurata*) following a challenge with the pathogen *Vibrio anguillarum*, suggesting a role for the antioxidant system in modulating the immune response (Montero et al., 2024).

Endogenous Antioxidant Defense Systems in Fish

Fish, like other organisms, possess endogenous antioxidant defense systems that help protect them from the deleterious effects of oxidative stress. These defense systems comprise a complex network of antioxidant enzymes and non-enzymatic antioxidants that work together to maintain cellular redox homeostasis and prevent oxidative damage to cellular components, such as lipids, proteins, and DNA (Song et al., 2023).

Enzymatic Antioxidants

Enzymatic antioxidants are proteins that catalyze reactions to neutralize reactive oxygen species (ROS) and convert them into less harmful molecules. The main enzymatic antioxidants in fish include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) (Subaramaniyam et al., 2023).

Superoxide dismutase (SOD) is the first line of defense against ROS, specifically superoxide anion (O_2). SOD catalyzes the dismutation of O_2 into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). In fish, there are three types of SOD: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD). The activity of SOD has been widely used as a biomarker of oxidative stress in fish exposed to various environmental stressors, such as temperature changes, hypoxia, and pollutants (Ahmed et al., 2023).

Catalase (CAT) is an enzyme that catalyzes the decomposition of H2O2 into water (H2O) and O2. CAT is mainly localized in the peroxisomes and plays a crucial role in protecting cells from the toxic effects of H_2O_2. In fish, CAT activity has been found to vary depending on the species, tissue type, and environmental conditions. For example, the activity of CAT in the liver of the Antarctic fish *Notothenia coriiceps* was higher than that of temperate fish species, suggesting an adaptive response to the extreme cold environment (Lattuca et al., 2023).

Glutathione peroxidase (GPx) is another important enzyme that catalyzes the reduction of H_2O_2 and organic hydroperoxides, using reduced glutathione (GSH) as a cofactor. GPx plays a crucial role in protecting cell membranes from lipid peroxidation. In fish, several isoforms of GPx have been identified, including cytosolic GPx, mitochondrial GPx, and phospholipid hydroperoxide GPx (PHGPx). The activity of GPx has been shown to be modulated by various factors, such as diet, temperature, and exposure to pollutants (Hu et al., 2024). Glutathione reductase (GR) is an enzyme that catalyzes the reduction of oxidized glutathione (GSSG) back to its reduced form (GSH), using NADPH as a cofactor. GR plays a crucial role in maintaining the cellular GSH/GSSG ratio, which is an important indicator of the cellular redox status. In fish, GR activity has been found to vary depending on the species, tissue type, and environmental conditions (Canosa and Bertucci 2023).

Non-enzymatic Antioxidants

Non-enzymatic antioxidants are low molecular weight compounds that can scavenge ROS or prevent their formation. In fish, important non-enzymatic antioxidants include glutathione (GSH), vitamin C (ascorbic acid), vitamin E (tocopherols and tocotrienols), carotenoids, and uric acid (Andrés et al., 2024).

Glutathione (GSH) is a tripeptide consisting of glutamate, cysteine, and glycine. GSH is the most abundant low molecular weight thiol in cells and plays a crucial role in maintaining cellular redox homeostasis. GSH can directly scavenge ROS, such as hydroxyl radicals (OH) and singlet oxygen ($1O_2$), and serve as a cofactor for GPx in the reduction of H_2O_2 and organic hydroperoxides. In fish, GSH levels have been shown to vary depending on the species, tissue type, and environmental conditions. Exposure to heavy metals, such as cadmium and copper, resulted in a significant decrease in GSH levels in the liver and gills of the freshwater fish *Oreochromis niloticus* (Kocalar et al., 2023).

Vitamin C (ascorbic acid) is a water-soluble antioxidant that can directly scavenge ROS, such as O_2, H_2O_2, and OH, and regenerate other antioxidants, such as vitamin E (Mahmood et al., 2023). Fish cannot synthesize vitamin C endogenously and must obtain it from their diet. Dietary vitamin C supplementation has been shown to enhance the antioxidant defense

system and protect fish against oxidative stress induced by various environmental stressors, such as high temperature, hypoxia, and pollutants (El-Sayed and Izquierdo 2022).

Vitamin E (tocopherols and tocotrienols) is a lipid-soluble antioxidant that plays a crucial role in protecting cell membranes from lipid peroxidation. Vitamin E can scavenge lipid peroxyl radicals and break the chain reaction of lipid peroxidation. In fish, dietary vitamin E supplementation has been shown to enhance the antioxidant defense system and improve the resistance to oxidative stress induced by various factors, such as high temperature, hypoxia, and pollutants (El-Sayed and Izquierdo 2022).

Carotenoids, such as astaxanthin and lycopene, are lipid-soluble pigments that possess antioxidant properties. Carotenoids can scavenge ROS, particularly singlet oxygen ($1O_2$), and protect cell membranes from lipid peroxidation. In fish, carotenoids are obtained from the diet and are known to contribute to the pigmentation of the skin and flesh. Dietary carotenoid supplementation has been shown to enhance the antioxidant defense system and improve the resistance to oxidative stress in various fish species (Taalab et al., 2022; Lim et al., 2023).

Uric acid is a water-soluble antioxidant that can scavenge ROS, such as OH and $1O_2$, and chelate transition metal ions, such as iron and copper, which can catalyze the formation of ROS. In fish, uric acid is produced as an end product of purine metabolism and has been shown to contribute to the antioxidant defense system (Nwizugbo et al., 2023).

Exogenous Antioxidants and their Potential in Fish

In addition to the endogenous antioxidant defense systems, fish can also benefit from exogenous antioxidants provided through their diet. Exogenous antioxidants are compounds that can be obtained from external sources, such as food or dietary supplements, and have the potential to enhance the antioxidant defense system and protect against oxidative stress (Monier 2020).

Natural Antioxidants

Natural antioxidants are compounds that are derived from natural sources, such as plants, algae, and microorganisms. These antioxidants have gained increasing attention in recent years due to their potential health benefits and consumer preference for natural products. In fish nutrition, several natural antioxidants have been investigated for their potential to enhance the antioxidant defense system and improve fish health and performance (Ahmadifar et al., 2021).

Plant-derived Antioxidants

Plants are rich sources of natural antioxidants, including phenolic compounds, flavonoids, carotenoids, and tocopherols. These antioxidants have been shown to possess potent free radical scavenging and metal chelating activities, making them promising candidates for use in fish nutrition. Phenolic compounds, such as phenolic acids and flavonoids, are among the most widely studied plant-derived antioxidants. These compounds have been found to exhibit strong antioxidant activities and have been shown to protect fish against oxidative stress induced by various factors, such as high temperature, hypoxia, and pollutants. Dietary supplementation with thymol, a phenolic compound found in thyme, significantly enhanced the antioxidant defense system and improved the growth performance of Nile tilapia (*Oreochromis niloticus*) under high temperature stress (Shourbela et al., 2021).

Carotenoids, such as astaxanthin and lycopene, are another class of plant-derived antioxidants that have been investigated for their potential use in fish nutrition. These compounds have been shown to possess strong singlet oxygen quenching and free radical scavenging activities. Dietary supplementation with carotenoids has been found to enhance the antioxidant defense system and improve the resistance to oxidative stress in various fish species (Meléndez-Martínez et al., 2022).

Algae-derived Antioxidants

Algae are another promising source of natural antioxidants for use in fish nutrition. Algae contain a wide range of antioxidant compounds, including carotenoids, phycobiliproteins, polyphenols, and sulfated polysaccharides (López-Pedrouso et al., 2020). These antioxidants have been shown to possess potent free radical scavenging and metal chelating activities and have been investigated for their potential to enhance the antioxidant defense system in fish. Among the algae-derived antioxidants, carotenoids, particularly astaxanthin, have received the most attention in fish nutrition.

Phycobiliproteins, such as phycocyanin and phycoerythrin, are water-soluble pigments that are found in cyanobacteria and red algae. These compounds have been shown to possess strong antioxidant and anti-inflammatory activities. Dietary supplementation with phycobiliproteins has been found to enhance the antioxidant defense system and improve the immune response in various fish species, such as rainbow trout and tilapia (Amer 2016; Vazirzadeh et al., 2020)

Microbial-derived Antioxidants

Microorganisms, such as bacteria and fungi, are also potential sources of natural antioxidants for use in fish nutrition. These antioxidants include carotenoids, enzymes, and exopolysaccharides. Microbial-derived antioxidants have been investigated for their potential to enhance the antioxidant defense system and improve fish health and performance.

Astaxanthin is not only produced by microalgae but also by several bacteria and fungi species, such as *Paracoccus carotinifaciens* and *Xanthophyllomyces dendrorhous* (formerly Phaffia rhodozyma) (Barredo et al., 2017). Microbial enzymes, such as superoxide dismutase (SOD) and catalase (CAT), have also been investigated for their potential use as antioxidant supplements in fish nutrition. (Gobi et al., 2018).

Synthetic Antioxidants

Synthetic antioxidants are chemically synthesized compounds that have been widely used in the food and feed industry to prevent lipid oxidation and extend the shelf life of products. The most commonly used synthetic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethoxyquin (EQ), and tert-butylhydroquinone (TBHQ). These antioxidants have been shown to possess strong free radical scavenging activities and have been used in fish feeds to prevent oxidative deterioration of lipids (Hossein et al., 2022).

However, the use of synthetic antioxidants in fish nutrition has been a subject of controversy due to concerns about their potential toxicity and consumer preference for natural products (Biller and Takahashi, 2018). Some studies have suggested that prolonged exposure to high levels of synthetic antioxidants may have adverse effects on fish health and performance. For example, a study by Nunes et al. (2015) demonstrated that high dietary levels of EQ (>150 mg/kg) can lead to reduced growth and feed efficiency in Atlantic salmon. There has been a growing interest in replacing synthetic antioxidants with natural alternatives in fish feeds. Natural antioxidants derived from plants, algae, and microorganisms have been shown to be effective in preventing lipid oxidation and enhancing the antioxidant defense system in fish, without the potential negative effects associated with synthetic antioxidants. However, further research is needed to optimize the use of natural antioxidants in fish nutrition and to evaluate their long-term effects on fish health and performance (Khan et al., 2017).

Antioxidant Supplementation in Fish Diets

The supplementation of antioxidants in fish diets has gained increasing attention in recent years due to their potential to enhance growth, immune response, stress resistance, and flesh quality. Antioxidants play a crucial role in maintaining cellular homeostasis and protecting against oxidative stress, which can have detrimental effects on fish health and performance (Shastak and Pelletier 2023).

Effects on Growth and Performance

Dietary supplementation with antioxidants has been shown to improve growth and performance in various fish species. The inclusion of antioxidants in fish diets can help alleviate oxidative stress, which can negatively impact feed intake, nutrient utilization, and growth. For example, astaxanthin significantly improved the growth performance and feed efficiency of juvenile large yellow croaker (*Larimichthys crocea*) (Zhu et al., 2024). The positive effects of vitamin E on growth performance have been attributed to its role in maintaining the integrity of cell membranes and protecting against oxidative damage (Rahman et al., 2023).

Effects on Immune Response

The fish immune system is highly susceptible to oxidative stress, and dietary antioxidants have been shown to play a crucial role in modulating immune responses. Antioxidants can help maintain the balance between pro-oxidants and antioxidants, which is essential for optimal immune function. Vitamin C has been shown to increase the activity of lysozyme, complement, and respiratory burst, which are important components of the fish innate immune system (Zhu et al., 2024).

Effects on Stress Resistance

Fish are exposed to various stressors in their environment, such as temperature fluctuations, hypoxia, and handling, which can lead to oxidative stress and compromised health. Dietary vitamin E supplementation has been found to improve the resistance of fish to oxidative stress induced by various factors, such as high temperature, hypoxia, and pollutants. Vitamin E supplementation significantly reduced the level of lipid peroxidation in the liver of grass carp exposed to high water temperature (Yao et al., 2024). Similarly, dietary vitamin C supplementation has been shown to enhance the resistance of common carp to oxidative stress induced by handling and transport (Labh 2024).

Effects on Flesh Quality

Flesh quality is an important aspect of fish production, as it directly affects consumer acceptance and market value. Oxidative stress can lead to the deterioration of flesh quality by promoting lipid and protein oxidation, which can result in off-flavors, discoloration, and texture changes. Dietary antioxidants have been shown to improve the flesh quality of fish by reducing oxidative damage and maintaining the stability of lipids and proteins. Dietary vitamin E supplementation has been found to improve the flesh quality of European sea bass (*Dicentrarchus labrax*) (Moroni et al., 2024). Vitamin E has been shown to reduce lipid oxidation and maintain the color and texture of fish fillets during storage (Nahavandi et al., 2024).

Challenges and Future Perspectives

While the supplementation of antioxidants in fish diets has shown promising results in enhancing growth, immune response, stress resistance, and flesh quality, there are still several challenges and knowledge gaps that need to be addressed. One of the main challenges in antioxidant supplementation is determining the optimal dietary levels and combinations of antioxidants for different fish species and life stages. The antioxidant requirements of fish can vary depending on factors such as species, age, size, physiological status, and environmental conditions. Therefore, it is essential to establish species-specific and life stage-specific recommendations for antioxidant supplementation. Moreover, the bioavailability and efficacy of different antioxidant sources can vary significantly. For instance, the bioavailability of astaxanthin from natural sources, such as microalgae and crustacean meals, has been shown to be higher than that of synthetic astaxanthin in Atlantic salmon and red sea bream. Therefore, future research should focus on identifying and characterizing novel antioxidant sources with high bioavailability and potency (Elbahnaswy and Elshopakey 2024).

Another challenge in antioxidant supplementation is understanding the interactions between antioxidants and other dietary components. Antioxidants can interact with other nutrients, such as lipids, proteins, and minerals, which can affect their bioavailability and functionality. Moreover, the presence of pro-oxidants, such as transition metals (e.g., iron and copper), in the diet can counteract the beneficial effects of antioxidants by promoting oxidative stress. Therefore, future research should aim to elucidate the complex interactions between antioxidants and other dietary components and develop strategies to optimize the antioxidant-nutrient balance in fish feeds (Tamta et al., 2024).

Potential Adverse Effects of Excessive Antioxidant Supplementation

While antioxidants are generally considered safe and beneficial, excessive supplementation can lead to potential adverse effects. High dietary levels of certain antioxidants, such as vitamin E, have been shown to impair growth performance and feed utilization in some fish species. This may be due to the pro-oxidant effects of antioxidants at high concentrations or their interference with the absorption and metabolism of other nutrients. Moreover, excessive antioxidant supplementation may also suppress the endogenous antioxidant defense system and increase the susceptibility of fish to oxidative stress. Therefore, it is crucial to establish safe upper limits for antioxidant supplementation in fish diets and monitor the potential adverse effects of long-term supplementation (Gopalraaj et al., 2024).

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Chapter 61

Current Trends in Vaccine Technologies: Innovations and Challenges

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ABSTRACT

Although vaccination is highly effective in decreasing or eliminating diseases caused by pathogens, there are still certain diseases and new infections that pose intrinsic challenges in the development of effective vaccines. Moreover, the process of creating vaccinations for persons who have weakened immune systems or pre-existing medical disorders poses substantial challenges. Emerging non-viral vaccine technologies offer fresh ways to get beyond the current barriers in vaccine production, in addition to conventional vaccinations such as live attenuated or deactivated vaccines, subunit vaccines, and viral vector vaccines. These technologies include viral-like particles and nanoparticle vaccines, DNA/RNA vaccines, and rational vaccine design. Our understanding of vaccine immunology has been considerably enhanced as a result of these breakthroughs, which have provided essential insights for the creation of vaccines against a wide range of diseases. These vaccines will be effective against a wide range of diseases, including newly developing infectious diseases such as COVID-19 and diseases that have previously shown resistance to vaccination. This chapter presents a complete overview of newly emerging non-viral technologies for vaccine production, as well as an examination of their potential uses in addressing the most critical problems in vaccine development.

KEYWORDS
Nanoparticle-based vaccinations, Non-viral DNA-RNA vaccinations, Infectious diseases, Vaccine development, Vaccinations for cancer

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INTRODUCTION

Since the late 18th century, when Edward Jenner discovered the smallpox vaccine, vaccination has been essential in preventing disease. In spite of immunizations' noteworthy successes in preventing and treating a wide range of illnesses, vaccine distribution and research still face significant challenges (Mohsen et al., 2017). Widespread and severely impactful diseases like HIV and influenza make it difficult to maintain successful immunization programs. In order to respond swiftly to outbreaks, it is necessary to hasten vaccine testing and licensing processes. The most recent worldwide COVID-19 pandemic, which was caused by the SARS-CoV-2 virus, provided direct evidence of this assertion. This pandemic resulted in a significant loss of life and had profound economic consequences. A multitude of vaccinations were expeditiously formulated and exposed to rigorous clinical studies (Dudek et al., 2006). Messenger RNA (mRNA) vaccines have demonstrated remarkable potential due to their artificial composition and ability to be manufactured with flexibility in terms of sequence (Dagan et al., 2021).

This enables the rapid and adaptable development and production of vaccinations. Given these advantages, Moderna, Inc. created and manufactured an mRNA vaccine (mRNA-1273) for SARS-CoV-2 human trials with remarkable speed and success (Condit et al., 2014). This astounding feat was completed in only 42 days after getting the target antigen's nucleotide sequence. These mRNA vaccines developed by Moderna and Pfizer/BioNTech for SARS-CoV-2 have demonstrated impressive effectiveness, with an efficacy rate of approximately 90% over a monitoring period of up to 6 months. This has been noted in the general population as well as in phase III clinical trials (Chackerian, 2007).

This is in addition to their straightforward design and manufacturing process. Both vaccines obtained extensive authorization for human utilization and started being dispensed in December 2020. Consequently, the widespread delivery

of such formulations to a significant population has significantly helped to the reduction in the incidence of COVID-19 cases and fatalities in countries which have established robust immunization strategies. The rapid and unmistakable success of mRNA vaccines has catapulted them into the spotlight, garnering attention from both the scientific community and the general public (Dhanwani et al., 2017).

Selecting the best suitable vaccination platform(s) for the development of pandemic vaccines can be difficult because each platform has its own advantages and downsides. The advantages and disadvantages of the conventional methods used in the production of vaccines, such as live attenuated and inactivated vaccinations and protein subunit vaccines, have been extensively discussed. On rare occasions, inactive immunizations may demonstrate inadequate capacity to generate an immune response or, in uncommon cases, result in exacerbated sickness symptoms (Clem, 2011). Live attenuated vaccinations pose the risk of regressing to a more virulent strain. Furthermore, it is frequently imperative to carry out clinical studies for pandemic vaccinations while an outbreak is ongoing in order to gather essential data regarding their efficacy and safety. However, this limitation narrows the pool of potential vaccines that are suitable for quick medical interventions (Barry, 2018).

Vector-borne virus platforms and non-viral vaccination methods are examples of recent developments in the field of vaccination. In order to transfer the antigen, viral vector vaccines use a virus that is not related to the specific infection. Researchers have been using a vaccinia viral vector for more than four decades in order to create the hepatitis B surface antigen (HBsAg) in order to protect chimpanzees from contracting hepatitis B (Grgacic and Anderson, 2006). This method has been successful in boosting immunity to the illness. Viral vectors have subsequently been effectively used to immunize various animal species. It is important to note that only one viral vector vaccine—rVSV-ZEBOV—has been officially approved for use in human immunization programs. Specifically, this vaccine is made to combat the Ebola virus (Zimmermann and Curtis, 2019).

Viral vectors originating from paramyxoviruses, herpesviruses, adenoviruses, flaviviruses, and retroviruses have all been used to create vaccines (Plotkin et al., 2014). The advantage of viral vectors is that they can stimulate a strong immune system response without the requirement for additional adjuvants. However, using replication-competent vectors increases the possibility that the compromised viral vector could revert to a hazardous condition (Kushnir et al., 2012). Researchers have looked into the usage of single-cycle as well as replication-deficient viral vectors as viable solutions to address this problem. In some cases, these options can still elicit a significant immune response while offering a higher degree of safety (Condit et al., 2014). There are several obstacles to effective vaccination, one of which is the wide variety of specific viruses (Bettschen et al., 2013; Townsend and Banks, 2020).

Furthermore, the efficacy and safety of vaccinations can be greatly impacted by underlying medical disorders and pre-existing immunity across vaccinated populations (Fahim et al., 2013). Furthermore, vaccinations are an unfeasible treatment due to the complexity of diseases like drug addiction and cancer as well as our poor understanding of the immune responses that offer defence against them. To optimize protection and prevent unfavorable consequences, it is important to determine the optimal balance between the cell-mediated response and antibody response for each particular ailment (Dhanwani et al., 2017). The immune response to vaccination may be influenced by a number of variables, including age, gender, genetic variations, and pre-existing medical disorders (Clem, 2011). Historically, immunizations have been administered using attenuated or inactivated vaccines. But developments in non-viral vaccine technology offer workable alternatives to get around barriers to vaccine production—especially in times of pandemics or outbreaks (Kollmann et al., 2012). This chapter explores novel approaches to creating non-viral vaccines and how they could be used to treat infectious and non-infectious diseases that are both emergent and well-established (Dagan et al., 2021).

Progress in the Development of Non-viral Vaccination Technologies

Virus-like Particle and Nanoparticle Subunit Vaccinations

Subunit vaccines are emerging as a more versatile and appealing alternative to whole-pathogen vaccinations because they can produce large amounts of specific antigens without the full virus (Dudek et al., 2006). However, subunit vaccinations often have a lower potential to induce an immunological response; hence, adjuvants and several administrations are required for maximal effectiveness. Several strategies have been implemented to address this problem, enhancing both the response of the immune system and the long-term effectiveness of subunit vaccines (Coutinho and Chapman, 2011). The production of vaccines involves the utilization of nanoparticles (NPs) and virus-like particles (VLPs). Vaccines containing virus-like particles are produced utilizing techniques that closely mimic the structure of authentic viruses. In both prokaryotic and eukaryotic systems, antigenic proteins can be synthesized and are capable of self-assembly (Mohsen et al., 2017).

