Chapter 64

From Lab to Leash: How Biotechnology is Transforming Animal Vaccines

Khadija Yasmeen[1], Najida Irfan[2], Iram Ilyas[2], Fazeela Arshad[2,3], Tehreem Tufail[2], Maryam Ashiq[2], Muhammad Asif[2], Imran Amin[2], Muhammad Naeem Riaz[1], Farhana Amin[1] and Fazal ur Rehman[1]

[1]National Institute of Genomics and Advanced Biotechnology (NIGAB), NARC, Islamabad
[2]National Institute for Biotechnology and Genetic Engineering (NIBGE-C), PIEAS, Faisalabad
[3]The Roslin Institute, University of Edinburgh, Easter Bush Campus, Scotland
*Corresponding author: najidairfan97@gmail.com

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

Received: 15-May-2024
Revised: 20-July-2024
Accepted: 15-Aug-2024

A Publication of Unique Scientific Publishers

Cite this Article as: Yasmeen K, Irfan N, Ilyas I, Arshad F, Tufail T, Ashiq M, Asif M, Amin I, Riaz MN, Amin F and Rehman F, 2024. From Lab to Leash: How Biotechnology is Transforming Animal Vaccines. In: Alvi MA, Rashid M, Zafar MA, Mughal MAS and Toor SI (eds), Complementary and Alternative Medicine: Immunization/Vaccinology. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 562-572. https://doi.org/10.47278/book.CAM/2024.474

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.