By combining protein antigens with carrier molecules, chemical crosslinking is used in nanoparticle (NP) vaccines to increase immunogenicity and prevent antigen degradation (Grgacic and Anderson, 2006). These carriers can be either organic (based on lipids) or inorganic (based on metals or polymers). The capacity of protein oligomer self-assembly to generate nanoparticles for the purpose of acting as carriers and transporters has been demonstrated. Although NPs and VLPs exhibit exceptional stability, NPs' potential to trigger the innate immune response is restricted. However, when it comes to ease of use, economy, consistency, and security, NPs outperform VLPs in a few areas (Becklund et al., 2016). This is so because using NPs does not need using several protein components. In order to compensate for NP vaccines' lower potency in eliciting an immune response in comparison to VLP vaccinations, the carrier can be tailored to the specific

antigen by considering attributes including size, electrical charge, arrangement, and water-repellent qualities (Wertheimer et al., 2014). Furthermore, antigens can be more effectively transferred via antigen-presenting cells (APCs) to other cells by using carriers to precisely transport nanoparticles (NPs) to immune cells (Barry, 2018).

DNA and RNA Vaccines

Because of their exciting potential, vaccines based on nucleic acids, such DNA and RNA vaccines, have attracted a lot of attention in the field of immunization research. These vaccines have benefits like durability, favorable biosafety characteristics, cost-effectiveness, and efficient production—the latter being especially true of DNA vaccines (Kushnir et al., 2012). The potential of nucleic acid vaccines for rapidly producing immunizations against newly emerging infectious diseases has received a lot of attention. Although there aren't many DNA vaccines approved for use in animals, research in lab settings and on small animal models has demonstrated that DNA vaccines can elicit an immune response and offer protection (Kogut et a*l*., 2012). DNA vaccines have not been able to trigger an immune response to the degree that was first predicted in either human or animal model (Zimmermann and Curtis, 2019). The fact that DNA vaccines given intramuscularly mainly stimulate cell-mediated immune responses offers one explanation for this phenomenon (Bialkowski et al., 2016). Fig 1 depicts the immune response and HPV vaccine design based on vector length polymorphisms.

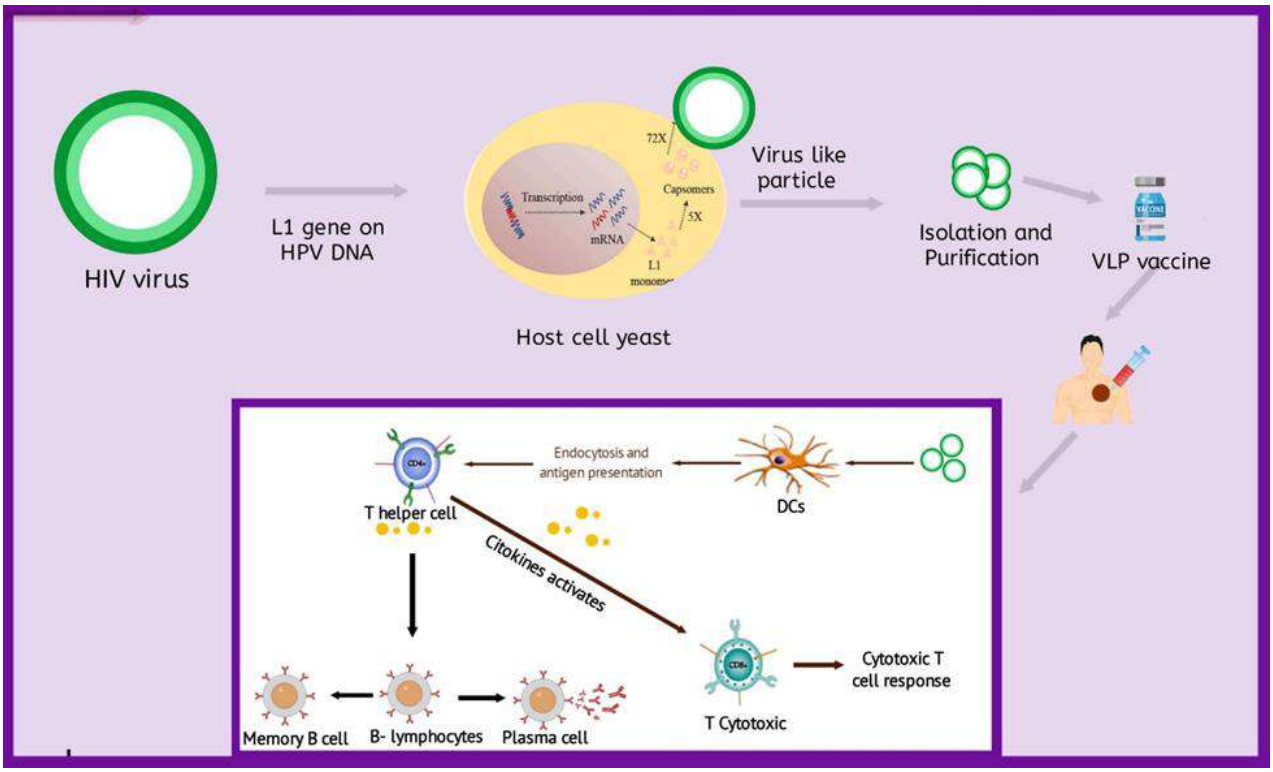

Fig. 1: The immune response and HPV vaccine design based on vector length polymorphisms.

The advancement of DNA/RNA vaccines poses numerous obstacles. While vaccinations based on microbes are generally associated with more safety issues, it is important to note that these vaccines also have their own distinct safety risks (Plotkin et al., 2014). Early studies indicated that DNA vaccines had the potential to occasionally integrate into chromosomes in a random manner (Angsantikul et al., 2017). However, further examinations demonstrated that this occurrence was much less frequent compared to spontaneous genetic alterations (Chackerian, 2007). However, a later analysis found no proof of chromosomal integration after DNA immunization, indicating that such an event did not occur (Isanaka et al., 2017). The possibility that DNA vaccination may introduce unwanted bacterial DNA components, like genes connected to antibiotic resistance, into the gut microbiota raises theoretical concerns (Kennedy et al., 2006). The World Health Organization (WHO) has categorized messenger RNA (mRNA) vaccines as a distinct therapeutic class due to their unique properties. However, there is currently insufficient empirical evidence to fully support this notion (Hobernik and Bros, 2018). To address vaccine safety issues, regulatory guidelines for clinical trials involving DNA immunization have been devised in both the United States and Europe (Li et al., 2017). The methods involved in synthesizing vaccinations using nanoparticles have been demonstrated in Fig 2.

On the other hand, immunizations based on mRNA offer benefits like the complete absence of chromosomal integration or prolonged expression (Khan, 2013). Adopting cell-free technologies for the production of vaccines has the additional benefit of reducing the likelihood of bacterial contamination throughout the process (Al-Halifa et al., 2019). However, because of the narrow scope of human studies pertaining to mRNA vaccines, there are currently no established

regulatory protocols that are expressly designed for this class of vaccines (Zhang et al., 2017).

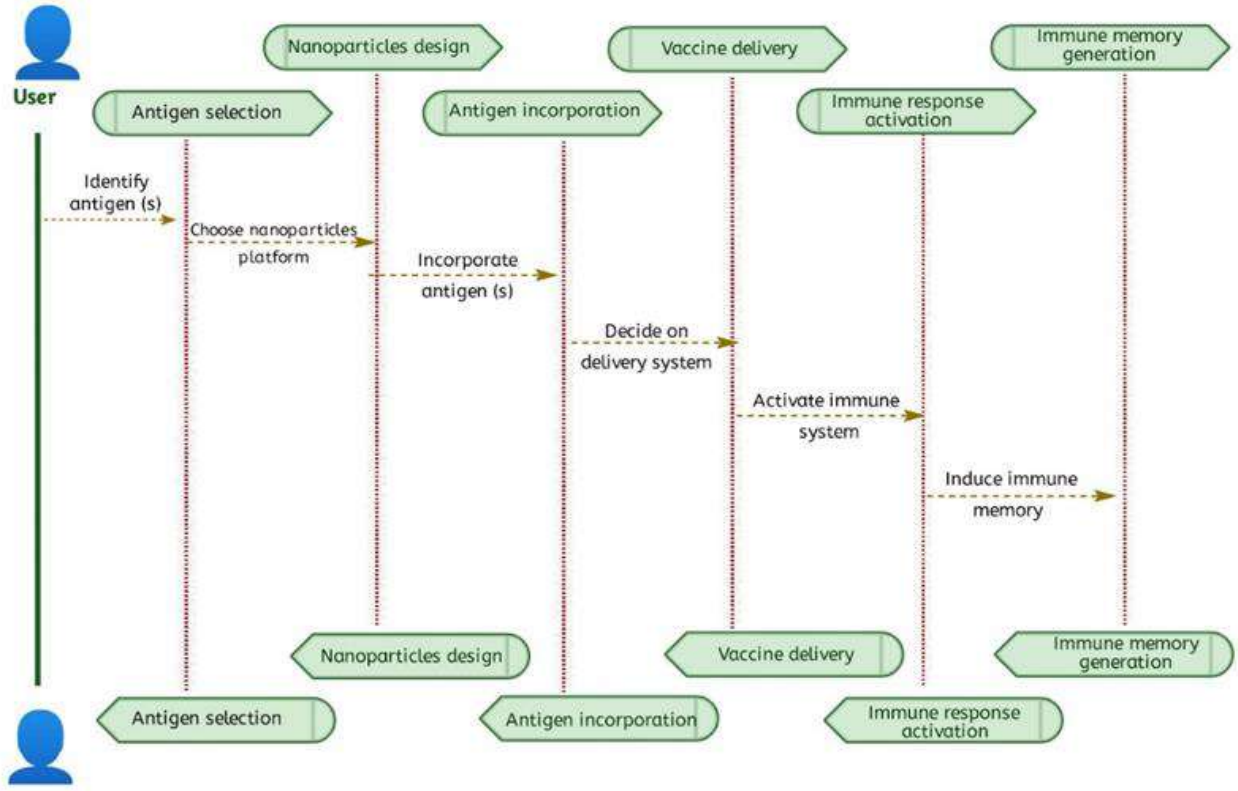

Fig. 2: The methods involved in synthesizing vaccinations using nanoparticles

Rationally Designed Vaccines

A critical first step in creating non-viral vaccines is figuring out which antigens will effectively elicit a protective immune response (Kitchin, 2011). Recent progress has brought forth novel techniques for the identification and development of antigens (Liljeroos et al., 2015). Conventional vaccinations are usually produced by attenuating or inactivating infections and using a small number of chosen antigens as vaccine constituents (Li et al., 2017). Reverse vaccinology is a method that involves sequencing the complete genome of a virus to recognize all of its antigens and assess their ability to stimulate protective antibody responses shown in Fig 3Scientists can successfully produce vaccine candidates that stimulate an immune response by combining reverse vaccinology with standard immunization procedures (Chackerian, 2007).

Progress in Non-viral Vaccine Systems

Vaccines for Immunosuppressed Individuals

Vaccination has a lower success rate among infants, the elderly, and persons with preexisting immunodeficiencies because their immune systems do not react well (Neek et al., 2019). Considering the unique immunosuppressive processes associated with each of these groups is essential for formulating the best immunization strategy (Sahin and Türeci, 2018). It is commonly assumed that young children, especially newborns and neonates, have weakened immune systems since their immune systems are still developing and becoming specialized (Rueckert and Guzmán, 2012). They are therefore more vulnerable to infections. This group has immunosuppression due to a multitude of factors. Decreased cytokine production that triggers Th17 cell responses through Toll-like receptors (TLRs) is one frequent outcome. In addition, neonates have elevated levels of anti-inflammatory cytokines, especially in preterm infants (Aurisicchio et al., 2018)

Conversely, Immunosenescence is a term used to describe the weakening of the immune system in older people. This state is distinguished by an intricate combination of alterations that results in a decline in both adaptive and innate immune responses, loss of lymphoid tissue structure, and an increase in proinflammatory cytokines and chemokines (Tagliamonte et al., 2014). Several changes have been documented, including a reduction in antigen absorption by dendritic cells, a decrease in macrophages' ability to engulf apoptotic cells, a loss in the population of naive T cells, and a decreased diversity of B cells. When it comes to diseases or drugs that lower immunity, the difficulties of immunization are different from those caused by natural ageing of the immune system (Maeng and Berzofsky, 2019). It is well-known that the medicine known as steroids promotes immunosuppression, and this fact has been the subject of much research. For example, dendritic cells can undergo a transition into tolerogenic dendritic cells when exposed to steroids. The development of regulatory T cells is greatly aided by these specific dendritic cells (Kramps and Elbers, 2017).

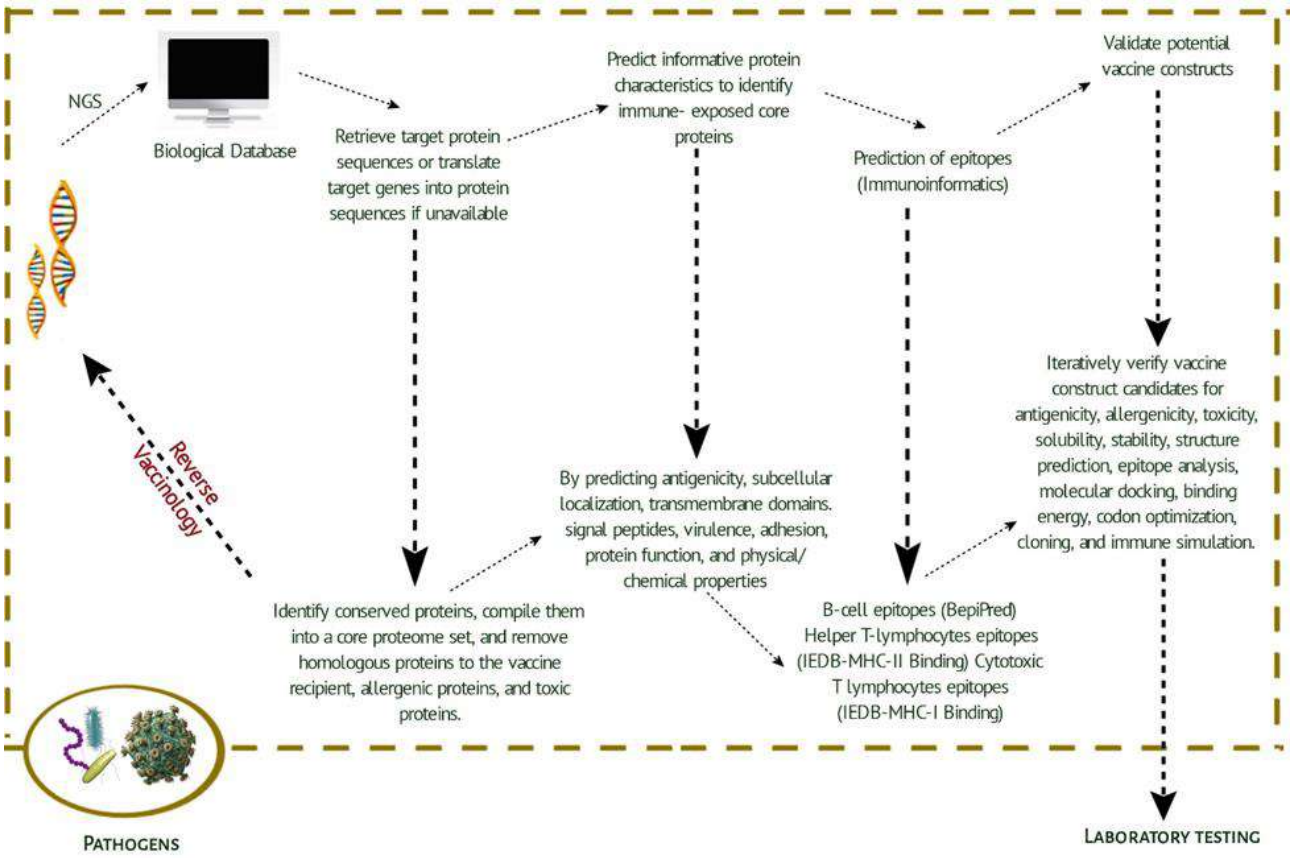

Fig. 3: The Reverse Vaccinology approach utilizes immunoinformatic, genome mining, and computer analysis to uncover and choose promising vaccine candidates

Vaccines with Non-traditional Antigens

When it comes to nonprotein antigens in particular, vaccinations made of nanoparticles (NP) or virus-like particles (VLP) provide a safer and more adaptable option. A diverse array of chemicals can be delivered as antigens by these vaccinations (Bahl et al., 2017). Vaccines utilizing NPs provide for greater design freedom by leveraging the bond between a therapeutic component and a chemical hapten carrier. Vaccines against drugs of abuse aim to trigger an immune response through antibodies that can block the effects of the drug before they enter the brain, so averting any potentially mind-altering effects. The purpose of hapten carriers, which are powerful B cell antigens, is to stimulate the immune system to react against particular drugs (Wu and Wong, 2007). Maintaining the vaccine's structural integrity and boosting B cell responses require the hapten and linker to be designed as optimally as possible (Myhr, 2017). It is also possible to augment T cell responses, especially CD4 T cell responses, which can improve B cell activity, by pairing the immunization with a protein carrier. Nevertheless, it remains uncertain whether an elevated CD4 Th2:Th1 ratio is directly associated with the efficacy of vaccinations that specifically target drugs of abuse. *(*El-Attar et al., 2009; (Boigard et al., 2017).

When designing immunizations against addictive drugs, it is crucial to determine whether the main emphasis should be on the drug itself or on its potentially more potent metabolites with psychoactive effects. This is because the latter compounds may offer a higher level of protection. Heroin immunizations are a perfect example of why this decision is extremely important. The most effective vaccinations are those that have a molecular structure similar to 6-acetylmorphine (6AM), the euphoric heroin metabolite (Pati et al., 2018). Published clinical trial results are available for vaccinations specifically designed to target nicotine and cocaine addictions (Pillay et al., 2010). Nevertheless, these vaccinations have demonstrated efficacy solely in a particular subgroup of patients who were capable of producing substantial quantities of neutralizing antibodies (Kübler et al., 2015; Lizotte et al., 2016). Additional in-depth analyses of the latest developments in immunization tactics for minimizing medication utilization can be located in alternative references (Franco et al., 2011).

Furthermore, toxoid vaccines use vaccine formulations containing VLP and NP to rapidly neutralize cytotoxic substances, like bacterial toxins, that are incapable of being produced in their whole and functional form. The DTaP inactivated subunit vaccine, which guards against pertussis, tetanus, and diphtheria, is a commonly known immunization. Preclinical research is currently being conducted to evaluate this approach, which has the potential to create effective immunizations against MRSA (Frietze et al., 2016).

Therapeutic Non-communicable Disease Vaccines

There has been a recent change in the utilization of immunizations for both the treatment of illnesses and the prevention of diseases. Improvements in vaccine research and manufacturing capabilities have accelerated the process. The development of effective immunization tactics depends on the discovery of unique protein markers connected to a particular disease phenotype. However, antigen-presenting cells (APCs) can miss these signals, which would hinder the start of an immune reaction. As a result of the difficulty in creating successful cancer treatments and the requirement for individualized treatment, cancer vaccines have garnered a significant amount of attention in the discipline of therapeutic vaccinations *(*Song et al., 2011).

The objective of these cancer vaccines is to stimulate the production of antibodies specifically targeting antigens found only on cancer cells. The development of cancer vaccines has proven to be difficult, especially when using viral vectors, due of the immunosuppressive effects of cancer. However, non-viral immunizations offer improved safety features, which bode well for the development of cancer vaccines (Dhanasooraj et al., 2016). To enhance the effectiveness of cancer vaccines, it is crucial to include an antigen that is specific to the genetic changes found in each type of cancer. This approach is particularly beneficial when used in conjunction with other treatments, as it helps overcome the body's natural resistance to immune responses (Zhou et al., 2011). Furthermore, non-viral vaccinations offer a more efficient way to encode antigens for nucleic acid vaccines or purify proteins for subunit vaccines, giving them an advantage over viral vaccinations in the creation of cancer vaccines (Garçon et al., 2007).

Vaccines against Rapidly Emerging Viral Diseases

The public health system has encountered substantial obstacles as a result of the advent of new and recurring diseases, such as the pandemic influenza virus, Ebola virus, Zika virus, dengue virus, and the ongoing global pandemic caused by SARS-CoV-2. Accelerated vaccine development and distribution are essential for effectively combating these diseases, including future outbreaks that the World Health Organization (WHO) has designated as "disease X". An ideal vaccination platform should be affordable, able to be developed quickly, and easily extensible for large-scale manufacture in order to meet global demand during a pandemic.

Furthermore, given the difficulties of preserving cold chain storage in less developed areas, the sensitivity to temperature becomes a crucial factor in the creation of vaccines. The development of heat stable vaccines is crucial in regions with poor transportation and refrigeration systems to offer efficient safeguarding against severe infections (Desai et al., 2010). This vaccine is an example of this. In view of the continual growth in the number of pandemics that are occurring all over the world, there is an imperative need to develop vaccinations that are not only cost-effective but also flexible and resistant to heat in order to combat these problems (Han et al., 2018). The present vaccine development process faces many obstacles related to resource availability and regulatory requirements. It is projected that the time and expense to produce a vaccine will range from 5 to 18 years, and it will cost between $250 million and $500 million.

Conclusion

According to modern medical research, vaccinations are the most cost-effective way to protect against infectious diseases. Thanks to immunizations, several diseases that formerly afflicted and killed tens of thousands of children and adults have been significantly reduced, and in some cases totally eradicated. In order to reduce the probability of a pandemic outbreak, vaccinations are the most efficient defense strategy. They will also be essential in combating any potential pandemic in the future. The capacity and effectiveness of traditional vaccination techniques have been significantly improved by recent developments in non-viral vaccination technology. Long-standing problems in the field of vaccination may be resolved with these innovative strategies by customizing safety, immunogenicity, protection breadth, scalability, and ease of production. Furthermore, new avenues for vaccination have been opened up by the advancement of non-viral vaccine technologies, including the use of immunizations to treat drug addiction and cancer. Additional research and vaccine development are required to address the difficulties highlighted by newly developing infectious diseases and noncommunicable illnesses. Multidisciplinary collaboration and ongoing evaluation of immunization schedules are required for this. Maintaining a leading position in scientific research will be essential to the development of vaccines for the foreseeable future.

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Chapter 63

Perception on Immune Checkpoint Inhibitor Vanquishing Standard Treatment Paradigm for Triple-negative Breast Cancer

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ABSTRACT

Triple-negative breast cancer/ TNBC has an apprehended challenge in the field of oncology because of its agonistic nature and limitation to its treatment. Some conventional treatments have efficacy to a limited extent and have a short durable return. In the context of this literal disease, the revolutionary immune checkpoint inhibitors have transformed the disease paradigm. The studies have concluded the potential of combining immunotherapy with several existing modules of treatments. The challenges regarding these algorithms are also discussed. Combining immune checkpoint inhibitors with taxanes, anthracyclines, and platinum therapy shows promise in treating Triple Negative Breast Cancer. This multi-agent approach enhances immune response, leading to improved outcomes, particularly in cases with BRCA mutations. Despite potential side effects, this comprehensive strategy offers an effective therapeutic intervention for TNBC. The amalgamation of vaccines with immune checkpoint inhibitors has ensured the enhanced activation of T cells to combat triple-negative breast cancer by incorporating DNA-based vaccines. Adjuvants are advanced strategic tools used to boost vaccine quality and functions. Certain adverse effects arise due to the suppression of T cells, referred to as immune-related adverse events which may induce immune toxicity. Progress in triple-negative breast cancer (TNBC) immunotherapy involves the exploration of predictive biomarkers, particularly focusing on TNBC subtypes like immune-modulatory (IM) and basal-like immune-activated (BLIA). Research is ongoing into TNBC subtypes for subgroup analysis. The dynamic nature of PD-L1 expression and the examination of CD274 amplification underscore the evolving landscape of TNBC immunotherapy. This article underscores the focal role of immunotherapy and ICI in comparison with standard treatment ideas. Its target is to get improved and effective outcomes for patients of Triple negative breast cancer (TNBC).

KEYWORDS
Triple-negative Breast Cancer, Treatment, Paradigm

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INTRODUCTION

Breast cancer is the second leading deadly cancer to occur in women. It is categorized into four types: hormone receptor-positive, HER2-positive, and triple-negative breast cancer (TNBC). Among them, Triple-negative breast cancer is a highly heterogeneous type of breast cancer with wide intertumoral and intratumor heterogeneity (Zhang et al., 2022). It has a high ratio of malignancy in most of the young women. From all breast neoplasms, TNBC accounts for 24% of the latest diagnoses (Rakha and Ellis 2009). TNBC is categorized by insufficient expression of Estrogen receptor (ER), progesterone receptor (PR), and amplification of antihuman epidermal growl factor receptor 2 (HER2) (Lin et al., 2019). Widespread interest in research on TNBC can be attributed to difficult prognosis along with limited therapeutic agents because hormone receptors and HER2 expression are not targetable (Wu et al., 2020). Only in the initial 5 years of disease diagnosis does a high mortality rate of 40% appear in patients (Bauer, et al., 2011; Gerratana et al., 2014). According to currently available evidence, TNBC is the term encompassing various entities with specifically unique genetics, histologic, clinical, and transcriptional changes. (Smith, et al., 2010) Breast cancer heterogeneity and genomic subtypes are revealed by looking at breast tumors from a molecular viewing approach (Sobolewski et al., 2019). The recently modified classifications of breast cancer based on the expression pattern of its gene differentiated the tumors in the breast into four

intrinsic subtypes namely: luminal androgen receptor(LAR) gene expression of estrogen receptor categorizes this, (basal-like BL1, BL2) tumor that is positive for myoepithelial and basal markers and fewer hormone receptors and HER2 gene amplification, HER2 subtype identified by HER2 gene amplification and lastly normal Breast like subtype including the phenotype of triple-negative. However, the cellular derivation is typical of normal breast epithelium (Krings and Chen, 2018). 45% of Patients with the mature stage of TNBC are with a high probability of metastasizing the TNBC to visceral organs and the brain (Claus et al., 2008). However, to high chances of malignancy, heterogeneity, drug resistance and increased risk of tumor recurrence of TNBC have contributed to convolution towards its treatment. TNBC patients will not benefit much from drugs like endocrine and HER-targeted drugs because of the absence of relative receptor markers. Many standard treatment therapies like surgeries, chemotherapy regimens, radiation therapy, combinational therapies, and conventional postoperative adjuvant chemotherapy remain incompetent due to toxicity, the resistance of some drugs, and the inability to identify molecular targets (Numan et al., 2021). Immunotherapy is, however, an emerging strategy for the treatment of TNBC. Immunotherapy uses the patient's immune system to defeat cancerous cells (Bertoni et al., 2016). Immunotherapy gives an efficient response by T- lymphocytes which provides CD8+ cytotoxic effectors. In addition to immunotherapy, a more favorable and modern phenomenon for treating TNBC is immune checkpoint inhibitors (ICI). They can relieve immunosuppression and anti-tumor effects of Tells. The microenvironment of the tumor is managed via high CD 4+ regulatory T cell levels (Diamandis et al., 2016).

In this article, we will discuss the prospects of immunotherapy with further advancements in immune response and the role of biomarkers. It is also important to highlight the challenges of immunotherapies and designing checkpoint blockages for TNBC.

TNBC Molecular Subtype Glossary

To design a specified therapeutic approach it is necessary to address molecular subtyping of TNBC with hallmarking causative factors of each of the types, specific chemotherapies for each type, their response to multiple treatments, and their complexities.

Clinical approaches and trials have shown that TNBC has molecular subtypes based on the specificity of the expression patterns. The subtypes are basal-like (BL1 and BL2), mesenchymal-stem-cell-like (MSL), immunomodulatory (IM), mesenchymal (M), and luminal androgen receptor (LAR) (Bauer et al., 2011; Li et al., 2012; Jovanović et al., 2016).

Basal-like (BL1) has greater expression of genes for proliferation signaling, DNA damage response, and cell cycle checkpoint loss. basal-like immunosuppressed (BLIS) subtype, has immunosuppressive molecule expression. BL2 has greater glycolysis/gluconeogenesis, growth factor [GF], and myoepithelial surface receptors. Thus, the BL1/2 subtype cells could be of either myoepithelial or basal origin. basal-like immune-activated (BLIA) subtype, exhibits signal transducer and transcription activators.

The M subtype has raised pathways of cell motility, extracellular matrix (ECM), cell differentiation pathways, and receptor interaction. The MSL subtype has the same expression as that of the M subtype, but also exclusively expresses the paths related to platelet-derived growth factor (PDGF), calcium (CA2+), G-protein-coupled receptor (GPCR), extracellular-related kinase (ERK 1 AND 2), GF signaling (inositol phosphate metabolism), epithelial-mesenchymal transition (EMT), and Wnt/B- catenin. There is a lowered gene expression for cell proliferation in the MSL subtype.

Both M and MSL subtypes have higher gene expression of genes in pathways of cell differentiation and growth factors.

IM subtype is engaged in the regulation of immune cells in a way by modulating cytokine signaling, T and B cell receptor signaling (immune cell signaling), antigen processing/presentation, and immune signal transduction.

Lastly, the LAR subtype has increased gene expression of pathways associated with hormone regulation, particularly with androgen receptor signaling (which could be about nine times the amount as the other TNBC subtypes) and AR antagonism (Finetti et al., 2006; Jovanović et al., 2016; Ghorayeb et al., 2022).

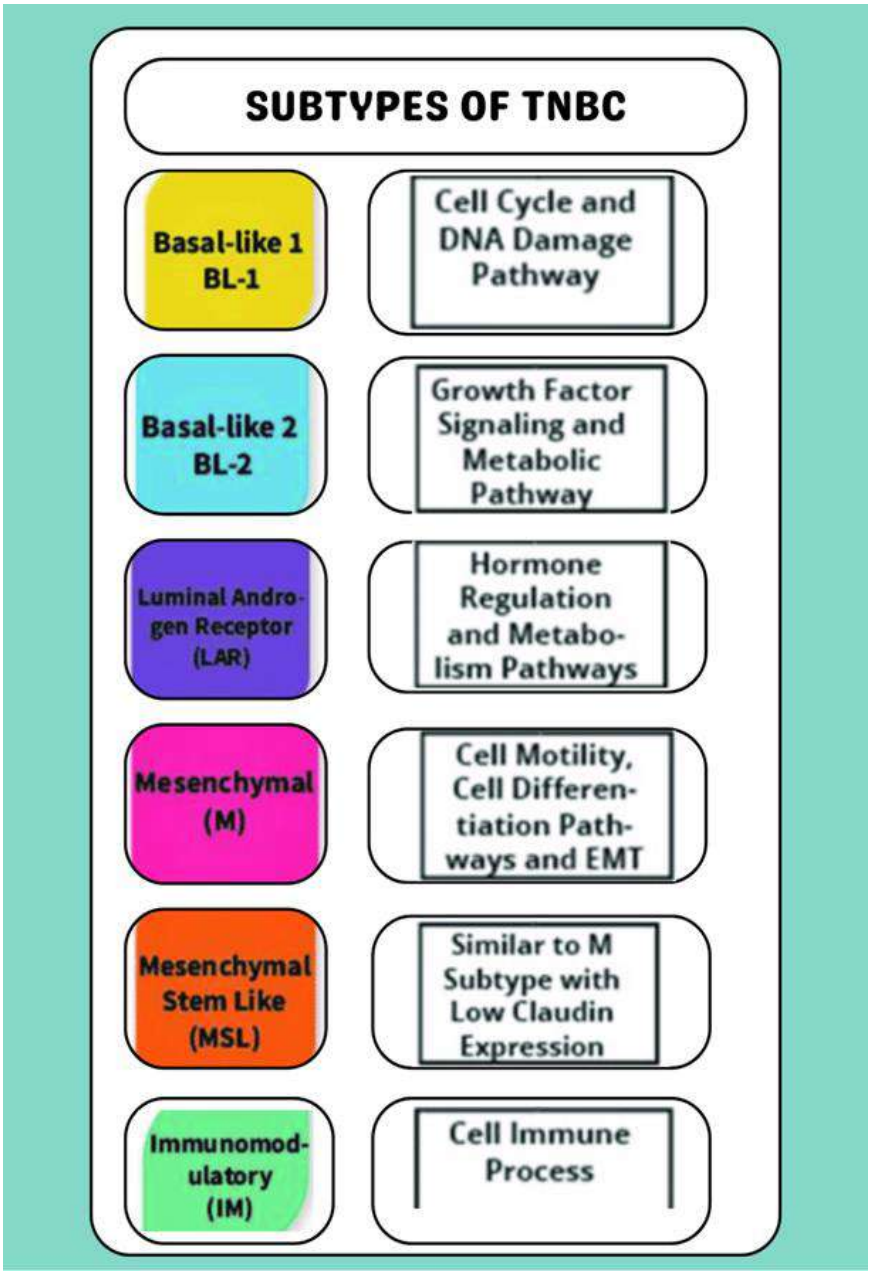
Introduction in Relevance with Immunotherapy and ICI

The tumor microenvironment consisting several components such as tumor cells, lymphocytes (B and T), NK cells, tumor-related macrophages, cytokines, chemokine, and fibroblasts interact with each other (Buisseret, et al., 2020). These interactions have the potential to change the internal environment, providing ease for developing resistance in TNBC. The tumor cells in combination with stromal cells aid metastasis, survival, and advancing tumor, drug resistance (De Angelis et al., 2022). Cancer-related fibroblasts promote tumor progression and resistance to chemokine, cytokines, etc. The cytokines secreted cause immunosuppression and proliferation of tumors (Jones et al., 2021; Wu et al., 2023). However, lipid-associated macrophages induce immunosuppression in TNBC (Gueguen et al., 2022). Macrophages activate PCAT 6 and secrete growth factor Vascular endothelial (VEGF) which enhances cancer metastasis and growth. Macrophage-enriched, neutrophil-enriched, myeloid cell subtypes have mechanisms for resistance to immunotherapy (Gao et al., 2019).

Furthermore, the failure of drug delivery, chemotherapy, and immunotherapy as treatment options is due to several reasons (Gibbons et al., 2020). The cancer tissues show serious fibrosis and extracellular matrix accumulation causing vascular compression and reduced passage of fluid. This leads to drug delivery failure (Fan and He, 2022). Altering the TME is important to versatile treatment options e.g. improving oxygen, blood flow to tumor sites/vacuolization, functional vasculature, the anti-tumor response through enhancing dendritic cells and TAM, activating immunosuppression (Nguyen et al., 2023).

The chemotherapeutic agents are far less effective against TNBC. Because cancer-associated fibroblasts and their

secretions including IL-6, IL10, and TGF- β act as a physical blockade. It inhibits the activity of CD 8+ and causes immunosuppression against cancer (Kellokumpu-Lehtinen et al., 2012). promotes extracellular matrix destruction. The TGF-β promotes CD8+ cells and destructs the extracellular matrix. Thereby, the effectuality of immunochemotherapy is increased due to immune cell infiltration and blood perfusion, hence, promoting drug delivery (Qin et al., 2022).



Gut microbiota plays a crucial role in enhancing immune checkpoint inhibitor (ICI) response by modulating innate and adaptive immunity, influencing anti-tumor immune responses. In TNBC, there is a subtype called IM, which subtype composed of more than 20% of cases and this also shows more commending prognosis and benefits from immunotherapy interventions. This subtype is characterized by the enhancement of Clostridium species also trimethylamine oxide (TMAO) in cancer cells, and TMAO also promotes anti-tumor immunity and increases CD8+ T cell infiltration (Yuan et al., 2022). Clinical trials using choline, a TMAO precursor, show promise for precision immunotherapy in TNBC. Despite the success of therapeutic strategies combining intestinal microbiome interventions with ICIs, (Zhou et al., 2021) the molecular mechanisms linking gut microbiota and their metabolites to ICI efficacy in TNBC require further exploration in future studies.

Interferons for Activation of Anti-tumor Immunity

Interferons in tumor immune cell therapy first activate CD8+ T cells by stimulating dendritic cells. However, long-term exposure has immunosuppressive effects and adverse feedback, which means that adjusting IFN responses may improve anti-tumor immunity (McGaha et al., 2017). Studies reveal that Interferon signaling in cancer cells hinders immune cell responses, while IFN signaling in innate and adaptive immune cells enhances the immune response (Johnson et al., 2019).

Type I IFNs (IFN-α, IFN-β) positively impact NK cells, promoting cytotoxicity and IFN-γ secretion, and aiding antigen-presenting cell differentiation, maturation, and migration. IFN-γ provides immunomodulatory purposes (Cardoso, et al., 2018). In TNBC, MYC overexpression inhibits the IFN signaling pathway, (Brambillasca, et al., 2022) leading to immunosuppression; targeting MYC may signal to reinstate MHC-I expression, CD8+ T cell infiltration, and enhance anti-PD-L1 responses.

CpG oligodeoxynucleotides, TLR9 agonists, stimulate IFN α and β production in plasmacytoid DCs, activating T cells and B cells, recruiting natural killer cells, and enhancing antibody-mediated (humoral) and cell-mediated immunity(cellular) (Yang et al., 2022). CpG-B, at low doses, significantly inhibits tumor growth and synergizes with PD-1 inhibitors, while CpG-C, even at higher doses, demonstrates greater efficacy in combination with anti-PD-1 suppressors (Hua et al., 2020). The difficulty in Triple-negative breast cancer treatment is successfully directing IFNs to re-adapt the immune microhabitat for rational combination treatment.

Addressing Resistance to Immunotherapy in Triple-negative Breast Cancer (TNBC)

ICI is beneficial for some Triple Negative Breast Cancer patients, but others show no betterment or develop resistance (Schoenfeld and Hellmann, 2020). The limited knowledge of resistance mechanisms hinders the development of next-gen immunotherapy.

p53 Delivery Combined with PD-1 Inhibitors

Several studies indicate that cancers with higher CD8+ T cell presence exhibit increased effectiveness in response to different PD-1 and PD-L1 inhibitors, exploring CD8+ T cells as potential prognostic and therapeutic indicators for anti-PD-1 therapy in the context of treatment (Zitvogel et al., 2019; Shrimali et al., 2019).

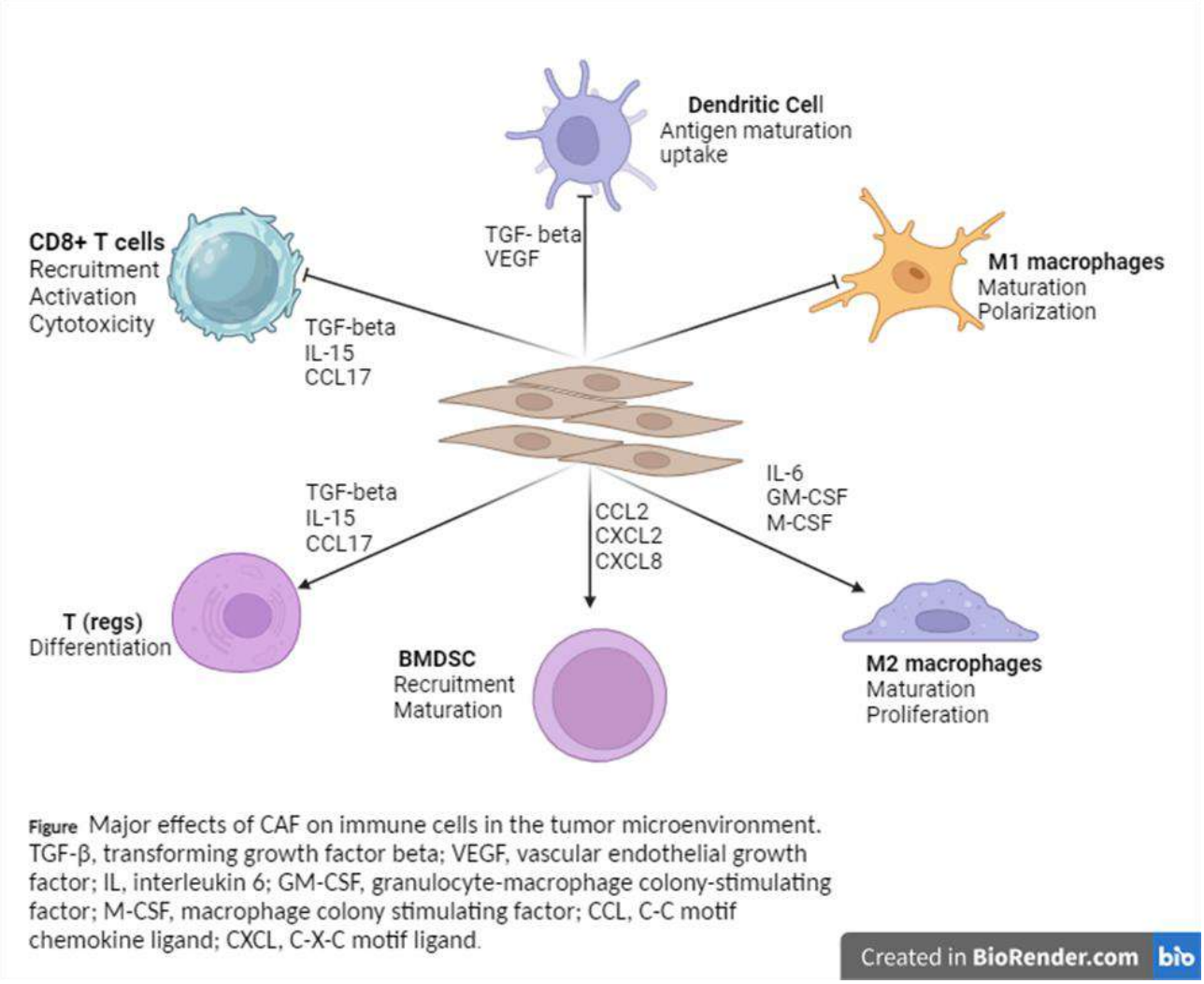


Figure Major effects of CAF on immune cells in the tumor microenvironment. TGF-β, transforming growth factor beta; VEGF, vascular endothelial growth factor; IL, interleukin 6; GM-CSF, granulocyte-macrophage colony-stimulating factor; M-CSF, macrophage colony stimulating factor; CCL, C-C motif chemokine ligand; CXCL, C-X-C motif ligand.

In advanced TNBC cases, which do not show CD8+ T cell infiltration, there has been observed resistance to inhibitors targeting PD-1/PD-L1. With a high mutation frequency of up to 80% in TNBC, the tumor suppressor protein p53 emerges as a potential biomarker (Synnott et al., 2018). Utilizing the natural Pos3Aa protein crystal in Bt bacteria as a carrier, the delivery of p53 restores its purpose in deficient tumor cells, overcoming immune escape. The combination of Pos3Aa-p53

crystals along with anti-PD-1 antibodies demonstrates safety, notably increases interferons and memory T cells, presenting a promising strategy for enhancing the efficacy of anti-PD-1 therapy in p53 mutant TNBC (Sun et al., 2022). Further studies and evidence-based clinical research are essential for future clinical applications

CXCR2 Inhibitor Combined with ICIs

TNBC, with heightened CXCR2 expression mainly in neutrophils (Jacot et al., 2020) faces drug resistance due to CSC stemness. Combining AZD5069 with doxorubicin reduces chemoresistance, and synergy with atezolizumab shows promising cytotoxic effects, emphasizing the need for clinical trials to assess potential breakthroughs in improving TNBC survival rates. This also suggests that beyond diminishing chemoresistance, CXCR2 inhibitors also improve the effectiveness of PD-L1 inhibitors (Eissa et al., 2022).

Revising Chemotherapy and Immune Checkpoint Inhibitors Combined with Chemotherapy

After analyzing the response ratio of immune checkpoint inhibitors as an independent treatment, there is needed to shift the focus to combined therapies. The motive is to induce an effective response to patients unresponsive to immunotherapies by using any additional agent that fabricates an inflamed tumor microenvironment (Emens and Middleton, 2015). Generally, chemotherapy is appealing due to its property to lessen the suppressive immune cells and upgrade response to PD-L1 by increasing the ratio of antigens affecting it (Zitvogel et al., 2017).

Patients with TNBC are usually treated with a mix of surgeries and chemotherapy since this is a very efficient way to eliminate cancer cells all over the body. The patient's condition and the tumor's stage determine the chemotherapy plan. The gold standard for treating Triple Negative Breast Cancer is systemic chemotherapy, which employs medications like taxanes (like paclitaxel) and anthracyclines (like doxorubicin) in the adjuvant or neoadjuvant (NACT) settings (Furlanetto and Loibl, 2020). To render inoperable tumors, the first NACT was carried out on patients with locally progressed breast tumors in the 1980s (Calabuig-Fariñas et al., 2021). Chemotherapy is considered to be the primary therapy for TNBC; however, tumor size should determine how it is administered, according to research. TNBCs that lack involvement of lymph nodes and have tumor diameters between 0.5 and 1.0 cm have decent future outcomes. (Gupta et al., 2020). Another team of researchers believed that there was no discernible benefit to treatment for TNBC tumors less than 1 cm in size (Vaz-Luis et al., 2014) Small tumors can attain full pathological cure with treatment alone. Durvalumab was found to have a greater pCR rate in those people with tumors grade IIA and above than placebo. This was shown in a phase 2 study that was randomized, double-blind, and inactive drug-controlled and examined the pCR rate of NACT in primary non-metastatic TNBC patients. The experiment included nab-paclitaxel accompanied by dose-dense epirubicin/cyclophosphamide with durvalumab against placebo (Loibl et al., 2019).