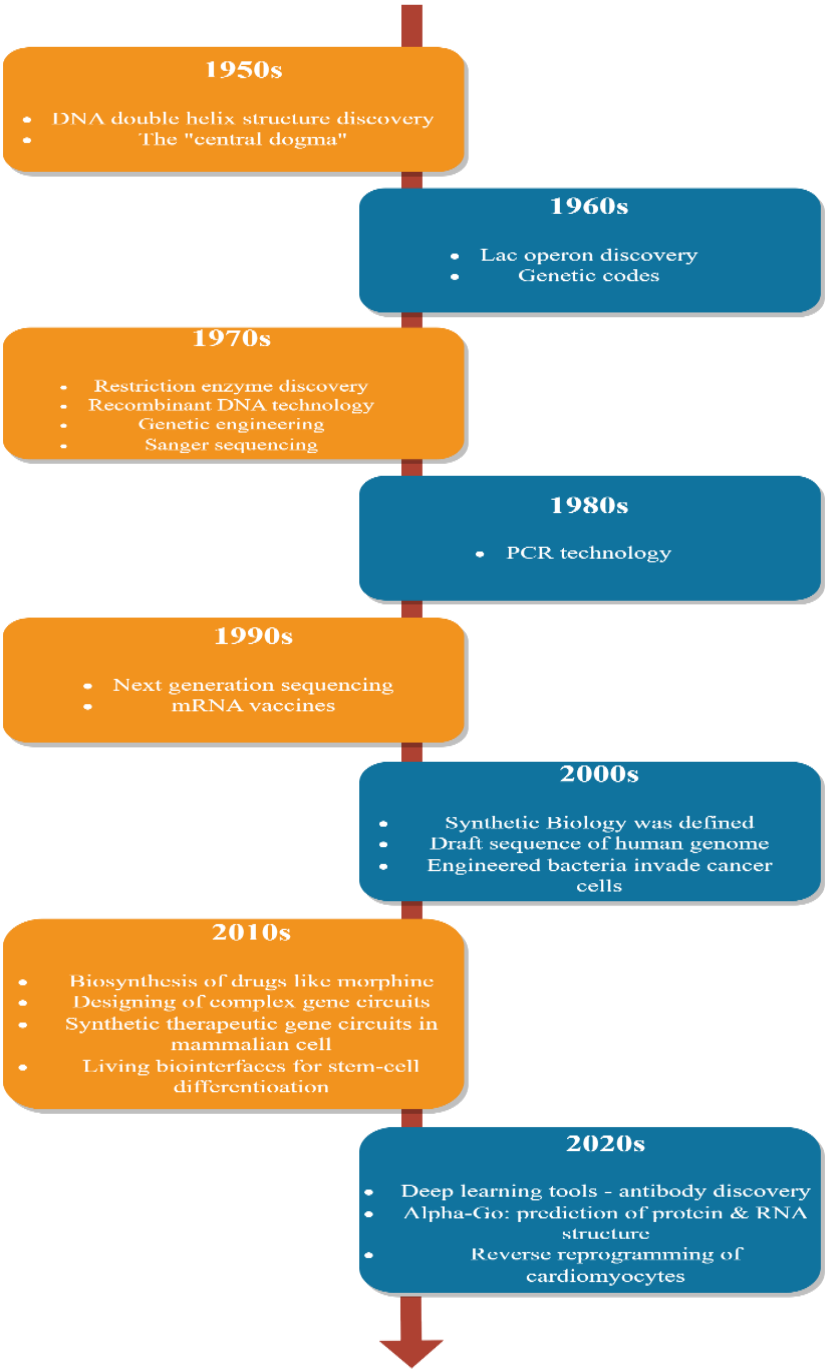

Fig. 1: Timeline of major milestones in Synthetic Biology. From 1950s to 2020s (Yan, et al., 2023)

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).

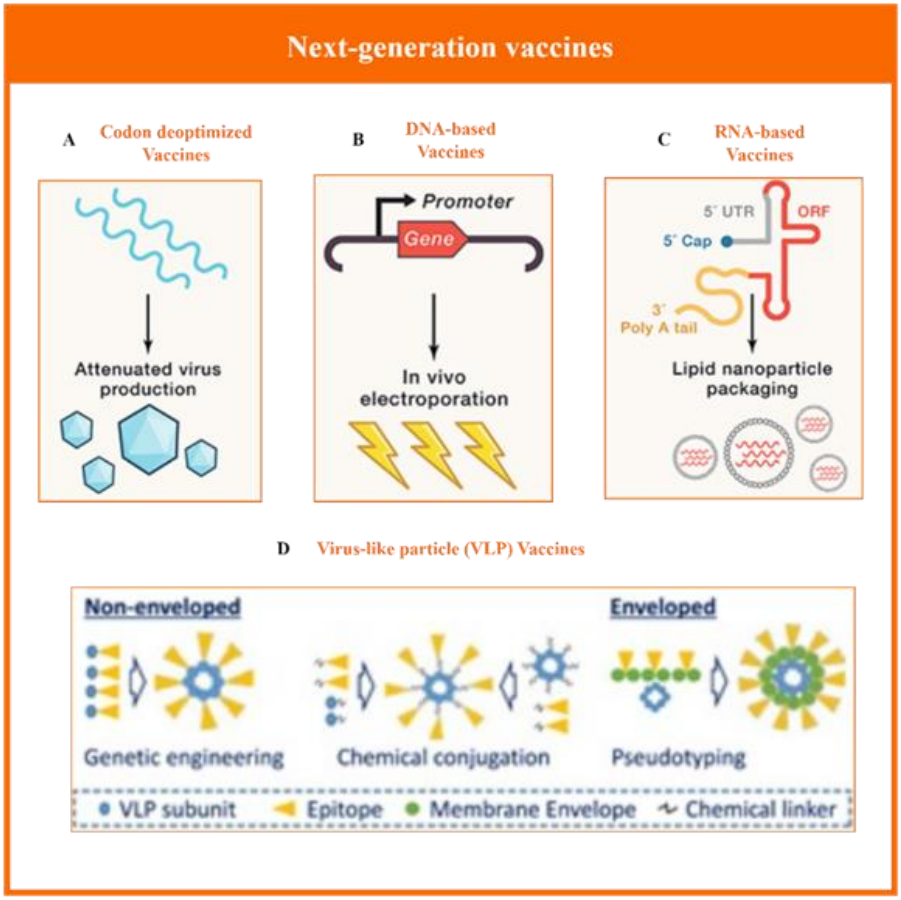

Fig. 2: Next-generation Vaccines (Data source (Charlton Hume, et al., 2019;Tan, et al., 2021))

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).