However, metastatic triple-negative breast cancer is treated by chemotherapy. The response ratio using only a single agent is less (10 to 30%) as compared to combined multi-agents (63 %) (Wang et al., 2015). The statistical analysis of early-stage TNBC patients showed the escalation in pathologic response rate induced by taxanes and anthracyclines. The immune checkpoint inhibitor in junction with chemotherapy is highly responsive and has less poor effects (Peng et al., 2021).

Taxanes and anthracyclines are often included in the conventional chemotherapy treatment for TNBC patients (Collier et al., 2020)Anthracyclines treat TNBC by destabilising DNA by insertion, which results in repaired DNA cascade degeneration (Yadav, Sharma et al., 2014) According to research, anthracycline effectively destroys cancerous tissues and stimulates the body's defence system by boosting CD8+ T cells (Katz and Alsharedi, 2017) Anthracyclines, an chemotherapeutic agent cause immunogenic cell death. As a result, the dendritic cells causing the multiplication of CD 8+ T cells are activated (Buqué, et al., 2017).

Anthracyclines, such as doxorubicin and epirubicin, have been demonstrated to improve cure rates and lifespan for longer periods. These medicines have shown advantages and increased responsiveness when administered either alone or as a neoadjuvant. The most common treatments for patients with ferine BRCA1/2 who have not had these medications administered in neoadjuvant or adjuvant contexts are taxane- or anthracycline-based treatments. Given the evidence that individuals may respond to anthracycline therapy, a lot of doctors refuse to retest their patients because of the accumulated cardiac damage they have seen (Sendi, et al., 2021)

Furthermore, the retaliation rate for anthracycline-based treatment is greater. However, its usage is limited due to its association with greater risks of reappearance and a low average survival rate. Short-term toxicity from the medication can also result in alopecia, permanent cardiopathy, myelosuppression, and nausea (Ghosh et al., 2021)

Taxanes are an important family of chemotherapy medications due to their distinct mode of activity and several uses in cancer treatment. Taxanes' primary molecular functions include suppression of new blood vessels, mitotic slippage, and rupture of the mitotic apparatus (Ilari et al., 2021) Taxanes, a class of chemotherapy, increase immune cells TIL (tumor-infiltrating lymphocyte), decrease T regulatory (Treg) and MDSC (Myeloid-derived suppressor cells). The Treg and MDSC suppress the immune response to tumors (Volm et al., 2001).In contrast with other subtypes of breast tumors, TNBC has proof of benefiting from either taxanes independently or in combination with anthracyclines, with pCR rates of no less than 40%. For therapeutic usage, three taxanes—paclitaxel, docetaxel, and cabazitaxel—have been authorized. among the oldest and most successful chemotherapy drugs for treating cancer is docetaxel, which was licensed in the 1980s. However, because of its negative impact on the body's natural microbiota, docetaxel may also contribute to resistant bacteria (Catalano et al., 2022).

Paclitaxel is one of the most well-liked chemotherapeutic drugs due to its selective, flexible, and saturable bonding characteristics to molecules and small tubules. Pneumothorax inhibits mitosis, which results in apoptosis in tumor tissues. It is an effective chemotherapeutic agent for several malignancies, including ovarian, and breast tumors (Blanchard, Paul et al., 2015). Treatments for many different cancers have all been shown to benefit from paclitaxel's antitumor properties (Reddy and Bazile, 2014). A combination of therapies is necessary for successfully treating Triple Negative Breast Cancer since PTX's cell death can have drawbacks, such as the cell's use-dependent tolerance and toxicity to normal cells (Cho, et al., 2020). Nab-paclitaxel, also known as albumin-bound paclitaxel, was created to enhance the medication's safety aspect.

Cyclophosphamide, being another chemotherapeutic drug causes suppression of T regulatory cells, boasts division of CD8+ T cells, and causes immunogenic death of cells. However, platinum therapy does the same but it additionally enhances the presentation of major histocompatibility complex Class 1 on tumor cells. This is for the downregulation of MDSC and activating T cells (Majewski et al., 2012).

Platinum agents have well-established effects on breaks in DNA and cell death. Because of their distinct mode of action, they are especially useful against cancerous cells that lack functional DNA repair systems, such as those harboring harmful BRCA mutations (Tovey et al., 2018). TNBC is typified by abnormalities in DNA repair. Since it is generally known that anthracyclines, platinum, and cyclophosphamide directly cause the degradation of DNA, these treatments have received a lot of attention in the treatment of malignant TNBC, particularly in patients with germline mutations of BRCA. The possibility that BRCA1/2 germline mutations are linked to susceptibility to platinum treatment has been the subject of several inquiries. Better results and higher susceptibility to chemotherapy are linked to gBRCAm and BRCAness statuses (Tutt et al., 2018) Tumours that have characteristics with BRCA-mutated tumors but do not contain hereditary mutations in BRCA1/2 are referred to as "BRCAness" (Mehanna et al., 2019). Individuals with homologous recombination abnormalities and BRCA1/2 mutant TNBC have demonstrated noteworthy advantages from platinum-based treatment plans (Sendi et al., 2021).

Combination of Immune Checkpoint Inhibitors with Vaccines

Breast cancer manifests numerous tumor-associated antigens, notably HER2 and mucin 1 (MUC1), serving as focal points in vaccine development (Cruz et al., 2019).

Preliminary vaccine trials demonstrated safe administration and elicited antigen-specific rise of immune responses, but the quality of clinical protocols seems appropriate. A significant constraint may arise from targeting common tumor antigens, suggesting a potentially more efficacious strategy of formulating vaccines incorporating mutation-specific antigens exclusive to the tumor. Furthermore, synergizing vaccines with checkpoint inhibitors holds promise in enhancing efficacy through heightened T-cell activation and mitigation of immunosuppressive pathways. a new approach to administrating DNA-based neoantigen vaccine has been introduced which involves gemcitabine and carboplatin along with nabpaclitaxel and durvalumab.

Immunizations have been instrumental in preserving millions of lives and safeguarding many others from illness. Throughout history, the efficacy of immunization practices in preventing infectious diseases has been evident. In ancient times, the utilization of these practices was based on empirical knowledge, as the protective mechanisms induced by early formulations were not fully understood, leading to occasional failures. (Jakubowska et al., 2015)

Adjuvants have been used as an effective strategic tool to obtain target-specific vaccines. These substances when incorporated into vaccines expedite the immunological activities by enhancing the functional efficacy and quality of vaccines. The therapeutic strategy of using peptide vaccines to target metastatic cancer seems to have low acceptance, so this challenge is sorted by using multi-peptide vaccines with a response rate of 9.9%. (Sukumar et al., 2015)

The amalgamation of vaccines with immune checkpoint inhibitors has proved to be a significant factor for the heightened immune response against tumor growth. The execution of clinical trials which are focused on determining the effectiveness of cancer vaccine and PD-L1 antibody combination is approved. A few trials are subjected to TNBC to unveil the efficacy results induced by the amalgamation of vaccines and pembrolizumab. incorporated vaccines are multi peptides and those that target p53 or WT1. In addition, the research trial involving the combination of dervalumab and multi-peptide (PVX-410)or neo-antigen vaccine (fused with atezolizumab) is under process.

The implementation of tumor vaccines in therapeutic measures for cancer prevention has assured the boosted immunological empowerment of the host. This advanced approach to tumor vaccines is much more efficient than the conventional therapeutic approaches used to combat cancer. as it offers high specificity and fewer side effects (DeCristo et al., 2017).

α-lactalbumin is a protein present in most TNBCs, although absent in normal and aging tissues post-lactation. The expression of this surplus protein is retarded with the activation of the innate immune system against it. It is done by amalgamation of vaccines with adjuvants that mediate targeted immune response and stop the advancement of tumors.

Immunotherapy Mediated Toxicity

Immune checkpoint proteins like PD-L1 play a role in inhibiting T-cell function. The use of immunotherapeutic techniques has successively reduced the suppression in T cell functionality, regulated by tumors. Such immune activations can mediate many associated toxicities as they are not solely targeted toward tumor sites in treated patients. Prevailed

toxicity raised by immunotherapy has caused inflammatory conditions in various organs of the patients. The significant contribution to obstructing the inhibitory pathways that terminate the immune response against the tumors is a notable function of immune checkpoint inhibitors. The disruption of inherent regulators for immunity is exhibited by inhibitors employed for the sake of anti-tumor effects (Zhou et al., 2019).

In regard to an analytical model, adverse events mediated by anti-tumor immune response posed genotoxicity in approximately 66% of the patients who were given treatments by PD-L1 therapy.

Immune-related adverse events are mediated by the activation of T cells. This activation notably produces the inflammatory cytokines along with autoantibodies generated by B cells. The genotoxicity stimulated in different organs of patients largely varies depending upon the type of therapeutic approaches employed. For example, anti-CTLA-4 therapy gives rise to colitis, and hypophysitis and anti-PD-1 therapy mediates pneumonitis and thyroiditis.

The cross-reaction build-up by tumoral antigenicity stimulates immune-related adverse events. Both normal and cancerous cells contain the T-cell antigens, which is proved by a study on treating non-small lung cancer using PD-1 therapy that mediated dermatologic toxicity that pinpointed the T-cell's presence in skin tumor cells. (Murakami et al., 2020).

Immune-related adverse events (IRAEs) tend to be more prevalent when immune checkpoint inhibitors (ICIs) are used in conjunction with cytotoxic chemotherapy. Despite this, the overall rates of IRAEs remain comparable between patients receiving combination therapy and those undergoing ICI monotherapy. Breast cancer patients when subjected to ICI and nabpaclitaxel are more prone to pneumonitis than the case of monotherapy (Fernandes et al., 2019).

Types of IRAEs

Acute IRAEs are those events that occur amid immune checkpoint inhibitor treatments, while the events manifested after completion of treatments are delayed IRAEs. Chronic IRAEs are supposedly mediated 12 weeks apart from the termination of ICI therapy. Examples of IRAEs with a high chance of chronicity are neurotoxicity, arthritis, and xerostomia.

The reasons behind varying susceptibility to immune-related adverse events (IRAEs) among individuals remain unclear, but it has been suggested that genetic factors could play a role.

Patients with autoimmune disorders are more vulnerable to immune-related adverse events. A study revealed that inheritance of autoimmune disorder led to rheumatic IRAEs in 10% of patients. The composition of microbiota and genetic changes may add to the risk of acquiring IRAEs. Nevertheless, the precise contribution of genetics to the susceptibility of developing IRAEs remains inadequately understood (Virassamy et al., 2018).

Therapy Type	Combination Treatments	Description
Chemotherapy	Anthracyclines, Taxanes, Albumin-PTX, Doxorubicin, Cisplatin, Eribulin	These chemotherapy drugs are combined with ICIs to enhance clinical responses in early-stage and metastatic TNBC.
Radiotherapy	Low-dose RT + Nivolumab RT + Pembrolizumab	These two combinations enhances anti-tumor efficacy in TNBC.
Target Therapy	Olaparib + Durvalumab	Combining Olaparib, a PARP inhibitor, with Durvalumab (an anti-PD-L1 therapy) shows favorable outcomes in TNBC.
Combination 1	Ipatasertib/Cobimetinib + Atezolizumab + Paclitaxel/Nab-Paclitaxel	Combining these drugs and ICIs with chemotherapy drugs like Paclitaxel enhances TNBC management.
Combination 2	Entinostat + Atezolizumab	This combination of a histone deacetylase inhibitor (Entinostat) and anti-PD-L1 therapy shows efficacy in TNBC.

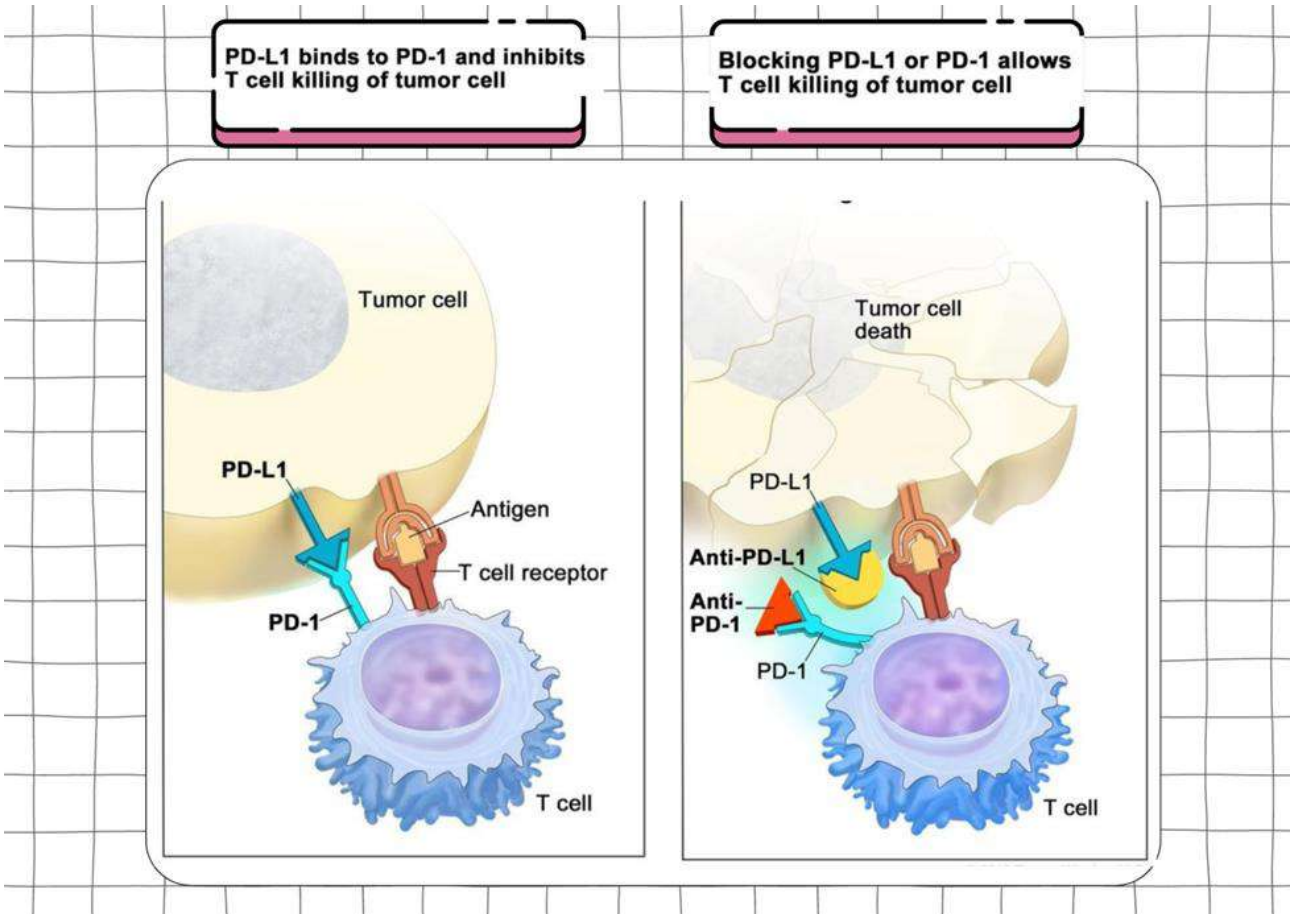
ICIs may lead to endocrine toxicities encompassing conditions such as hypothyroidism, hyperthyroidism, thyroiditis, and insulin-dependent diabetes mellitus. The specificity of the agent and type of endocrinopathy influence the period of endocrine IRAEs. Hormone replacement therapy is permanently employed to target permanent endocrine toxicity. Other IRAEs can be treated and resolved. (Shah et al., 2018)

Immune checkpoint blockade (ICB) may lead to toxicities as a result of an exaggerated immune response against normal tissues. In severe cases, oral corticoids, high-dose steroids, and additional immunosuppressants are used for suppression of the immune system to manipulate IRAEs. For cases refractory to steroids, such as severe IRAEs unresponsive within 48–72 hours, additional immunosuppressants or plasmapheresis, guided by a disease-specific specialist, can be initiated.

Combination 3	Ladiratuzumab Vedotin + Pembrolizumab	A combination that improves TNBC treatment outcomes by pairing an antibody-drug conjugate with Pembrolizumab.
Combination 4	Trastuzumab Deruxtecan + Durvalumab	Combining these drugs, which target HER2-positive cells, with Durvalumab offers promising results in TNBC

Introduction to Biomarkers for immunotherapy in TNBC

Over time, progress in cancer immunotherapy has made the prognosis of many patients with a range of malignancies much easier. Treatment and its response are currently under observation. Predictive biomarkers are being researched to increase ICI efficiency. This is important to reduce toxic side effects and financial strain caused by the treatments (De Angelis et al., 2022). To develop the predictive biomarkers, TNBC heterogeneity is taken into consideration.



Some TNBC subtypes are better candidates for strategies of immunotherapy as demonstrated by using predictive markers. It has been shown that PD-1/PD-L1 ICI was not predictive for response when it was used as a neoadjuvant treatment in early TNBC. However, the response was predicted when it was used in combination with chemotherapy in the patients having metastatic TNBC (Cortes et al., 2020) PD-L1 expression was also affected by the tumor microenvironment

as it changes with regards to the stage of disease and organs associated. Approved agnostic biomarkers include Microsatellite instability (MSI) and high tumor mutational burden (TMB-H). These are used in association with ICI to treat solid tumors (Prasad and Addeo, 2020). TMB is widely known as a surrogate for the neoantigen and T cell activation biomarker (Litchfield et al., 2017).

But TMB-H and MSI are both uncommon markers in TNBC, therefore few TNBC patients are considered suitable for ICI therapy. Studies that covered past responses and future potential were evaluated comprising of patients who were treated with ICIs in combination with chemotherapy or in monotherapy. Among the subtypes, the IM and BLIA are significant due to increased immune gene expression and immune checkpoints that are potential targets so they are linked to improved prognosis (Venet et al., 2018)

There are only two available biomarkers for customized therapy in TNBC including PD-L1 IHC staining for immunotherapy and germline BRCA1/2 mutations for PARP inhibitors. Current studies are looking into TNBC subtypes as biomarkers for subgroup analysis (Liu et al., 2021). Other limitations include the absence of a consensus between different classification systems and that no one has been validated in a metastatic setting. PD-L1 IHC was related to a greater response rate to ICI monotherapy in phase 1-2 trials in metastatic TNBC. Patients with PD-L1-positive tumors were included in these studies (Loi et al., 2019). The phase 3 IMpassion 130 and KEYNOTE 355 trials evaluated a combination of an anti-PD-L1 ICI and chemotherapy as the forefront option of therapy. This depicted the predictive value of PD-L1 IHC and its relation to better outcomes in metastatic TNBC (Molinero et al., 2021).

PD-L1 is not considered the best biomarker. Response to ICI treatment is also noticed in patients with tumors that are negative for PD-L1 and all patients with PD-L1 tumors that are positive derive benefit from immunotherapy. This difference in result can be due to tumor heterogeneity or inconsistency in PD-L1 assessment methods (Zerdes, et al., 2021). Another reason for different results can be attributed to the dynamic expression of PD-L1 as depicted by the change in PD-L1 status after neoadjuvant chemotherapy (Bhalla, et al., 2021).

The non-uniformity in the PD-L1 predictive value between early and metastatic settings can also be attributed to the ability of cancer cells to perform immunoediting processes when evolving from a primary tumor to a metastatic lesion and the ongoing development of TME more conducive to immune invasion. In that case, the combination of a greater immunosuppressive TME and the appearance of less immunogenic tumor in the metastatic lesions might make ICI response prediction more dependent on biomarkers such as PD-L1 expression (Thompson et al., 2016). Hence, the PD-L1 biomarker has numerous flaws to overcome after testing. It should be limited to metastatic TNBC for now.

The distribution of CD274 amplification in the context of the 4-type molecular TNBC classification was investigated. They found a generally low prevalence with the M subtype showing the highest incidence and the BL2 subtype following a little less. Notably, M tumors exhibited increased methylation in the CD274 gene promoter, leading to reduced expression of PD-L1 on the cell surface. The study suggested that neoadjuvant chemotherapy might contribute to the selection of CD274 amplification in TNBC, resulting in elevated PD-L1 expression. The SAFIR02 BREAST IMMUNO phase 2 trial supported these findings, indicating that CD274 gain or amplification predicted the benefit of durvalumab in metastatic breast cancer. The assessment was performed using comparative genomic hybridization arrays (CGH arrays) (Schwarz et al., 2016).

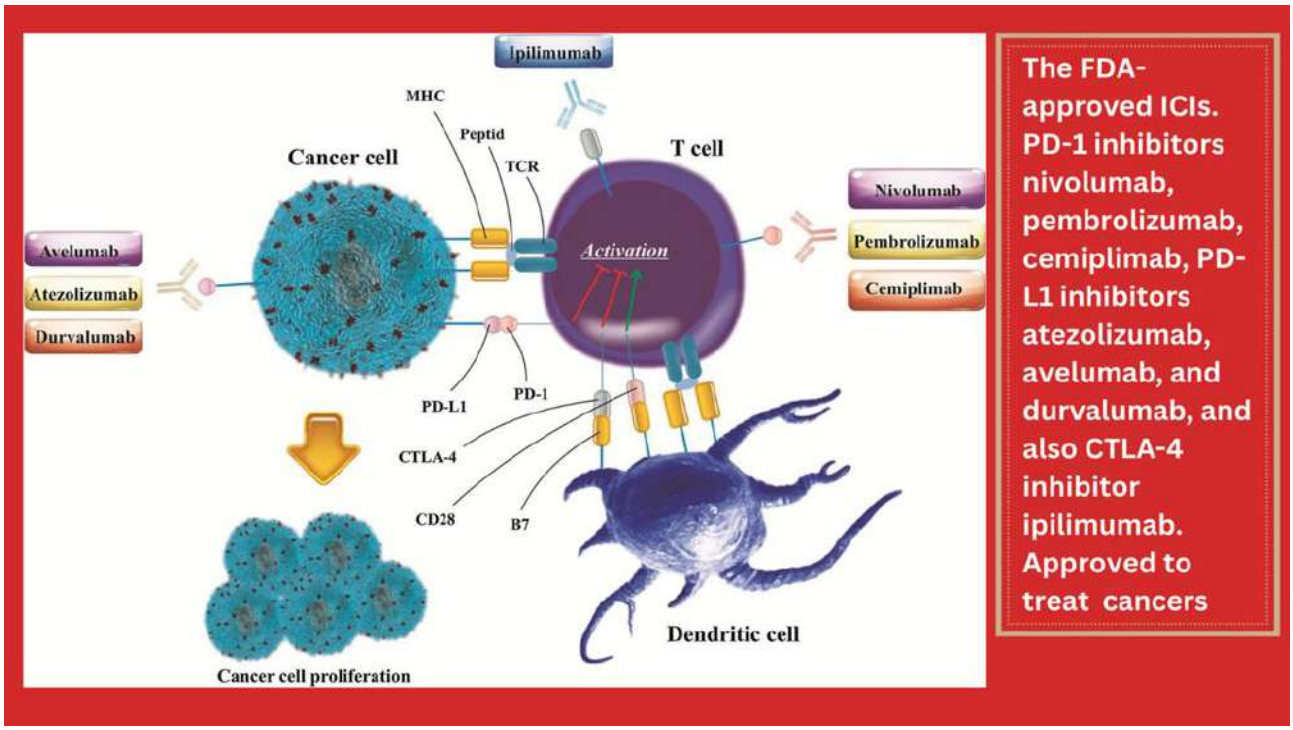
Immune Checkpoint Blockade

Traditional therapies such as surgery, radiation, and especially chemotherapy are proving to be ineffective options. If all subtypes are to be targeted then the latter option is insufficient and condensed chemotherapy is a better alternative. It still poses an economic challenge since it requires growth factors. Additionally, chemotherapeutic drug response is difficult to tackle (Dewanjee et al., 2022). Hormone therapy is also not a promising non-targeted treatment option due to its harmful effects (Masoud and Pagès, 2017). Various subtype targets are handled using a targeted therapy approach. Despite this, the efficacy of target tissues needs to be critically checked in clinical conditions. Likewise, immunotherapy is considered a possible therapy for TNBC. Modifying the immune system is being looked into as a treatment approach. (Fortis et al., 2021). HER2+ tumor gene expression in TNBC showed influence due to immune factors in a microarray-based study (De Caluwé et al., 2023). The immunotherapeutic approach varies from patient to patient on the basis of subtype. Thus, prognostic biomarkers enable customized therapies. The most effective of these is Programmed death-ligand 1 (PD-L1) in TNBC. The tumor mutational burden (TMB) is a designated marker for foreignness and immunogenicity (Hubbard-Lucey et al., 2019). Tumor-infiltrating lymphocytes (TILs), interferon Y (IFN-Y), programmed cell ligand-1 (PD-L1), and human leukocyte antigen-I (HLA-I) are also considered as predictive markers.

For specific subtypes of cancer, the effector TILs in trace amounts in TME form a barrier for therapy based on T cells. Hence, this kind of approach seems promising. Inducing hyperthermia for the TME offers a direct approach to killing tumor cells (Toraya-Brown and Fiering, 2014). This aids in exposing cancer cells to natural killer (NK) cells and CD8+ cells in human leukocyte antigen-I (HLA-I) in a manner dependent on the polypeptide. Anti-estrogenic factors could instigate the immunotherapeutic drug action because estrogen is responsible for suppressing HLA-I. Hence, HLA-I expression has an important role in immunotherapeutic drugs.

Both T-cell activation and tolerance are included in immune checkpoints. In usual conditions, these are crucial for maintaining homeostasis. Immune escape of tumor antigens can occur from immune inhibitory signals from tumors. PD-1/PD-L1 axis as well as cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) act as inhibitory signals to suppress the

immune response of T-cells (Stefanovic et al., 2017). A key role is played by CTLA-4 to ensure that T-cell response can be prevented from reinitiating. Whereas, PD-1 plays a greater role afterward by abstracting T-cell activity in the immunological setting of TME. CTLA-4 binds to those markers present on dendritic cells such as CD86 and CD80 thereby, reducing the effect of immune reaction caused by T cells. On the flip side, PD-L1 activating PD-1 causes inhibition of immune activity by T-cells, activation of T-cell death, pro-inflammatory cytokine production suppression, and antigen tolerance induction. Immune checkpoints are inhibited by blocking the CTLA-4 or PD-1/PD-L1 axis could as a result lower tumor cell immune escaping and present a potential immunotherapeutic approach. (Miao et al., 2021). Some immune checkpoints are being developed and others such as AntiPD-1, anti-PD-L1, or anti-CTLA-4 monoclonal antibodies are currently used in clinical practice. Certain types of Anti-PD-1 antibodies such as pembrolizumab, atezolizumab, and anti-CTLA-4 including tremelimumab and ipilimumab have shown potential for therapy and are approved for treating certain malignant tumors solid in nature.



PD/PD-1 Blockers as Immunotherapeutic Agents

The immunotherapeutic significance of the PD1/PDL1-based pathway has grown, making it a crucial immune checkpoint. The targeting mechanism of PD1 and PDL1 provides ample information regarding immunotherapy-based combinations and problem diagnosis. Inhibitors like specific antibodies blocking PD1 or PDL1 exhibit clinical efficacy, enhancing T-cell responses and mediating antitumor activity across various tumors.

CD28 family contains PD1 protein specified as a checkpoint inhibitor. The activation factors for regulating the function of this protein are the stimulus received after antigen recognition and cytokines synthesis (Lawani et al., 2013).

Situated advantageously, it can control T cell activity within dendritic cells and (APCs) Along with the elimination of tumor cells by T cells, the recognition of PD1 protein by tumor cells triggers an upregulation of PDL1 protein. Apoptosis is mediated by the attachment of PD1 to PDL1 in T cells (Taube et al., 2016).

The binding of PD1 to PDL1 hampers T cell-mediated immune surveillance, causing a lack of immune response and potential T cell apoptosis. Additionally, tumor infiltration is retarded and results in reduced cytokine levels, including tumor necrosis factor. This paves the way for cancer cells to evade immune reactions (Sage et al., 2010).

For complete T cell activation, a stimulatory signal requires the binding of CD80, and CD40 on APC surfaces to ligands on T lymphocytes, such as CD28, and CD40 ligands. Simultaneously, an inhibitory signal deactivates T lymphocytes post-activation, safeguarding against excessive immune reactions and cell damage (Yano et al., 2019).

CTLA4 Blockers as Immunotherapeutic Agents

T cells are known to contain a molecule called CTLA4 having a crucial role immune checkpoint inhibitory molecule. Its principal role involves modulating early-stage T-cell activation intensity. CTLA4 shares ligands, CD80 (B7.1) and CD86 (B7.2), with the co-stimulatory receptor CD28] (Bakker et al., 1996; Lo and Abdel-Motal, 2017).

T cells are activated by the CTLA4 expression on its surface as these molecules overpower the + co-stimulatory signals released from the CD28 molecule. The reduction in T cell proliferation and IL-2 production is seen because CTLA4, CD80/CD86 interact, and negative signals dominate (Taylor et al., 2009).

Foxp3 enhances and stabilizes CTLA-4 expression. It is evident in typical post-TCR stimulation of T-cells. Moreover,

CTLA-4 initiates reverse signaling via B7, inducing indoleamine-2,3-dioxygenase (IDO). This prompts the catabolism of tryptophan, inhibiting T-cell proliferation (Ribas, 2010).

Anti-CTLA-4 therapy involves the enhancement of co-stimulation such as using irradiated tumor cells expressing GM-CSF. Preclinical studies show that CTLA-4 blockade strengthens therapeutic immunity against cancer. Currently, the use of antibodies like ipilimumab and tremelimumab greatly enhances the anti-tumor effect, and malignant diseases are targeted effectively (Chen et al., 2018).

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Chapter 64

From Lab to Leash: How Biotechnology is Transforming Animal Vaccines

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ABSTRACT

Vaccines are proven to be very effective in reducing the prevalence and transmission rates of infectious diseases like polio and smallpox. Recently they have also shown promising results in immune-oncology. The influence of Biotechnology has proven to be a transformative force in revolutionizing animal vaccine development. Use of Biotechnology has enabled the development of more effective and innovative vaccination platforms including the development of recombinant proteins, nucleic acid therapeutics, synthetic biology and nanotechnology that offer enhanced efficacy and safety. Various biological research sectors have attained rapid developments due to the use of biotechnology-based approaches. Limitations of traditional vaccines such as delayed manufacturing and limited applicability in cancer-like non-infectious disorders have now been addressed by these technological advancements. This chapter reviews the potential applications and advancements of Biotechnology in the revolution of veterinary vaccines and their importance in maintaining animal health. The potential for developing highly effective vaccines against the most prevalent infections of domestic animals is tremendous due to better understanding of pathogenesis and microbe-host responses to the infections and the immense progress in genetics and biochemical techniques.

KEYWORDS
Veterinary Vaccines, Traditional Animal Vaccines, Nanotechnology, Synthetic Biology, Next Generation Vaccines, Bioinformatics Approaches

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INTRODUCTION

Importance of Animal Vaccines in Agriculture, Food Security and Veterinary Medicines

Biotechnology is defined as the biological process involved in the agricultural, medicinal, and industrial applications to the manipulation of microorganisms and production of genetically modified organisms (Khan, 2020). The veterinary vaccine plays a significant role in protection against animal infection and improving their health. Food-related diseases are a major problem worldwide because they contribute to high rates of mortality and disability in human beings. Some parasites are considered as highly ranked foodborne parasites including *Echinococcus granulosus, Trichinella spiralis, Cryptosporidium spp, and Toxoplasma gondii.* They commonly affect domestic livestock and cause huge risks to human health and food production (Sander, et al., 2020). Animal vaccines ultimately reduce the need for antibiotics to control foodborne infections (Kolotilin, et al., 2014). Moreover, animal vaccines prompted the consumer's interest in food production without chemical residues in milk, eggs and meat and also fostered the development of novel livestock vaccines (Joachim, 2016). Veterinary vaccine development has great importance over human vaccines because it can conduct successful experiments on living organisms. Most vaccines against bacterial and viral diseases are produced by veterinary industries (Jorge, et al., 2017). The chemically produced synthetic subunit vaccines are easy to preserve without prolytic enzymes and contaminations. However, it has an ability to develop immunity while avoiding side effects of the elements present in pathogenic microorganisms (Nascimento, et al., 2012).

Traditional Animal Vaccine Development

Animal health and welfare are seriously threatened by infectious diseases, which must be effectively controlled to protect agronomic health, ensure food security, and reduce poverty in rural areas. Traditionally, empirical trial-and-error

methods were used to develop veterinary vaccines to simulate the immunity brought on by a natural infection. A variety of bacterial and viral diseases can be cured or prevented by the traditional "isolate, inactivate, or kill and inject" method (Delany, et al., 2014). Currently, licensed veterinary vaccines are mostly delivered as toxoids, live-attenuated vaccinations, or inactivated (killed) vaccines. Traditional vaccinations are more costly to make and require repeated administration to achieve maximum immunity. The goal of traditional vaccine development tends to mimic the immune response that is elicited by an infection that occurs naturally. Nevertheless, this approach could not be considered the most effective durable immunity against many infections. Surpassing innate immunity levels while reducing harmful effects associated with inflammation may be necessary to achieve robust and lasting immunity. This requirement is most apparent in situations involving chronic infections, in which the pathogen coexists with the host's immune system for a long period despite the host's maximal efforts to eliminate it (Jorge, et al., 2017). The most significant and widely used traditional vaccines are the toxoid, Live-attenuated vaccines, Subunit vaccines and inactivated or killed vaccines usually called traditional vaccines.

a) Live-attenuated Vaccines

Live-attenuated vaccines have been around since 1950s and are derived from pathogens like viruses and bacteria. They proliferate in immunized animals, but they rarely or never cause disease, however they intend to trigger a humoral and cell-mediated immune response that resembles an actual infection. Attenuated vaccines are now considered extremely safe, highly immunogenic, and capable of inducing a long-lasting protective immune response with just one dose when maternal antibodies are absent (Moreira Jr, et al., 2016).

b) Subunit Vaccines

Synthetic subunit boosters use a short, non-infectious pathogen protein that is unable to multiply in the host. The recombinant immunization can be administered as a safe, non-replicating vaccination. Both producers and consumers are safer when antigen expression occurs in a heterologous system because immunity is induced without needing a toxic or partially harmful bacterium (Weiner, et al., 2018).

c) Inactivated or Killed Vaccine

A vaccine that involves the growth of bacteria, viruses, or other pathogens in culture, followed by their destruction to render them incapable of causing disease. They may not be as effective as attenuated vaccines, but they are safer than typical (Ghattas, et al., 2021).

d) Toxoid Vaccinations

Toxins are dangerous substances that are produced by bacteria that cause disease and are used in toxoid vaccinations. Rather than developing immunity against the bacteria themselves, they do so against the portions of the bacteria that cause illness. The primary factor causing the disease's symptoms is the entry of toxins into the bloodstream. Immunity is induced by neutralizing protein-based toxins (Shuja, et al., 2022).

Evolution and Milestones in the Development of Traditional Animal Vaccines

The history of the vaccination began in 400 B.C. when Hippocrates discussed diphtheria and mumps for the first time. It was an inefficient process for a while, but in the middle of the 18th century, smallpox, cholera, and yellow fever vaccinations were discovered (Abdaal, et al., 2024). The Evolution of virology is followed by scientific advancements and ground-breaking research from various disciplines like biochemistry, microbiology, and genetics. Having started with the smallpox vaccine in 1796 and progressing to COVID-19 in the 20th century milestones have experienced remarkable progress (Zuo, et al., 2024). Smallpox pus pustules were applied to skin tissue as the first smallpox prevention technique, known as variolation, which may have originated in China or India (Matić, et al., 2022). However, English physician Edward A. Jenner noted in 1796 that milkmaids who had contracted cowpox were resistant to smallpox. He vaccinated a youngster with pus from a cowpox blister, demonstrating the efficacy of immunization and contributing to the widespread usage of the smallpox vaccine. The first effective rabies vaccination was developed by Louis Pasteur in 1885. A major step forward in the prevention of this fatal disease was made when the vaccine was created using the spinal cords of rabies-infected rabbits. Formaldehyde was found by Alexander Glenny in 1923 to be an efficient way of combating the tetanus toxin. This innovative approach was then utilized in 1926 to produce a diphtheria vaccine. Additionally, the pertussis vaccine took longer to develop, and the first approved whole-cell vaccination was released in the US in 1948 (Cavaillon, 2022). Due to its repeated outbreaks, polio became the most feared disease in the world in the late 1800s and early 1900s. More than 2000 people died in a serious polio outbreak that struck New York City in 1916, and more than 3000 people suffered in the deadliest polio outbreak in American history in 1952. By growing the polioviruses in human tissue in 1949, Enders, Weller, and Robbins eventually won a Nobel Prize. Soon after this discovery, in 1953, Jonas Salk created the first effective polio vaccine, which was then tested on 1.6 million children in the USA, Canada, and Finland by 1954.

The United Nations (UN) estimates that immunization saves up to three million lives yearly and is a successful and affordable public health strategy. There has been a noticeable decline in childhood illnesses and fatalities from avoidable causes since the Expanded Programme on Immunization (EPI) was introduced in 1974 to vaccinate everyone against six

diseases (Mantel, et al., 2020). The FDA authorized the Gardasil vaccination in 2006, which was created by Merck and was the first HPV vaccine. In the meanwhile, the European Medicines Agency approved the GSK-created Cervarix® vaccine in 2007 and the FDA approved it in 2009 (Cheng, et al., 2020). Around 200 candidates were produced in the early 2020 global race among scientists to develop a safer and more effective COVID-19 vaccine. Before the end of 2020, the Pfizer-BioNTech partnership made history by creating the first COVID-19 vaccine to be authorized, making it one of the fastest-acting vaccine development successes to date (Saleh, et al., 2021).

Limitations and Challenges of Traditional Vaccines

The rapid alterations and genomic diversity of certain pathogens are obstacles to the development of vaccines and could contribute to vaccine evasion, which reduces the potency of existing vaccinations. The emergence of novel diseases with increased transmissibility, fatality rate, or potential for immune evasion poses one of the most significant obstacles to public health. The development of vaccines against infections that evade the immune system, such as HIV has not shown to be successful and remains an ongoing concern (Ghattas, et al., 2021). Developing vaccines for many important public health pathogens is difficult due to their evolving nature. These obstacles include a lack of comprehension about the development of immunity, genetic heterogeneity in both hosts and pathogens and a rise in public fear of the safety (Kennedy, et al., 2020).

Advancements in Nanotechnology for Targeted Vaccine Delivery and Antigen Presentation

A likely novel approach for vaccine development is nanotechnology, whilst scientists have been focusing progressively on nanoparticles for displaying antigens and precise vaccination administration. By tailored administration in vivo, nanomaterials stimulate immune responses by providing monitored features like diameter, zeta-potential, surface structure, and antigen loading efficacy (Gheibi Hayat, et al., 2019). Subunit vaccines containing particular antigens that provoke tailored immune responses account for the majority of nano vaccines, in contrast to traditional vaccinations that contain inactivated microorganisms (Zhang, et al., 2019). However, subunit vaccinations lack pathogen-associated molecular patterns (PAMPs), they are less immunogenic but safer, demanding added adjuvants or nanomaterial delivery systems for maximum effectiveness (Tandrup Schmidt, et al., 2016). By limiting unwanted immune reactions from exposing antigens in systemic circulation, nanomaterials ensure optimal antigen protection till they reach their intended location. A wide variety of nanomaterial delivery methods, comprising liposomes, metallic nanoparticles, polymer-based nanoparticles, inorganic nanoparticles, and composited nanoparticles, have been investigated for use in nano vaccine development (Cai, et al., 2020).

As smaller diameters permit easier internalization by antigen-presenting cells (APCs) using a variety of delivery pathways, the size of nano vaccine plays a substantial role in their immunogenicity. Particle diameter and immunogenicity interplay in an intricate manner that is regulated by delivery routes, particle types, and doses. This is due to the reason that smaller nanoparticles exhibit a tendency to drain to lymphatic veins and aggregate in lymph nodes (Kijanka, et al., 2018). Immunogenicity is modulated by immuno-particle's shape, which also affects immune cell formation and bio distribution. The most rapid endocytosis rate is observed in spherical nanoparticles, which are followed in order by cubic, rod, and disk-shaped nanoparticles. Multiple endocytosis pathways lead to various levels of internalization and trends in biodistribution (Shao, et al., 2017).

Phagocytic cell intake, circulatory time, and hydrophilicity are all affected by the surface coating, another vital nanoparticle attribute. When a surface coating called PEGylation is administered repeatedly, it can lead to the generation of anti-PEG antibodies in animals, thereby lowering the potency of the treatment. Hyaluronic acid and poly(sarcosine) surface coatings (Rao, et al., 2020), on the other hand, demonstrate modified coronal protein composition and diminished immunogenicity, particularly affecting the immunogenicity of nanoparticles. Furthermore, novel avenues for the development of nano vaccines are offered by biomimicking approaches that employ cell membrane-based strategies. These approaches take advantage of tumor-specific proteins and APC surface proteins to boost immune responses. Owing to their ease of manufacturing and safety, genetic nano vaccines, such DNA and mRNA vaccines, are also being investigated for the treatment of cancer (D'amico, et al., 2021). With all factors taken into account, nanotechnology offers novel opportunities to improve vaccination safety, effectiveness, and cellular transportation. Scientists from multiple fields must collaborate to develop next-generation vaccines with better qualities, such as increased immunogenicity, long-lasting protection, and reduced potential for pathogenicity (Yenkoidiok-Douti, et al., 2020).

Synthetic Biology: Engineering Tomorrow's Vaccines

Synthetic biology is a multidisciplinary field connected to various fields like molecular biology, biotechnology and biomaterials, for the synthesis of new biological systems, parts or individuals and assists in providing disciplines and methodology guidelines to these diverse fields. It designs and constructs various biological circuits to efficiently produce high-value-added pharmaceutical intermediates and products by providing a sustainable, robust, feasible and scalable alternative to excessive cultivation of medicinal plants. (Yan, et al., 2023). Le Duc (1914) proposed the concept of synthetic biology in 1910s. Figure 1 contains a summary of developments in synthetic biology, listing important events from the 1950s to 2020s. From its origins in chemical biosynthesis, fields like medical treatments, environmental conservation, chemical engineering, pharmaceutical research, agriculture and food have also been included in its applications.