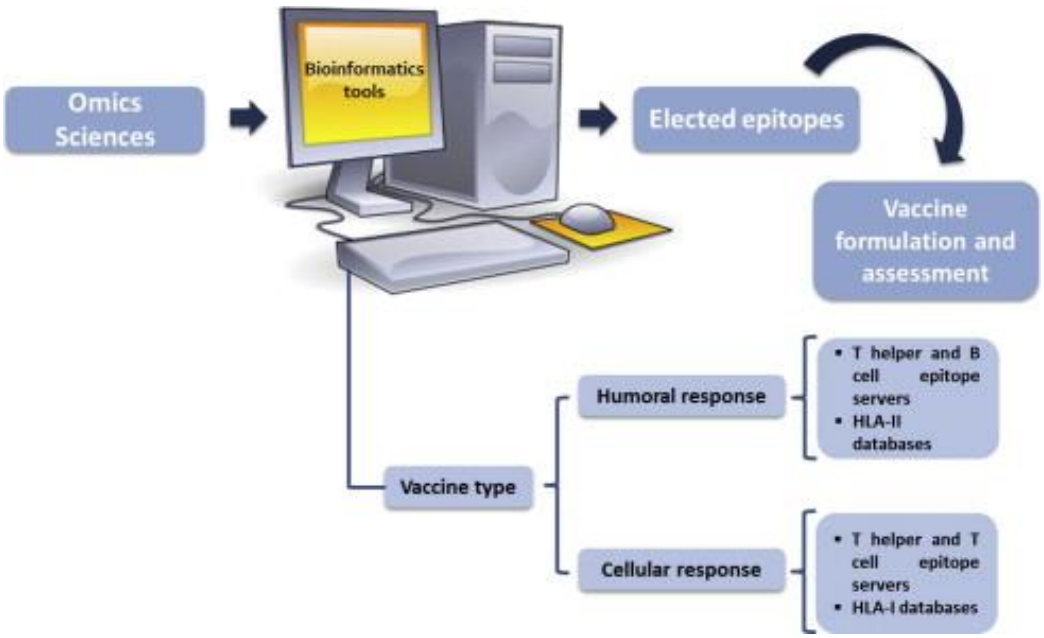

Fig. 3: Workflow schematics of vaccine development (Soria-Guerra, et al., 2015)

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.

REFERENCES

Abdaal, K., Batool, A., Navid, M., Ahmed, S., Qazi, A., Safdar, W., Ali, H., Rashid, M. and Rafaqat, S. (2024). Advancements in Vaccination Strategies: From Historical Milestones to Modern Innovations in Viral Disease Prevention and Public Health. *Research Journal of Veterinary Practitioners*, 12(1), 11-21.

Abdelaziz, K., Helmy, Y. A., Yitbarek, A., Hodgins, D. C., Sharafeldin, T. A. and Selim, M. S. (2024). Advances in Poultry Vaccines: *Leveraging Biotechnology for Improving Vaccine Development, Stability, and Delivery. Vaccines*, 12(2), 134.

Cai, Z., Xin, F., Wei, Z., Wu, M., Lin, X., Du, X., Chen, G., Zhang, D., Zhang, Z. and Liu, X. (2020). Photodynamic Therapy Combined with Antihypoxic Signaling and Cpg Adjuvant as an in Situ Tumor Vaccine Based on Metal–Organic Framework Nanoparticles to Boost Cancer Immunotherapy. *Advanced Healthcare Materials*, 9(1), 1900996.

Cavaillon, J.-M. (2022). From Bacterial Poisons to Toxins: The Early Works of Pasteurians. Toxins, 14(11), 759.