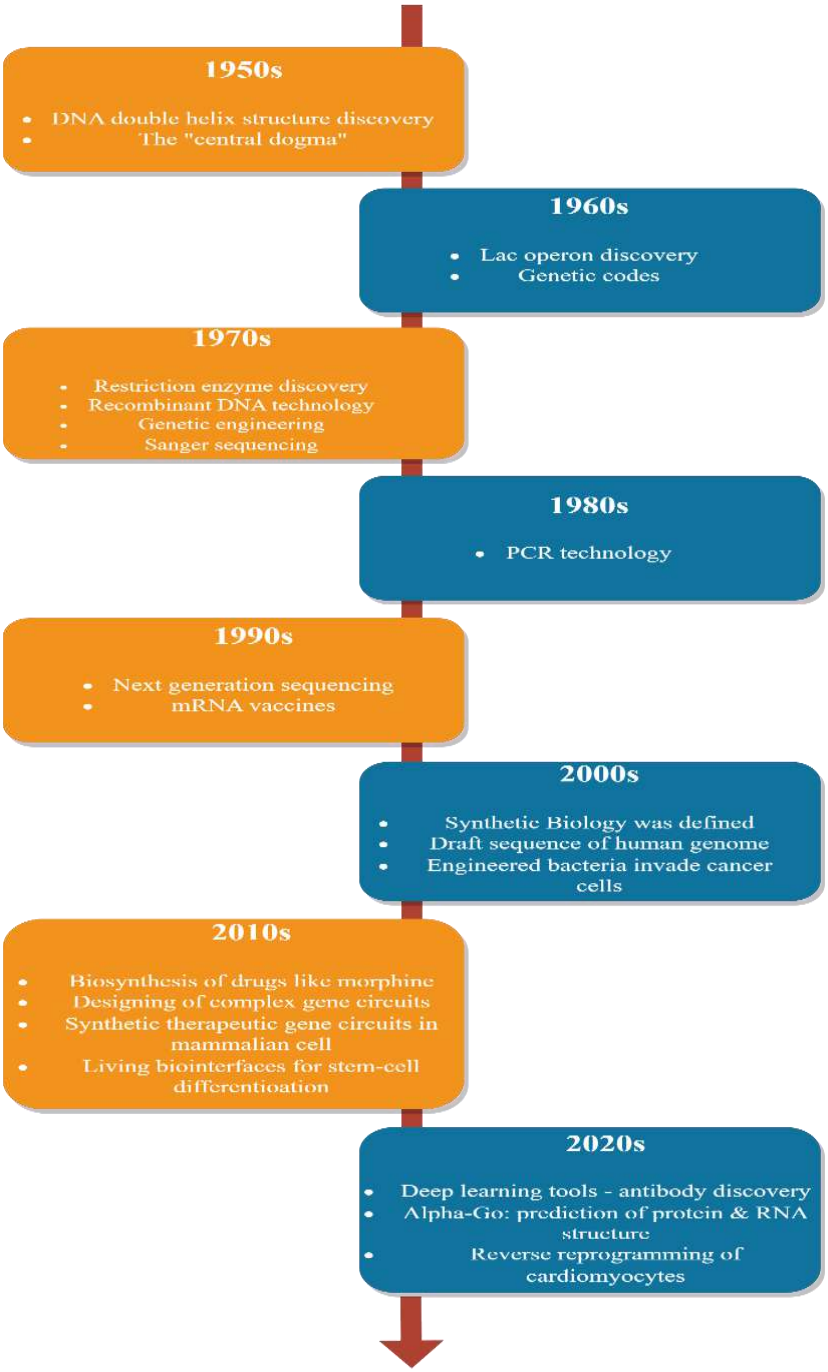

Fig. 1: Timeline of major milestones in Synthetic Biology. From 1950s to 2020s (Yan, et al., 2023)

Production of Next-Generation Vaccines Using Synthetic Biology Tools

Vaccination, an effective disease control and prevention strategy, has resulted in the eradication or control of once-catastrophic pandemics like measles, smallpox, poliomyelitis etc. However, the synthesis of vaccines continues to be an extremely challenging step. With the integration of computational analysis and biological data, synthetic biology has the ability to enhance the safety and efficacy of vaccines and lower production times (Charlton Hume, et al., 2019;Tan, et al., 2021). Synthetic biology uses various technologies such as pathway design, expression fine-tuning, genetic circuits, protein and molecular engineering, machine learning and CRISPR/Cas systems for the promotion of design-build-test-learn cycle of cell factory construction (Yan, et al., 2023). Various techniques for the large-scale manipulation of nucleic acid are mentioned in the following section (figure 2).

Genomic Codon De-Optimized Vaccines

Synthetic biologists can re-engineer viral genomes using large-scale synonymous mutations due to advances in low-cost nucleic acid synthesis. This approach of viral inactivation uses the non-random frequencies of codon pairs and degeneracy of triplet codons that exist in many species. As a result, an infectious virus with severely attenuated virulence is

produced. Codon deoptimization technique has several benefits including robust vaccine synthesis and long-lasting protective immunity. However, culture conditions, handling, storage, refrigeration and compromised immune system are some of the shortcomings of using this strategy for virus attenuation (Tan, et al., 2021).

DNA-Based Vaccines

DNA vaccines induce robust cellular and humoral immunity by delivering plasmid-free dsDNA of viral components into the cell nuclei where transcripts are cytoplasmically translated. DNA vaccines have several advantages such as higher thermostability, prolonged antigen expression (upto 1.5 years), rapid design and ease of manufacturing (Tan, et al., 2021). Recently DNA-based vaccines have been developed for Ebola (Tebas, et al., 2019) and SARS-CoV-2 (Smith, et al., 2020).

RNA-Based Vaccines

RNA-based vaccines are designed to introduce pathogen-specific antigens in the form of synthetic RNA that triggers immune responses in individuals. They provide advantages over conventional vaccines in terms of higher immunogenicity, safety, speed of development and scalability (Pfeifer, et al., 2023). mRNA vaccines have been developed against a variety of infectious diseases such as SARS-CoV-2 (Kashte, et al., 2021), dengue virus (Wollner, et al., 2021), influenza (Chivukula, et al., 2021), Zika virus (Essink, et al., 2023), rabies (Li, et al., 2022), herpes simplex virus type 2 (HSV-2) (Latourette Ii, et al., 2020) and Mycobacterium tuberculosis (M.tb) (Larsen, et al., 2023). Treatment of prostate cancer (Kübler, et al., 2015) and breast cancer (Jiang, et al., 2023) using mRNA vaccines has also shown promising results by inducing immune responses against these cancer cells.

Viral Vector-Based Vaccines

Viral vectors have the ability to induce antigen-specific immunity by expressing heterologous antigens without requiring any exogenous adjuvants. The first virus developed as a vaccine vector was vaccinia virus (Moss, et al., 1984). Several vectors are currently undergoing clinical trials such as novel adenovirus (Phase II), cytomegalovirus (Phase I), measles virus (Phase I) and vesicular stomatitis virus (Phase III) (Humphreys, et al., 2018).

Virus-like Particle (VLP) Vaccines

VLPs are self-assembling, recombinant viral structures that are noninfectious but exhibit immune-protective characters of native viruses. Porcilis PCV®, Gardasil®, Hecolin® and Cervarix® are prophylactic VLP vaccines that are licensed, effective and safe. Besides their applications in vaccinology, they are also efficient biodegradable delivery agents for drugs and gene therapy (Charlton Hume, et al., 2019).

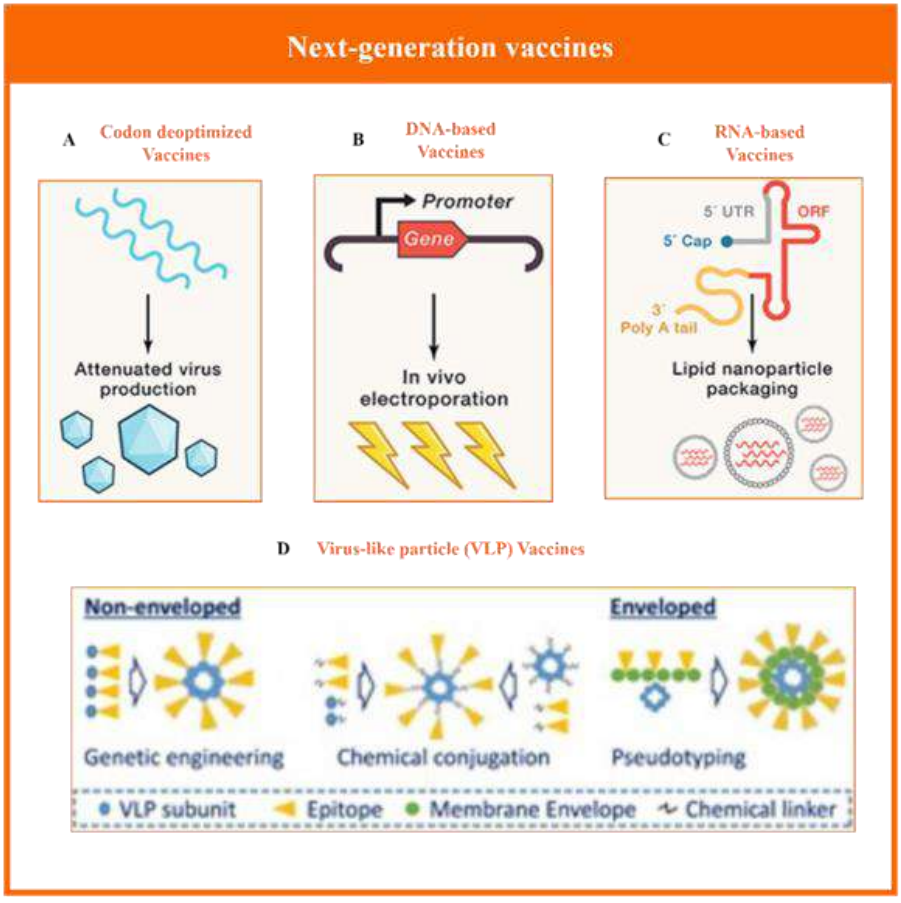

Fig. 2: Next-generation Vaccines (Data source (Charlton Hume, et al., 2019;Tan, et al., 2021))

Next-Generation Adjuvants and Immune-Modulators

When administered in conjunction with an antigen, adjuvants are substances, mixtures, or macromolecules that augment non-specific immunity and modify the nature of the immune reaction in the body, but their toxicity and potential need to be controlled. With nanoparticles having a higher prospect of adjuvant activity than microparticles, nano-carriers provides an appealing platform for immune activation and antigen delivery. By better overcoming biological barriers, nano-adjuvants precisely target antigen-presenting cells (APCs) and enable tailored antigen delivery (Nooraei, et al., 2023). Next generation vaccine adjuvants are summarized below.

a) Bacterial Derivatives

Lipopolysaccharides (LPSs) and cholera toxin are typical bacterial derivatives that serve as adjuvants in vaccines, boosting immune responses. As adjuvants that elicit humoral and cell-mediated immunity, Bacterial Ghosts (BGs) and Outer Membrane Vesicles (OMVs) from Gram-negative bacteria have been studied. Additionally, components of Poly-α-L-Glutamine (PLG) and flagellin exhibit as adjuvants, enhancing vaccination efficacy and stimulating robust immune responses (Li, et al., 2020).

b) Liposomes

Liposomes offer an innovative way of trapping both hydrophilic and lipophilic antigens in vaccines to boost immune responses owing to their lipid bilayer composition. By modifying liposome parameters including size, charge, and membrane fluidity, one may maximize the targeting of APCs and influence the immune response. Current clinical research and commercially accessible liposomal vaccines illustrate their potential for both prevention and treatment of infectious illnesses (Karunakaran, et al., 2023).

c) Nanosomes

Similar to liposomes, lipid-based nanoparticles called nanosomes are used as adjuvants in vaccinations because they can better transfer antigens to APCs by packaging them. Nanosomes, which are smaller (20–50 nm) lipid bilayers, have been integrated into vaccine formulations to enhance cellular and antibody immune responses and provide a shield against viral infections (Moni, et al., 2023).

Bioinformatics Approaches for Antigenic Epitope Prediction for Vaccine Candidates

Vaccines were formerly created to prevent infectious diseases caused by different infectious agents but now different autoimmune diseases (Zhang, et al., 2018), degenerative and for cancer vaccines (Safavi, et al., 2019) are used as new vaccine technology. Conventional vaccines are not very effective for diseases where complex immune pathways are involved. Therefore, with advancements in vaccine production area there is needed to develop new generation vaccines such as epitope-based vaccines. These vaccines have minimum side effects and are more effective and target oriented.

Bioinformatics approach is now used for vaccine designing. Reliable data regarding genome and protein is available on NCBI website (www.ncbi.nlm.nih.gov) which is freely available and easily accessible. This database provides comprehensive information about nucleotides, genes, proteins, assembles and transcripts. The PDB (www.rcsb.org) provides complete information about proteins their structures, crystallography, three-dimensional structure, fiber diffraction, powder diffraction and associated molecules (Rose, et al., 2012).

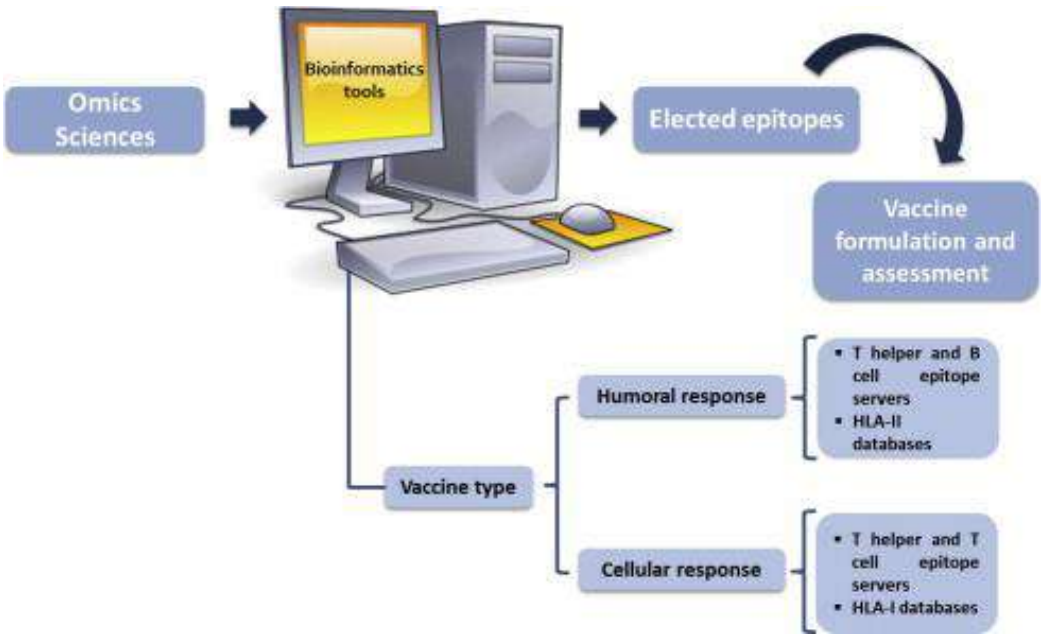

Fig. 3: Workflow schematics of vaccine development (Soria-Guerra, et al., 2015)

After genomic and protein data analysis from database. Comparative analysis and homology modeling can be performed on 3D structures. MODELER is used for protein modeling and homology-derived spatial restraints are created (Janson, et al., 2019). Protein docking is performed which predicts ligand binding. First ligand binding sites are determined and then score them and select highest score for complex structures. Molecular docking is performed to check interactions of candidate epitope with immune cell receptors such as toll-like receptors (TLR), TLR2, TLR3 and TLR4. Also, interactions with major histocompatibility complex (MHC) I and MHC II are analyzed (Kar, et al., 2020).

Antigenicity prediction is very important and includes Welling method and Kolaskar-Tongaonkar method. In welling method presence of specific amino acids on antigenic site and in protein is determined. While in Kolaskar-Togaonkar method presence of hydrophobic amino acids such as leucicne, valine and cysteine are determined and check whether peptide is antigenic or not (Kolaskar, et al., 1990). This method has 75 % accuracy. Epitopes which are recognized by B-cells are of two types, Continuous epitopes and discontinuous epitopes. B-cells are basically mediate humoral immune response and produce antibodies to kill or neutralize the antigens. Continuous epitopes are short peptides which are specific to antibodies and recognized by antibodies while discontinuous epitopes are complex. B-cells mostly recognize discontinuous epitopes.

Table 1: B-cell epitope prediction tools (Yurina, et al., 2022)

Tools	Description	URL
ABCpred	Based on sequence with ANN	http://crdd.osdd.net/raghava/abcpred/
BEPITOPE	Based on sequence to predict continuous epitope	http://bepitope.ibs.fr/
BCPREDS	Predicting linear B-cell epitopes using the subsequence kernel	http://ailab-projects1.ist.psu.edu:8080/bcpred/index.html
Bepro	Based on antigen structure to predict discontinuous epitope	http://pepito.proteomics.ics.uci.edu/
CEP	Based on structure to predict continuous and discontinuous epitopes	http://bioinfo.ernet.in/cep.htm
COBEpro	Based on B-cell epitope primer sequence. Secondary structure and solvent accessibility are also responsible for increasing prediction accuracy	http://scratch.proteomics.ics.uci.edu/
DiscoTope	Based in sequence and structure for predicting continuous and discontinuous epitopes	http://www.cbs.dtu.dk/
Ellipro	Based on solvent accessibility and protein flexibility	http://tools.immuneepitope.org/tools/ElliPro/iedbinput
EMT	Based on phage display to predict continuous and discontinuous epitopes	elro@novozymes.com

T-cells epitopes can be predicted more accurately and is easier. T-cells epitopes are short linear peptides of about 9-15 amino acids in length. T-cells epitopes are recognized by T-cells receptors and MHC I and MHC II. These epitopes bind with MHC parts with van der waals interactions, hydrogen bonds and electrostatic interactions. In-silico studies are not very complex but these approaches are less time consuming and give more accurate results for determining new vaccine targets and vaccine designing. To improve the accuracy of epitope prediction, it is necessary to analyze multidimensions of proteins to validate the binding of antibodies to receptors and increase protein-protein interactions. By improving accuracy in this manner, the effectiveness of vaccines is also expected to be improved.

Advantages of Bioengineered Vaccines

The development of novel and next-generation vaccines as well as their distribution to clinical settings can be sped up by using tools that aid in the in silico prediction of immune responses to biothreats and emerging infectious illnesses. African swine fever, PCV2, and swine influenza A are among the viral diseases of pigs that have been the subject of animal vaccine applications so far. Adjuvants with higher efficacy and innovative delivery methods could promote timely vaccination adoption (Celis-Giraldo, et al., 2021). Compared to traditional vaccine development methods, the integration of bioinformatics and immunogenetics has transformed vaccine design and improved specificity and thermodynamic stability. A recombinant thermostable NDV vector vaccine that expresses multiple epitope cassette of the IBV is developed using the reverse genetics method. This vaccine can be sprayed on and given through drinking water, eliminating the need for a cold chain during administration, storage and distribution (Abdelaziz, et al., 2024).

Commercial trivalent vaccines, including an HVT vector expressing IBDV antigen with either NDV or LTV antigen, have been approved for use in immunizing chicks. However, current research is investigating its potential use in the creation of multi-epitope vaccines that target parasitic pathogens like Eimeria species in poultry, viral pathogens like IBD and chicken anemia virus, and bacterial pathogens like M. gallisepticum, C. jejuni, and C. perfringens (Fulber, et al., 2022). The fast growth rate and minimal needs of the algal expression system have made it possible to be a potential platform for producing proteins at a reasonable cost (Cid, et al., 2021). Engineering yeast and bacteria can reduce manufacturing costs and times while improving the yields of heterologous protein expression (Legastelois, et al., 2017). The construction and equipment requirements for a vaccine manufacturing facility are streamlined by the versatility and ease of scaling up that come from using insects as single bioreactors (Francis, 2018).

Future Prospects and Challenges

The development of safe and effective immunotherapy for a wide range of pathogens and species will be accelerated by the successful integration of in silico immunoinformatics tools, ex vivo/in vitro, and in vivo immune system technologies across the entire vaccine development pipeline. This will allow developers to predict and assess the safety, toxicity, efficacy, quality, and performance of vaccines (De Groot, et al., 2020).

The main objectives of veterinary vaccinations are to enhance animal health, boost livestock productivity in an economical way, and prevent the spread of diseases from domestic animals and wildlife to humans. It will be crucial for researchers and medical practitioners to continue collaborating with animals to adapt new technology, provide animal models of sickness, and combat newly discovered infectious diseases (Kahn, 2006) In addition to fully knocking out virulence factors, modification in genetic makeup or expression of desired gene products can be done. These approaches range from simple yet efficient whole-pathogen preparations to molecularly specified subunit vaccines, chimeras or genetically altered organisms, vector antigen formulations, and naked DNA injections. The ultimate effective result of vaccine research is development of a product that will be sold or utilized in the field to accomplish desired results (Meeusen, et al., 2007). Developing new vaccines and enhancing the quality of those that already exist has been made possible by the application of advanced technologies such as proteomics and genetic engineering (Shams, 2005).

Personalized vaccine is developed through a complex interplay between environmental, genetic, and other factors that affect the host's immune system. Therefore, identifying these genetically encoded limitations presents a chance to improve clinical decision-making while also advancing research by using the knowledge to create more effective vaccinations and improved algorithms for administering vaccines (Poland, et al., 2008). The high variability of microorganisms poses a challenge to the development of such vaccines. The serotype determines the immunological response in the majority of cases. Rather than employing multivalent vaccination mixtures, the cross-protective ability of vaccine strains could be enhanced by increasing the immunogenicity of the conserved antigens (Nagy, et al., 2008).

The public's acceptance of bioengineered vaccines is the primary obstacle, as some believe that genetically modified items are bad for the environment and society. Since there is a possibility of cross-contamination during pollination between genetically modified and non-genetically modified plants in molecular farming, careful observation is necessary while manufacturing bioengineered vaccines (Rasool et al, 2023). Pharmaceuticals may unintentionally find their way into the human food chain and have an impact on wildlife. Bioengineered vaccines have advantages over side effects that make them worthwhile to pursue, and they have the potential to usher in a new era of improved control over infectious illnesses.

Conclusion

This chapter provides a comprehensive overview of a highly innovative and revolutionary biotechnological approach to veterinary vaccine development, which effectively caters to the urgent and dire requirements of the animal health industry. In today's globalized world, the surge of diseases like avian flu in poultry, foot-and-mouth disease virus (FMDV) in cattle, and a multitude of immunodeficiency-syndrome-related viruses poses colossal threats to the global economy and the very sustenance of animal agriculture. Perturbingly, the currently available vaccines predominantly rely on conventional technologies and suffer from significant drawbacks. They showcase limited efficacy in terms of duration, lack specificity, and often manifest harmful side effects, potentially perpetuating the very diseases they aim to combat if not implemented alongside stringent control measures. However, a magnificent breakthrough in the form of genetically engineered peptide vaccines has emerged, offering a prodigious solution to this predicament. These groundbreaking vaccines exhibit an unprecedented ability to precisely target the T-cell response, an unreachable feat for traditional vaccines. The improved efficacy and broad-spectrum protection provided by these peptide vaccines render them an unparalleled option when combating the emergence, reemergence and pandemics of diseases. Leveraging this cutting-edge technology, researchers employ a traditional yet immensely powerful technique to construct multiepitope vaccines. By ingeniously designing an intricately woven amino acid sequence derived from a multitude of peptides sourced from diverse genes or obtained from a vast pool of random peptides, the stage is set for a quantum leap in veterinary vaccine development. Hence, the future of animal immunization seems promising, with biotechnology establishing possibilities for safer, targeted, and more effective vaccination strategies.

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Chapter 65

Characterizing Stability of Fish Vaccine Antigens Encapsulated in Plant-Based Nanoparticles

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ABSTRACT

Vaccination is essential for aquaculture in prophylaxis maintenance or preservation of fish health and productivity; however, the classical vaccine delivery systems have certain limitations. Here we review nanoparticle-based antigen delivery systems. The plant-derived nanoparticles are distinct as they have been shown to be bio-compatible, biodegradable and capable of targeted delivery making them attractive candidates. Bacterial, viral and parasitic antigens are regular targets used in fish vaccines; these who have their effective ability influenced by parameters such as pH of the environment, temperature and chemical oxidation. Some plant-based nanoparticle e.g. zein and chitosan types exhibit potential resistance to immune cell degradation which is beneficial for targeted delivery. Encapsulation efficiency can be improved by offering a wide range of antigen encapsulation techniques like emulsion, ionic gelation and coacervation. Advances in targeting for the delivery of Ag-loaded NPs require a comprehensive characterization which entails particle size, morphology as well as exclusion/conformation to some antigen loading and techniques such circular dichroism which show the conformational stability. Studies in vitro exhaustively analyze various facets such as the release kinetics, bioavailability and bioactivity besides assessing interactions with antigen-presenting cells while those in vivo examine immunogenicity, bio-distribution and protective activity using appropriate fish models to establish effectors having greatest therapeutic relevance. They can be turned into multivalent vaccines, formulated with adjuvants and targeted delivery. Further areas of research include using omics, modeling tools needs to be explored alongside stabilizing strategies and the concept around manufacturing (scale-up) and safety. Therefore, conferment of enhanced antigen stability and bioavailability as well as improved immunogenicity for fish vaccine antigens by encapsulation in plant-based nanoparticle formulations could promote effective vaccination strategies sustainable in aquaculture.

KEYWORDS
Aquaculture vaccination, Antigen delivery, Fish immunology, Plant-based nanoparticles, Vaccine formulation

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INTRODUCTION

Aquaculture, or the farming of aquatic organisms around the world has grown rapidly to meet global demand for seafood. This, however, may increase the chances of disease outbreaks on fish health and productivity. Furthermore, vaccination is key to reducing these risks and ensuring the sustainability of aquaculture. Vaccination is the best possible option for combating infectious diseases in aquaculture, thereby reducing antibiotic usage and also minimizing economic returns. Vaccines confer extended efficacy against key bacterial, viral and parasitic pathogens that increase the survival of fish throughout growth or production periods (Liu et al., 2024). Effective vaccination programs provide substantial savings by reducing the costs of disease outbreak which include mortality losses, growth retardation and treatment expenditures. This cost-efficiency is of particular importance in aquaculture where disease outbreaks can lead to significant economic losses for farmers (Mukaila et al., 2023). Fish vaccination is essential to avoid the need for antibiotics which nowadays has

become a serious problem in many parts of the world concerning resistance. The more that antibiotics are employed in aquaculture, the greater the probability for multiple drug-resistant bacteria to manifest and this is by no means innocuous as it pertains both to animal health but human wellbeing too. In turn, this could likely to lower the requirements for antibiotic treatments by way of disease outbreaks and make a significant contribution towards sustainability in the aquaculture practices (Imtiaz et al., 2023).

Vaccination also benefits fish welfare and product quality and the vaccinated fish usually grow more, consume their food better and present themselves with higher product quality than unvaccinated siblings. This is not only of benefit for the farmers but also adds to a better image in general and acceptance of aquaculture products by consumers (Miccoli et al., 2021). Fish vaccination has proven advantageous but the creation of safe and affordable vaccines for different aquaculture species is still facing a number of difficulties. Both of these barriers can be overcome via ongoing research and creativity, which contribute to the overall sustainability as well as financial success within aquaculture (Priya and Kappalli 2022). Vaccination of fish is an important tool to avoid infections in aquaculture and has the potential to increase animal health, welfare, product quality minimize use of antibiotics. The future sustainability and resilience of aquaculture operations depend on implementing effective vaccination strategies as the industry continues to grow (Flores-Kossack et al., 2020).

Conventional ways of vaccine delivery in aquaculture like injection and immersion confront several barriers. The problem with injection vaccination is that it works, but also laborious (handles the fish several times), causes stress to the fish and high incidence of adverse reactions or handling-related injuries. Injection vaccination have the tendency to cause side effects like adhesion, pigmentation and tumor near where vaccinations are injected thereby leads death sometimes. Immersion vaccination, in contrast, rarely achieves commercial success due to the low efficacy of antigen intake and limited immune response. It can be more efficient but still dependent on the type of antigen, to immersion time not say about water quality and therefore protection levels also vary enormously (Du et al., 2022).

As a potential alternative to the conventional vaccination approaches, nanoparticle-mediated antigen delivery systems have recently been researched. These nanoparticles can shield antigens from degradation, enhance their bioavailability and selectively deliver them to immune cells providing an edge for the development of more efficient vaccines (Bezbaruah et al., 2022). Nanoparticle drug delivery system has certain advantages, such as better antigen stability and release of mucosal adjuvant that could reduce the injection burden of vaccination remarkably (Elumalai et al., 2024). The plant based nanoparticles have been the most attractive among various nanoparticle platforms as vaccine carriers in aquaculture. Recently, plant-based nanoparticles received increasingly more attention in the drug delivery research community due to their biodegradability and potential lower immunogenicity with the ability of synthetic polymer degradation products might induce innate immunity (Mondal and Thomas 2022).

Typically, one of the most common plant based nanoparticles include zein (corn), gliadin (wheat) and chitosan which are derived from shellfish. It is possible to use these nanoparticles as carriers, either by forming a protective shell around an antigen-loaded core or surfacing the nanoparticle with antigens (in this case without protecting them from myriad proteases in solution) thus targeting particles for uptake by immune cells (Nguyen et al., 2022). Plant-based nanoparticles, especially those with proteinaceous nature have been evaluated as potential vaccine carriers for fish (Table 2). Zein nanoparticles loaded with a viral antigen were able to serve as an entry for inducing active immunity against nervous necrosis virus in European sea bass (Angulo et al., 2022). Additionally, improved immunity response and increased survival against Streptococcus iniae disease was also observed upon incorporating chitosan nanoparticles encapsulating a bacterial antigen in Nile tilapia (Suwanbumrung et al., 2023). The attractive advantages of the plant-based nanoparticles are evident, but several daunting challenges need to be overcome before exploring these tiny versatile platforms further and employ more advanced polyanhydrides such as copolymers in MN design including fine tuning of antigen loading/releasing kinetics, long-term stability issues like solvent compatibility during synthesis/storage and scaling up for high volume manufacturing (Basu et al., 2021).

Fish Vaccine Antigens

In fact, vaccine antigens are the key components that induce protective immune responses against particular pathogens. In the area of fish vaccination, a great number of antigens from bacteria/pathogens and also parasites have been attempted for use in vaccine formulae (Schijns et al., 2021).

Common Bacterial, Viral and Parasitic Antigens used in Fish Vaccines

Bacterial Antigens

Gram-negative bacterial antigens have probably been the most extensively studied and applied in fish vaccines for the control of diseases caused by these microorganisms. The bacterial pathogens which are targeted by phage biocontrol products include some of the Vibrio species (V. anguillarum, V. salmonicida), Aeromonasspp (A. salmonicida, A. hydrophila) and Yersinia ruckeri responsible for substantial economic losses incurred in aquaculture industry [6]. The latter may be derived from outer membrane proteins, lipolysacharides or extracellular products and have been used successfully in fish vaccines (Singh et al., 2023).

Viral Antigens

Viral infection can be one of the most threatening and as a result, vaccination has mainly targeted viral pathogens causing diseases such as infectious pancreatic necrosis virus (IPNV), Infectious hematopoietic necrosis virus (IHNV) and viral haemorrhagic septicaemia virus (VHSV) in salmonids or betanoda virus for marine species. Glycoproteins and capsid proteins present on the surface of virus whether in recombinant subunit vaccines or by using whole-virus preparation (such as inactivated) have been evaluated extensively (Mugimba et al., 2021).

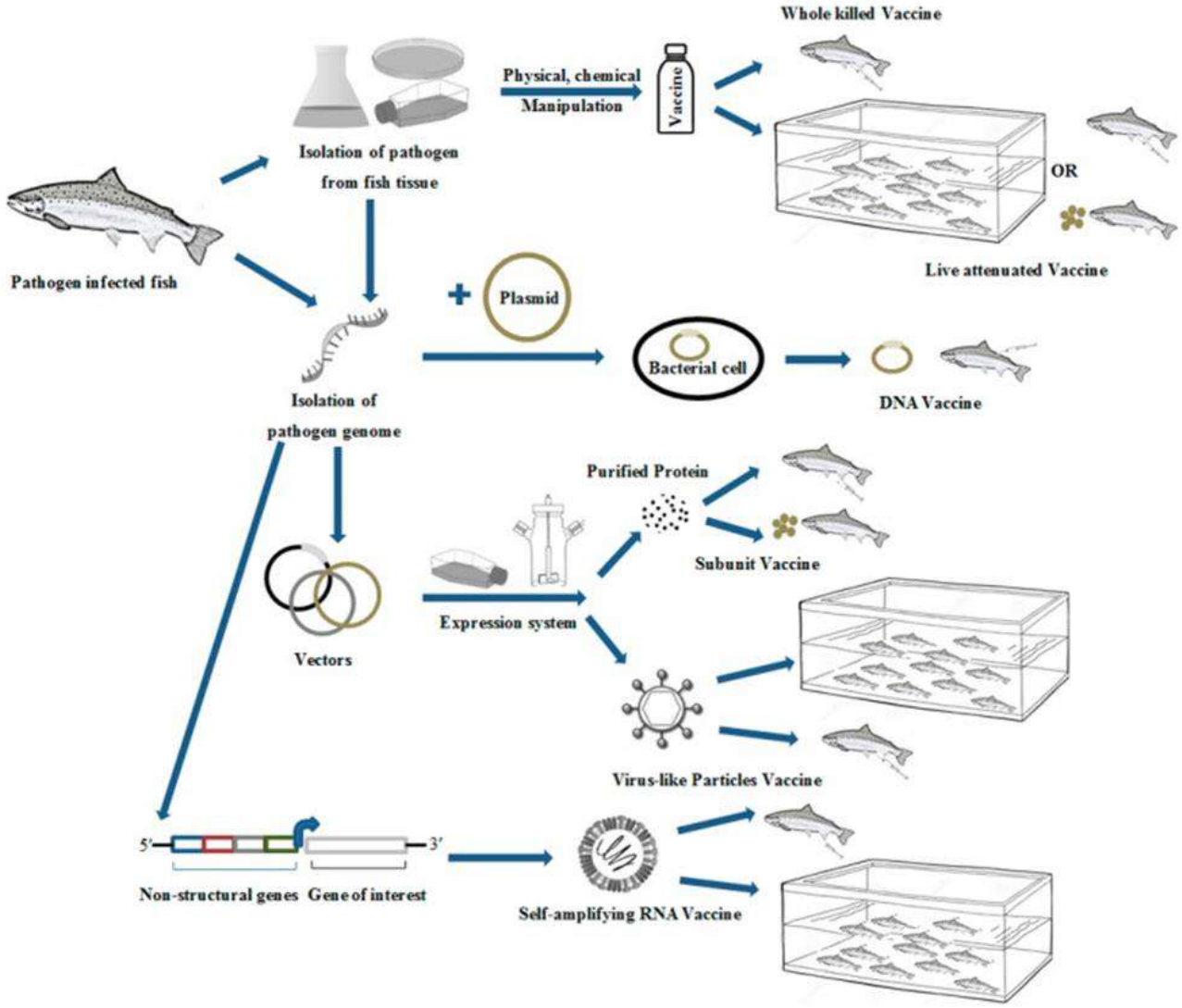

Fig. 1: Various approaches for fish vaccine development (Ma et al., 2019)

Parasitic Antigens

Parasitic diseases also pose a challenge for aquaculture and vaccine development has included protozoan and metazoan parasites. Antigens from Ichthyophthirius multifiliis, a ciliated protozoan have been investigated, using vaccination research for white spot disease in freshwater fish as an example. Antigens of the monogenean parasite Gyrodactylus salaris to be used for prophylaxis against salmonid gyrodactylosis have also been examined (see below) (Buchmann 2022).

Structural and Functional Properties of Antigens

Antigens are usually proteins or polysaccharides which can be recognized by the immune system and that induces either a specific antibody response, sometimes referred to as humoral immunity. Antigenic properties are essential in determining the structural and functional characteristics of antigens, hence antigen design is necessary for effective vaccine development (Kapingidza et al., 2020).

Protein Antigens

Proteins are antigens belonging to almost all bacterial, viral and parasitic strains; they perform various structural forms and have specific functional regions. These features may alter the level of immunogenicity, stability and mode-of-action. Examples of this include the selection and testing of surface-exposed molecules or those involved in

pathogenesis/virulence as vaccine antigens, because it has been hypothesized that host immunity against these bacterial proteins may prevent invasive infections caused by bacteria expressing them (Mishra et al., 2020).

Antigen-Polysaccharide

Lipo-polysaccharides (LPS) from gram−bacteria capsular polysaccharides from some pathogens carbandoglycans are able to induce the production of antibodies against protein antigens, without being as immunogenic as other proteins in fish (Gao et al., 2023).

Table 1: Commercial vaccines used in fish against various pathogens

Vaccine name	Administration	Pathogen	Target Fish Species
Bacterial pathogens			
ALPHA JECT® micro 4	injection	*Aeromonas salmonicida, Vibrio anguillarum, Vibrio salmonicida*	Atlantic salmon (*Salmosalar*)
Alpha ERM Salar	injection	*Yersinia ruckeri*	Atlantic salmon (*Salmosalar*)
fiALPHA JECT LiVac® SRS	injection	*Piscirickettsia salmonis*	Atlantic salmon (*Salmosalar*), Rainbow trout (*Oncorhynchus mykiss*) Coho salmon (*Oncorhynchus kisutch*)
ALPHA JECT® micro 1 Tila	injection	*Streptococcus agalactiae*	Nile tilapia (*Oreochromis niloticus*)
ALPHA JECT® Panga 2	injection	*Aeromonas hydrophila, Edwardsiella ictaluri*	Iridescent shark (*Pangasianodon hypophthalmus)*
ALPHA JECT® 5–3	injection	*Aeromonas salmonicida, Vibrio salmonicida, Vibrio anguillarum, Moritellaviscosa*	Atlantic salmon (*Salmosalar*)
ALPHA JECT® 3000	injection	*Aeromonsa salmonicida, Vibrio anguillarum*	Atlantic salmon (*Salmosalar*)
ALPHA JECT® 2000	injection	*Vibrio anguillarum, Photobacterium damsela*	Sea bass (*Dicentrarchus labrax*)
Aeromonasveronii vaccine	injection	*Aeromonas veronii*	Sea bass (*Dicentrarchus labrax*)
ALPHA DIP® Vib	immersion	*Vibrio anguillarum*	Sea bass (*Dicentrarchus labrax*)
Alpha ERM Salar	immersion	*Yersinia ruckeri*	Atlantic salmon (*Salmosalar*)
ALPHA DIP® Vibrio	immersion	*Vibrio anguillarum*	Sea bass (*Dicentrarchus labrax*)
ALPHA DIP® 2000	immersion	*Vibrio anguillarum, Photobacteriumdamsela*	Sea bass (*Dicentrarchus labrax*)
AQUAVAC® Strep SA	injection	*Streptococcus agalactiae*	Nile tilapia (*Oreochromis niloticus*)
AQUAVAC® Strep Sa1	injection	*Streptococcus agalactiae*	Nile tilapia (*Oreochromis niloticus*)
AQUAVAC® Strep Si	immersion /injection	*Streptococcus iniae*	Nile tilapia (*Oreochromis niloticus*), Sea bass (*Dicentrarchus labrax*)
AQUAVAC® RELERA™	immersion	*Yersinia ruckeri*	Rainbow trout (*Oncorhynchus mykiss*)
AQUAVAC® VIBRIO	injection/ immersion	*Vibrio anguillarum*	Rainbow trout (*Oncorhynchus mykiss*)
AQUAVAC® Vibrio Pasteurella	injection	*Photobacterium damsela*	European sea bass (*Dicentrarchus labrax*)
AQUAVAC® ERM	immersion/oral	*Yersinia ruckeri*	Rainbow trout (*Oncorhynchus mykiss*)
BLUEGUARD® SRS ORAL	oral	*Piscirickettsia salmonis*	Atlantic salmon (*Salmosalar*), Rainbow trout (*Oncorhynchusmykiss*), Coho salmon (*Oncorhynchuskisutch*), *Chinook salmon* (*Oncorhynchus tshawytscha*)
BLUEGUARD® SRS INYECTABLE	injection	*Piscirickettsia salmonis*	Atlantic salmon (*Salmosalar*), Rainbow trout (*Oncorhynchus mykiss*), Coho salmon (*Oncorhynchus kisutch*), Chinook salmon (*Oncorhynchus tshawytscha*)
Viral pathogens			
ALPHA JECT micro® 1 PD	injection	Salmonid alphavirus 3	Atlantic salmon (*Salmosalar*)
ALPHA JECT® micro 1 ISA	injection	Infectious salmon anaemia virus	Atlantic salmon (*Salmosalar*)

ALPHA JECT® 1000	injection	Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*), Rainbow trout (*Oncorhynchus mykiss*)
ALPHA JECT micro® 1 Noda	injection	Nervous necrosis virus	Sea bass (*Dicentrarchus labrax*)
AQUAVAC® IridoV	injection	Iridovirus	Asian sea bass (*Lates calcarifer*), Nile tilapia (*Oreochromis niloticus*)
BLUEGUARD® IPN ORAL	oral	Infectious pancreatic necrosis virus	Atlantic salmon *(Salmosalar*), *Rainbow trout* (*Oncorhynchus mykiss*), Coho salmon (*Oncorhynchus kisutch*), Chinook salmon (*Oncorhynchus tshawytscha*)
BLUEGUARD® IPN INYECTABLE	injection	Infectious pancreatic necrosis virus	Atlantic salmon *(Salmosalar*), Rainbow trout (*Oncorhynchus mykiss*), Coho salmon (*Oncorhynchus kisutch*)
Combined vaccines against bacterial and viral pathogens			
ALPHA JECT® IPNV-Flavo 0,025	injection	*Flavobacterium psychrophilum*, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*)
ALPHA JECT micro® 7 ILA	injection	*Aeromonas salmonicida, Vibrio anguillarum, Vibrio salmonicida, Moritella viscosa*, Infectious salmon anaemia virus, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*)
ALPHA JECT micro® 6	injection	*Aeromonas salmonicida, Vibrio anguillarum, Vibrio salmonicida, Moritellaviscosa*, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*)
ALPHA JECT® micro 3	injection	*Vibrio ordalii*, *Piscirickettsia salmonis*, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*)
ALPHA JECT® micro 2	injection	*Piscirickettsia salmonis*, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*), Coho salmon (*Oncorhynchus kisutch*), Rainbow trout (*Oncorhynchus mykiss*)
ALPHA JECT® 6–2	injection	*Aeromonas salmonicida, Vibrio salmonicida, Vibrio anguillarum, Moritellaviscosa*, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*)
ALPHA JECT® 5–1	injection	*Piscirickettsia salmonis, Vibrio ordalii*, *Aeromonas salmonicida*, Infectious pancreatic necrosis virus, Infectious salmon anaemia virus,	Atlantic salmon (*Salmosalar*)
ALPHA JECT® 4–1	injection	*Piscirickettsia salmonis, Vibrio ordalii*, *Aeromonas salmonicida*, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*)
ALPHA JECT® 2–2	injection	*Aeromonas salmonicida*, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*)
BLUEGUARD® SRS + IPN INYECTABLE	injection	*Piscirickettsia salmonis*, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*), Rainbow trout (*Oncorhynchus mykiss*), Coho salmon (*Oncorhynchus kisutch*), Chinook salmon (*Oncorhynchus tshawytscha*)
BLUEGUARD IPN + SRS+ As+Vo + ISA INYECTABLE	injection	*Piscirickettsia salmonis, Vibrio ordalii, Aeromonas salmonicida*, Infectious pancreatic necrosis virus, Infectious salmon anaemia	Atlantic salmon (*Salmosalar*)
Viral pathogens			
ALPHA JECT micro® 1 PD	injection	Salmonid alphavirus 3	Atlantic salmon (*Salmosalar*)
ALPHA JECT® micro 1 ISA	injection	Infectious salmon anaemia virus	Atlantic salmon (*Salmosalar*)
ALPHA JECT® 1000	injection	Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*), Rainbow trout (*Oncorhynchusmykiss*)

ALPHA JECT micro® 1 Noda injection	Nervous necrosis virus	Sea bass (*Dicentrarchuslabrax*)
AQUAVAC® IridoV injection	Iridovirus	Asian sea bass (*Lates calcarifer*), Nile tilapia (*Oreochromis niloticus*)
BLUEGUARD® IPN oral ORAL	Infectious pancreatic necrosis virus	Atlantic salmon *(Salmosalar*), *Rainbow trout* (*Oncorhynchus mykiss*), Coho salmon (*Oncorhynchus kisutch*), Chinook salmon (*Oncorhynchus tshawytscha*)
BLUEGUARD® IPN injection INYECTABLE	Infectious pancreatic necrosis virus	Atlantic salmon *(Salmosalar*), Rainbow trout (*Oncorhynchus mykiss*), Coho salmon (*Oncorhynchus kisutch*),
Combined vaccines against bacterial and viral pathogens		
ALPHA JECT® injection IPNV-Flavo 0,025	*Flavobacterium psychrophilum*, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*)
ALPHA JECT injection micro® 7 ILA	*Aeromonas salmonicida, Vibrio anguillarum, Vibrio salmonicida, Moritellaviscosa*, Infectious salmon anaemia virus, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*)
ALPHA JECT injection micro® 6	*Aeromonas salmonicida, Vibrio anguillarum, Vibrio salmonicida, Moritellaviscosa*, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*)
ALPHA JECT® injection micro 3	*Vibrio ordalii*, *Piscirickettsia salmonis*, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*)
ALPHA JECT® injection micro 2	*Piscirickettsia salmonis*, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*), Coho salmon (*Oncorhynchus kisutch*), Rainbow trout (*Oncorhynchus mykiss*)
ALPHA JECT® 6–2 injection	*Aeromonas salmonicida, Vibrio salmonicida, Vibrio anguillarum, Moritellaviscosa*, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*)
ALPHA JECT® 5–1 injection	*Piscirickettsia salmonis, Vibrio ordalii*, *Aeromonas salmonicida*, Infectious pancreatic necrosis virus, Infectious salmon anaemia virus,	Atlantic salmon (*Salmosalar*)
ALPHA JECT® 4–1 injection	*Piscirickettsia salmonis, Vibrio ordalii*, *Aeromonas salmonicida*, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*)
ALPHA JECT® 2–2 injection	*Aeromonas salmonicida*, Infectious pancreatic necrosis virus	Atlantic salmon (*Salmosalar*)

Factors Affecting Antigen Stability

Antigen stability is vital to vaccine potency and effectiveness. Antigen stability can be impacted by a variety of factors, including:

pH

Antigens, especially proteins can degrade their structure and functionality due to a simple pH modification. Denaturation, aggregation or degradation of antigens from pH conditions that are either too high or too low and will reduces the immunogenicity. One strategy in vaccine formulation and storage is to maintain the associated antigen stability within a suitable pH range (Delfi et al., 2021).