Celis-Giraldo, C. T., López-Abán, J., Muro, A., Patarroyo, M. A. and Manzano-Román, R. (2021). Nanovaccines against Animal Pathogens*: The Latest Findings. Vaccines*, 9(9), 988.

Charlton Hume, H. K., Vidigal, J., Carrondo, M. J., Middelberg, A. P., Roldão, A. and Lua, L. H. (2019). Synthetic Biology for Bioengineering Virus-Like Particle Vaccines. *Biotechnology and Bioengineering*, 116(4), 919-935.

Cheng, L., Wang, Y. and Du, J. (2020). *Human Papillomavirus Vaccines: An Updated Review. Vaccines*, 8(3), 391.

Chivukula, S., Plitnik, T., Tibbitts, T., Karve, S., Dias, A., Zhang, D., Goldman, R., Gopani, H., Khanmohammed, A. and Sarode, A. (2021). *Development of Multivalent Mrna Vaccine Candidates for Seasonal or Pandemic Influenza. npj Vaccines*, 6(1), 153.

Cid, R. and Bolívar, J. (2021). Platforms for Production of Protein-Based Vaccines: From Classical to Next-Generation Strategies. *Biomolecules*, 11(8), 1072.

D'Amico, C., Fontana, F., Cheng, R. and Santos, H. A. (2021). Development of Vaccine Formulations: Past, Present, and Future. *Drug Delivery and Translational Research*, 11(2), 353-372. https://doi.org/10.1007/s13346-021-00924-7

De Groot, A. S., Moise, L., Terry, F., Gutierrez, A. H., Hindocha, P., Richard, G., Hoft, D. F., Ross, T. M., Noe, A. R. and Takahashi, Y. (2020). *Better Epitope Discovery, Precision Immune Engineering, and Accelerated Vaccine Design Using Immunoinformatics Tools. Frontiers in Immunology*, 11, 442.

Delany, I., Rappuoli, R. and De Gregorio, E. (2014). Vaccines for the 21st Century. *EMBO Molecular Medicine*, 6(6), 708-720.

Essink, B., Chu, L., Seger, W., Barranco, E., Le Cam, N., Bennett, H., Faughnan, V., Pajon, R., Paila, Y. D. and Bollman, B. (2023). The Safety and Immunogenicity of Two Zika Virus Mrna Vaccine Candidates in Healthy Flavivirus Baseline Seropositive and Seronegative Adults: The Results of Two Randomised, Placebo-Controlled, Dose-Ranging, Phase 1 Clinical Trials. *The Lancet Infectious Diseases*, 23(5), 621-633.

Francis, M. J. (2018). Recent Advances in Vaccine Technologies. Veterinary Clinics: *Small Animal Practice*, 48(2), 231-241.

Fulber, J. P. and Kamen, A. A. (2022). *Development and Scalable Production of Newcastle Disease Virus-Vectored Vaccines for Human and Veterinary Use. Viruses*, 14(5), 975.

Ghattas, M., Dwivedi, G., Lavertu, M. and Alameh, M.-G. (2021). Vaccine Technologies and Platforms for Infectious Diseases: *Current Progress, Challenges, and Opportunities*. *Vaccines*, 9(12), 1490.

Gheibi Hayat, S. M. and Darroudi, M. (2019). Nanovaccine: A Novel Approach in Immunization. *Journal of Cellular Physiology*, 234(8), 12530-12536.

Humphreys, I. R. and Sebastian, S. (2018). *Novel Viral Vectors in Infectious Diseases. Immunology*, 153(1), 1-9.

Janson, G., Grottesi, A., Pietrosanto, M., Ausiello, G., Guarguaglini, G. and Paiardini, A. (2019). Revisiting the "Satisfaction of Spatial Restraints" *Approach of Modeller for Protein Homology Modeling. PLoS Computational Biology*, 15(12), e1007219.