Temperature

You can make antigen stability a high-risk item here as temperature changes will considerably affect the will of a nucleotides. Proteins unfold and/or aggregate and may lose biological activity at high temperatures, but freeze-thawing can promote structural changes that cause denaturation (Ma et al., 2020).

Oxidation

Oxidative stress can alter the protein structure and potentially inactive antigens, namely those with cysteine residues or other amino acids that are easily oxidized. Proper antioxidants or packaging materials can thus help in controlling oxidation and maintaining antigen quality (VasileandBaican 2021).

Proteolytic Degradation

Protein-based antigens are often unstable and become degraded by proteases where these enzymes may be present in the formulation or secreted to process endocytosed antigen. Protease inhibitors or stabilizing agents will likely be required to protect against premature degradation (Pishesha et al., 2022).

Adjuvants and Excipients

Vaccine formulations also differ in the adjuvant (immunologic stimuli) and excipient (inactive ingredient that stabilizes the antigen) (Qi and Fox, 2021). Some adjuvants or excipients can impact antigens in a way that changes their structure or potency. It is essential to know the antigens stability which effect its effective and stabile fish vaccines. It is lethal to the virus, however it also keeps antigen conformation and retains optimal vaccine function with suitable formulation strategies such as stabilizers, lyophilization or encapsulation techniques (Du et al., 2022). Addressing the issues of antigen stability, and including relevant bacterial, viral or parasitic antigens in vaccines may allow researchers to deliver effective fish vaccines that ensure long-term success for a sustainable aquaculture industry (Sahoo et al., 2020).

Plant-Based Nanoparticles for Antigen Encapsulation

Over the few last years, plant-based nanoparticles (NPs) have appeared to be an innovative antigen delivery system in vaccine formulation especially for aquaculture species. The nanoparticles described in this work are bio-sourced from different types of plant-based biomaterials and present several benefits over the synthetic ones (Stander et al., 2022).

Types of Plant-based Nanoparticles

Protein-based Nanoparticles

Plant proteins, including zein (corn origin), gliadin wheat or soy protein are widely investigated for the synthesis of nanoparticles. Zein nanoparticles have been recently investigated in detail for their considerable biocompatibility, degradability and also controlled antigen encapsulation/release capabilities (Martínez-López et al., 2020).

Lipid-based Nanoparticles

Plant derived lipids like those found in oils and waxes can be formed into nanoparticles, called nanostructured lipid carriers (NLCs) or solid lipid nanoparticles (SLNs). Efforts at exploring the encapsulation and delivery of antigens within vaccine formulations by lipid-based nanoparticles (Xu et al., 2022).

Advantages and Limitations of Plant-based Nanoparticles

Advantages

Biocompatibility and biodegradability: Plant-based nanoparticles are capable to reduce the risk of adverse reactions and facilitate their clearance from organism due to biocompatibility/biodegradability (Kučuk et al., 2023). Nanoparticles are used as delivery systems for antigens, and they provide protection against antigen degradation while allowing controllable release to increase bioavailability of the antigen in immune cells (Luzuriaga et al., 2021). Adjuvant properties as a side note, some plant nanoparticles like chitosan has an inherent immune stimulating property which can acts as adjuvants to potentiate the desired response (Nordin et al., 2023). Plant-derived nano-technology may facilitate the mucousal or oral administration of vaccines, which can be useful for aquaculture (Ortega-Berlanga and Pniewski 2022).

Limitations

- Batch-to-batch variability: Plant-derived materials may inherently be variegated in composition and performance, which could influence the reproducibility of various nanoparticles formulations (Majeed et al., 2024).
- Scalability and manufacturing challenges: Scaling up plant-based nanoparticle production while maintaining quality and reproducibility is a battle in itself (Zhu et al., 2021).
- Potential immunogenicity: How bad is the potential immunogenicity of plant-derived materials which can be hardly useful for some applications (Umeoguaju et al., 2021).
- Regulatory implications: Although the use of plant-based nanoparticles in vaccine formulations is feasible, this approach may necessitate additional regulatory approvals and/or safety testing (Venkataraman et al., 2021).

Antigen Encapsulation Techniques and Efficiency

Several techniques have been explored for encapsulating antigens within plant-based nanoparticles including:

Emulsion-based techniques: the antigen is dissolved or dispersed within an organic solvent and mixed in emulsification with a water phase containing plant material. The subsequent elimination of the organic solvent produces nanoparticles loaded with antigen (Jamir et al., 2024).

Ionic gelation Antigens may also be encapsulated into nanoparticles via ionic interactions between oppositely charged polymers e.g. chitosan with tripolyphosphate (TPP). This ionic gelation leads to formation of nanoparticles with antigen entrapment (Di Santo et al., 2021).

Coacervation: a technique for physically separating an aqueous liquid into two immiscible phases in which one is enriched with plant based materials and the other contains antigen. The solvent can then be removed, leading to the nanoparticles with entrapped antigens (Yusree et al., 2021). The efficiency of antigen encapsulation relies on different factors such as nature of the antigen and plant matrix, method used for encapsulating and processing parameters. Since encapsulation efficiencies are governed by the same parameters it is of utmost importance that these have to be optimized such that not only can antigens withstand process stresses but also remain thermostable and bioreactive after being loaded (Klojdová et al., 2023). Therefore, through integrating the potential benefits of plant-based nanoparticles with specific encapsulation technologies, fish vaccine antigen delivery systems would reach higher efficacy levels and will drive both researches and manufacturers from academia into small companies. Nevertheless, mitigation of the shortcomings and legal aspects is important in order to ensure such nanoparticle-based vaccines can effectively be translated into realistic applications within aquaculture (Ahmed et al., 2023).

Characterization of Antigen-Loaded Nanoparticles

It is important that the loading antigen and chemistry of the nanoparticulate material being tested are accurately characterized as this information can impact their fluency, stability and immunological potential. Various methods are used to characterize these nanoparticles that include (Dong et al., 2021).

Particle Size, Polydispersity and Zeta Potential

The most valuable parameter is the particle size and its distribution which significantly affects bioavailability, biodistribution as well as cellular uptake of nanoparticles. Based on the size and PDI, DLS is a common technique used to measure hydrodynamic sizes of nanoparticles in suspension (Wang et al., 2020). Zeta potential, which provides information about the surface charge of nanoparticles is also an important parameter that influences their stability and interactions with biological systems as well as ability for antigen encapsulation and release. Electrophoretic light scattering or laser doppler electrophoresis is the method of choice for zeta potential measurements (Rasmussen et al., 2020).

Morphological Analysis (SEM, TEM, AFM)

Morphological analysis is important to recognize the shape, surface topography and internal structure of NPs which may results in different biological activity as antigen carrier. The surface morphology and topography of nanoparticles were analyzed using scanning electron microscopy (SEM) which gives high-resolution images (Zhang et al., 2021). Transmission electron microscopy (TEM) can render nanoparticle size, shape and internal structure at higher magnifications to identify probable defects or irregularities (Mast et al., 2020). Three-dimensional topographic images of nanoparticles are obtained by atomic force microscopy (AFM) at high resolution, which provides the analysis on surface features and roughness (Lutter et al., 2020).

Antigen Loading Capacity and Encapsulation Efficiency

Antigen loading capacity and encapsulation efficiency are the two essential parameters related to how well nanoparticle vaccine delivery systems perform. Antigen loading capacity: It is the measure of how much antigen was loaded or associated with the NPs, usually it's expressed as weight ratio of Ag/NP (Hong et al., 2020). It is the proportion of content in percentage that was encapsulated or associated with the nanoparticles compared to original antigen amount (Ryu et al., 2021). Antigen loading and encapsulation efficiency are quantified using a number of techniques, by:

Quantification by direct methods: the amount of antigen present in nanoparticle formulation can be quantified using techniques such as UV-Visible spectroscopy, Fluorescence spectroscopy or ELISA (Tabatabaei et al., 2021).

Methods for Indirect quantification: includes the separation of non-encapsulated or free antigen from nanoparticle suspension and then, evaluation of this separated part using conventional techniques such as centrifugation, ultrafiltration and size exclusion chromatography (Giordani et al., 2023).

These studies reveal the importance of determining the particle size, surface charge value and morphological characteristics as well as antigen loading/encapsulation efficiency to assess whether there are properties that can be associated with enhanced performance of vaccine delivery using these antigens loaded nanoparticles. These analyses give particularly useful insight into designing nanoparticle formulations, maintaining batch-to-batch consistency and predicting their fate in biological systems (Alqahtani et al., 2020). Additionally to meet regulatory compliance and support the quality and reproducibility of nanoparticle-based vaccine products through comprehensive characterization. Using the correct techniques of characterization and creating defined physicochemical parameters, researchers can help to enable the development and translation of these novel approaches for vaccine delivery systems in aquaculture (and other applications) by industrial producers (Ramos et al., 2022).

Stability Studies of Encapsulated Antigens

The antigen encapsulation stability of these types of delivery vehicles is an important parameter that ensures the

potency and efficacy of nanoparticle-based vaccine formulations. Stability studies: encapsulated antigens shall be evaluated for thermal, pH as well storage and conformational stability under various conditions (Diaz-Arévalo and Zeng 2020).

Thermal Stability (DSC, TGA)

The integrity and bioactivity of the encapsulated antigens are affected by thermal stability during storage, transportation or administration. Thermal properties of antigen loaded nanoparticles were investigated using thermal analysis techniques including: differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) (Cao et al., 2024). DSC thermal stability and compatibility of the encapsulated antigen with nanoparticle matrix are obtained from heat flow measurements associated to such transition temperature, whether it refers a melting point, glass transition or protein denaturation (Kaur et al., 2021), measures the sample mass loss vs temperature allowing you to provide thermal degradation temperatures or identify any potential dehydration, and/or possible decomposition (Wang et al., 2021).

pH Stability

Antigens, such as proteins are very sensitive to pH changes inducing structural rearrangement or denaturation or aggregation. The pH stability studies consist of incubating antigen-loaded nanoparticles in buffers at selected values and measuring the retained amount prior/after time points (Zhang et al., 2021One would then quantify how much of the intact antigen remains with different pH treatment using techniques such as size-exclusion chromatography, SDS-PAGE or enzyme-linked immunosorbent assay (ELISA) (Lieu et al., 2021).

Storage Stability (accelerated and real-time)

Storage stability studies are required for the calculation of shelf-life and long-term activity retention in case of antigen-loaded nanoparticles. These studies could be real-time or but not limited to accelerated. When assessing real-time stability, nanoparticle formulations should be stored under desirable conditions (e.g. refrigerated and room temperature) with an antigen release profile monitored at predetermined intervals for a extended period. Accelerated stability studies: These are the studies that include high temperatures and humidity conditions in order to fasten probable degradation processes. Estimation of shelf life: The data may be subjected to kinetic modeling and extrapolation methods (Reinhart et al., 2023). Size exclusion chromatography, ELISA or bioassays can be employed to follow the integrity and stability of antigen during storage studies across time (Le Basle et al., 2020).

Conformational Stability (CD, FTIR)

The preservation of antigenic, especially protein antigens in their native conformation is indispensable for any further development and utilization. Circular dichroism (CD) and Fourier-transform infrared spectroscopy (FTIR) are used, among others that help to elucidate the conformational stability of the encapsulated antigens (Parlar et al., 2021). CD spectroscopy can differentiate in the absorbance of left and right circularly polarized light inducing by optically active molecules.it gives an information about secondary andtertiary structure of proteins (Subadini et al., 2022). FTIR spectroscopy can be used to detect the vibrational modes of chemical bonds of a peptide bond, make it possible for different secondary structures such as α-helix and β-sheet specific identification in proteins modulate their conformational shifts (Yang et al., 2022). A deeper insight is gained into the thermal, pH and storage stability of antigens in NP formulations with the help of different analytical methods used during NPs-antigens incubation under varied conditions. Such studies are critical to optimize formulation parameters, develop appropriate storage conditions as well as maximize the long-term stability and potency of vaccine products based on nanoparticle (Shi and McHugh 2023)In addition, stability data are an essential prerequisite for regulatory approval and market release of these vaccine preparations. To provide a comprehensive demonstration of stable encapsulation and the potential for freeze drying, we believe that demonstrating these properties in closed studies will help increase the chances to translate innovative delivery system vaccines into practical use both at field (aquaculture or other) levels (Ingle and Fang 2023).

Factors Influencing Antigen Stability

Several factors, as the type of nanoparticles and their properties composition in plant-based nanoparticles, have impact on it; even consumption method how antigen is packedratio or modifying pharmacological compounds are related to (Kyriakoudi et al., 2021). Nanoparticles composition and surface properties: The type of protein, polysaccharide or lipid used to produce plant-based nanoparticles can have a major influence on the stability of encapsulated antigens. For example, zein nanoparticles have shown higher protective effect to antigens compared with protein based because of their hydrophobic nature and high ordered structure (Paliya et al., 2023).

Encapsulation Method and Conditions

Depending on both the specific method used to encapsulate antigens in plant-derived nanoparticles and conditions during the process of encapsulation, antigen stability can be dramatically affected. The use of organic solvents or harsh processing conditions (e.g. high temperatures, shear forces) for emulsion-based encapsulation techniques may negatively affect antigen stability, hence resulting in the loss/modifications/enhancement/neutralization of some epitopes on the

encapsulated antigens during formulation development. In contrast, gentle encapsulation methods such as ionic gelation or coacervation may protect antigen stability more effectively by excluding the material from denaturing conditions (Ramos et al., 2022). The way an antigen interacts with the nanoparticle matrix can greatly affect its stability. The presence of antigen surrounding particles has been suggested to stabilize the biologics by stronger electrostatic, or hydrophobic interactions between the antigen and nanoparticle components providing a protective environment so that protein cannot de-nature/denigrate. Conversely, weak or unfavorable interactions might result in antigen release or denaturation before the particles have been internalized. Such interactions are important for rational vaccine design and thus an understanding is required to formulate nanoparticles that can retain the antigenicity suitable for vaccines (Gomes et al., 2022).

In Vitro and *In Vivo* Evaluations

More detailed assessment by in vitro and in vivo studies of the performance and efficacy of a nanoparticle-based fish vaccine formulation is needed as well. These evaluations generally deal with effects of the carrier on release kinetics and bioavailability of encapsulated antigens, their preservation or modulation by respect to free forms, as well as uptake and processing by antigen-presenting cells (Attaya et al., 2021). The in vitro release studies are carried out to assess the kinetics of antigen release from nanoparticles prepared using plant repository and also for comparing it with animal origin materials. These studies give us an idea of the release profiles that may affect bioavailability and, thus, adjuvanticity or dangers posed by encapsulated antigens. Release studies are typically carried out by dialysis or diffusion based methods in which the nanoparticle formulation is loaded into a dialysis membrane/insert and released antigen is measured over time (Gómez-Mascaraque et al., 2021). The pH value, temperature or presence of enzymes and biological fluids were not always the same in every study for a given physiological environment (Yadav et al., 2023). Preserving the bioactivity and immunogenicity of antigens that are encapsulated with carriers is critical to induce effective immune responses in fish. For assessment of the biological activity such as potential antigens dance released to antigen–antibody fit or effect on immune cells, in vitro assays are performed. Miscellaneous in vivo immunogenicity studies such as adaptive immunity, cellular immune response or to determine whether the nanoparticle-encapsulated antigens can elicit a protective responses against pathogens infecting fish models are also documented (Zhang et al., 2021). Indeed, studies previously conducted with this vaccine format usually included immunizations that followed by monitoring the development of antigen-specific antibodies and cytokines or challenge exams right after stimulating the fish together with live pathogens to verify its protective role. Antigen presenting cells (APCs), such as dendritic cell and macrophages are crucial in the initiation of adaptive immune responses, having an impact on both antigen uptake ability and processing efficiency. Nanoparticle-encapsulated antigens are used to study uptake and intracellular trafficking in vitro with fish APCs or cell lines (Wang et al., 2021).

Flow cytometry, confocal microscopy and immunofluorescence assays techniques could be used to visualize or quantify the uptake of nanoparticles (with a fluorophore) or antigens by professional APCs. Second, APC activation and co-stimulatory molecules or cytokines are examined following administration of nanoparticle-encapsulated antigens. In vivo studies in fish models can additionally help to identify the bio-distribution and uptake of nanoparticle-encapsulated antigens by APCs from different lymphoid organs or tissues, pointing out the potential induction of a systemic or mucosal inflammatory profile (Zhao et al., 2024). Such in vitro and in vivo studies provide detailed information on the performance of different parameters i.e., release kinetics, bioavailability, bioactivity immunogenicity as well as possible fish immune system-interactions associated with nanoparticle-based vaccine formulations for application to protect against early marine pathogen infections. This is essential for the formulation optimization, assessing their efficacy and guaranteeing a high verticalization of this innovative vaccine delivery systems in aquaculture.

Challenges and Future Prospects

Plant-based nanoparticles have shown, unique and promising attributes for effective fish vaccine antigen delivery; nonetheless several inherent challenges need to be tackled along with exploring potential research directions in future that may steer their successful translation and practical implementation. The major bottleneck in the development of plant-based nanoparticle-like vaccines is scale-up and manufacturing. The move from small-scale laboratory fabrication to large volume commercial manufacturing may be complex, demanding a degree of product consistency and batch-to-batch uniformity. Furthermore, the bio-based nature of plant derived materials can result in compositional and property variance necessitating that future material are subject to strict quality control and standardized protocols. Solving these issues through the implementation of strong and scalable manufacturing platforms is an essential requirement for a successful commercialization process around those vaccine products.

The field of regulation for nano-particulate-based vaccines is ever-growing and requires guidelines or framework to affirm their safety as well as the efficacy. These concerns are also why a perfusion-based safety evaluation is needed for nanoparticle toxicity, because the bio-distribution and environmental issues related with nanoparticles. In addition, the regulatory authorities may demand further data and evidence to confirm that plant-derived nanoparticles are suitable for vaccine carriers especially on their immunogenicity, long stability and compatibility with the current process of producing vaccines. One of the attractive aspects about plant-based nanoparticles is their capability to deliver multivalent or combined vaccines. Synthetic vaccines using these nanoparticles may be engineered to include or surface-display multiple antigens, creating the ability to vaccinate against more than one pathogen at a time. Moreover, plant-based nanoparticles

could be combined with other vaccine entities such as immuno-stimulants or adjuvants to potentiate the immune response and offer multi strain protection. These combination strategies can offer insights into designing more effective and holistic vaccine formulations for aquaculture utilization. Current studies are investigating new plant-derived substrates and also different types of nanoparticle formulations for antigen delivery. It also explores the potential of using plant-based polysaccharides, lipids and biopolymers as a viable matrix for nanoparticles. Researchers are also investigating the evolution of targeted nano delivery systems, attaching ligands or antibodies onto nanoparticles to come into contact with immune cells for mucosal access.

Intercalation of advanced characterization methods, such as omics-based profiling and in silico modeling will enable a better comprehension to the interactions among nanoparticles-antigens-immune system leading toward rationalized design pathways for highly effective vaccine formulation. Future research will additionally focus on challenges associated with the stability, time-released manner and bioavailability of encapsulated antigens using new strategies to encase these vaccines and surface modifications in addition to including stabilizers or adjuvants. With the resolution of these challenges and an increasing trend in new research directions, it will be possible to maximize on the potential delivery systems for fish vaccine antigens based on plant-based nanoparticles leading to safe, effective and sustainable vaccination strategies predominantly intended towards maintaining healthy aquatic environments.

Summary of Key Findings

Proteins, polysaccharides and lipids are the key plant-based materials used to generate nanoparticles that not only show good biocompatibility but also have bio-degradation properties with an added advantage for targeted delivery of antigens. Such nanoparticles can protect antigens from degradation, increase their bio-availability and enable controlled release, hence improving the immune response. First the exhaustive researches were dedicated efforts, plant-based nanoparticles loaded with antigen characterization, stability studies under changing conditions, release kinetics bioactivity and immunogenicity. Properties as nanoparticle composition, method of encapsulation and the presence of stabilizers or adjuvants do play a key role in stability assessment. The application of plant-derived nanoparticles demonstrates great promise as sustainable oral vaccines for fish, due to their capacity to encapsulate a variety of bacterial, viral and parasitic antigens which can be directed against different infectious diseases that affect aquaculture species. Moreover, their multivalent and combination vaccine delivery potential as well as the versatility regarding routes of administration including mucosal/oral immunizations makes these nanoparticle-based formulations highly appealing.

Outlook and Future Research Opportunities

The progress in the plant-based nanoparticle based fish vaccines development and implementation can be defined as a significant one so far, but there are still numerous challenges. These formulations are postulated to translate well into scale-up and manufacturing, however further work needs to be done in addressing regulatory aspects of these platforms alongside safety considerations. Future directions of research should include the discovery of alternative plant sources, design and engineering targeted delivery systems incorporating modern methods for characterization along with omics based approaches. These efforts should also focus on the enhancement of stability, controlled-release and bio-availability properties by using innovative encapsulation procedures (micro or nano) as well as surface modifications allowing for their release in a specific target dictionary. This will require interdisciplinary collaborations and stakeholder partnerships between researchers, industry and regulatory authorities to address these obstacles together which are crucial for future success of plant-based nanoparticles in the delivery of effective preventive fish vaccines on a broad scale. This highlights the potential applications as well challenges faced by plant-based nanoparticles in aquaculture and provides insights for enabling research direction to harness advantages of nano-carriers towards economically viable, safe vaccination procedures optimized against major pathogens on which improvements are needed for achieving sustainability along with growth goals.

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