Jiang, X.-t. and Liu, Q. (2023). Mrna Vaccination in Breast Cancer: Current Progress and Future Direction. *Journal of Cancer Research and Clinical Oncology*, 149(11), 9435-9450.

Joachim, A. (2016). Vaccination against Parasites–Status Quo and the Way Forward. *Porcine Health Management*, 2(1), 30.

Jorge, S. in Dellagostin, O. A. (2017). The Development of Veterinary Vaccines: A Review of Traditional Methods and Modern Biotechnology Approaches. *Biotechnology Research and Innovation*, 1(1), 6-13.

Kahn, L. H. (2006). Confronting Zoonoses, Linking Human and Veterinary Medicine. *Emerging infectious diseases*, 12(4), 556.

Kar, T., Narsaria, U., Basak, S., Deb, D., Castiglione, F., Mueller, D. M. and Srivastava, A. P. (2020). A Candidate Multi-Epitope Vaccine against Sars-Cov-2. *Scientific Reports*, 10(1), 10895.

Karunakaran, B., Gupta, R., Patel, P., Salave, S., Sharma, A., Desai, D., Benival, D. and Kommineni, N. (2023). Emerging Trends in Lipid-Based Vaccine Delivery: A Special Focus on Developmental Strategies, *Fabrication Methods, and Applications. Vaccines* 2023, 11, 661. V.

Kashte, S., Gulbake, A., El-Amin III, S. F. and Gupta, A. (2021). Covid-19 Vaccines: Rapid Development, Implications, *Challenges and Future Prospects. Human cell*, 34(3), 711-733.

Kennedy, R. B., Ovsyannikova, I. G., Palese, P. and Poland, G. A. (2020). Current Challenges in Vaccinology. *Frontiers in Immunology*, 11, 541543.

Khan, F. A. (2020). Biotechnology Fundamentals Third Edition (prevajalec, Trans.). *CRC Press*.

Kijanka, G., Bee, J. S., Korman, S. A., Wu, Y., Roskos, L. K., Schenerman, M. A., Slütter, B. and Jiskoot, W. (2018). Submicron Size Particles of a Murine Monoclonal Antibody Are More Immunogenic Than Soluble Oligomers or Micron Size Particles Upon Subcutaneous Administration in Mice. *Journal of Pharmaceutical Sciences*, 107(11), 2847-2859.

Kolaskar, A. S. in Tongaonkar, P. C. (1990). A Semi-Empirical Method for Prediction of Antigenic Determinants on Protein Antigens*. FEBS Letters*, 276(1-2), 172-174.

Kolotilin, I., Topp, E., Cox, E., Devriendt, B., Conrad, U., Joensuu, J., Stöger, E., Warzecha, H., McAllister, T. and Potter, A. (2014). Plant-Based Solutions for Veterinary Immunotherapeutics and Prophylactics. *Veterinary Research*, 45, 1-12.

Kübler, H., Scheel, B., Gnad-Vogt, U., Miller, K., Schultze-Seemann, W., Vom Dorp, F., Parmiani, G., Hampel, C., Wedel, S. and Trojan, L. (2015). Self-Adjuvanted Mrna Vaccination in Advanced Prostate Cancer Patients: A First-in-Man Phase I/Iia Study. *Journal for Immunotherapy of Cancer*, 3, 1-14.

Larsen, S. E., Baldwin, S. L. and Coler, R. N. (2023). Tb Vaccines Update: Is an Rna-Based Vaccine Feasible for Tb? *International Journal of Infectious Diseases*.

LaTourette II, P. C., Awasthi, S., Desmond, A., Pardi, N., Cohen, G. H., Weissman, D. and Friedman, H. M. (2020). Protection

against Herpes Simplex Virus Type 2 Infection in a Neonatal Murine Model Using a Trivalent Nucleoside-Modified Mrna in Lipid Nanoparticle Vaccine. *Vaccine*, 38(47), 7409-7413.

Le Duc, S. (1914). The Mechanism of Life (prevajalec, Trans.). *Rebman Company*.

Legastelois, I., Buffin, S., Peubez, I., Mignon, C., Sodoyer, R. and Werle, B. (2017). Non-Conventional Expression Systems for the Production of Vaccine Proteins and Immunotherapeutic Molecules. *Human Vaccines and Immunotherapeutics*, 13(4), 947-961.

Li, J., Liu, Q., Liu, J., Wu, X., Lei, Y., Li, S., Zhao, D., Li, Z., Luo, L. and Peng, S. (2022). An Mrna-Based Rabies Vaccine Induces *Strong Protective Immune Responses in Mice and Dogs. Virology Journal*, 19(1), 184.

Li, M., Zhou, H., Yang, C., Wu, Y., Zhou, X., Liu, H. and Wang, Y. (2020). Bacterial Outer Membrane Vesicles as a Platform for Biomedical Applications: An Update. *Journal of Controlled Release*, 323, 253-268.

Mantel, C. and Cherian, T. (2020). New Immunization Strategies: Adapting to Global Challenges. *Bundesgesundheitsblatt-Gesundheitsforschung-Gesundheitsschutz*, 63(1), 25-31.

Matić, Z. and Šantak, M. (2022). Current View on Novel Vaccine Technologies to Combat Human Infectious Diseases. *Applied Microbiology and Biotechnology*, 106, 25-56.

Meeusen, E. N., Walker, J., Peters, A., Pastoret, P.-P. and Jungersen, G. (2007). Current Status of Veterinary Vaccines. *Clinical Microbiology Reviews*, 20(3), 489-510.

Moni, S. S., Abdelwahab, S. I., Jabeen, A., Elmobark, M. E., Aqaili, D., Ghoal, G., Oraibi, B., Farasani, A. M., Jerah, A. A., Alnajai, M. M. A. and Mohammad Alowayni, A. M. H. (2023). Advancements in Vaccine Adjuvants: *The Journey from Alum to Nano Formulations. Vaccines*, 11(11), 1704. https://www.mdpi.com/2076-393X/11/11/1704

Moreira Jr, C., da Cunha, C. E. P., Moreira, G. M. S. G., Mendonça, M., Salvarani, F. M., Moreira, Â. N. and Conceição, F. R. (2016). Protective Potential of Recombinant Non-Purified Botulinum Neurotoxin Serotypes C and D. *Anaerobe*, 40, 58-62.

Moss, B., Smith, G. L., Gerin, J. L. and Purcell, R. H. (1984). Live Recombinant Vaccinia Virus Protects Chimpanzees against Hepatitis B. *Nature*, 311(5981), 67-69.

Nagy, G., Emo, L. and Pál, T. (2008). Strategies for the Development of Vaccines Conferring Broad-Spectrum Protection. *International Journal of Medical Microbiology*, 298(5-6), 379-395.

Nascimento, I. P. and Leite, L. (2012). Recombinant Vaccines and the Development of New Vaccine Strategies. *Brazilian Journal of Medical and Biological Research*, 45, 1102-1111.

Nooraei, S., Sarkar Lotfabadi, A., Akbarzadehmoallemkolaei, M. and Rezaei, N. (2023). Immunogenicity of Different Types of Adjuvants and Nano-Adjuvants in Veterinary Vaccines: A Comprehensive Review. *Vaccines*, 11(2), 453. https://www.mdpi.com/2076-393X/11/2/453

Pfeifer, B. A., Beitelshees, M., Hill, A., Bassett, J. and Jones, C. H. (2023). Harnessing Synthetic Biology for Advancing Rna Therapeutics and Vaccine Design. *NPJ Systems Biology and Applications*, 9(1), 60.

Poland, G. A., Ovsyannikova, I. G. and Jacobson, R. M. (2008). Personalized Vaccines: *The Emerging Field of Vaccinomics. Expert Opinion on Biological Therapy*, 8(11), 1659-1667.

Rasool, N., Farooq, A., Asrar, R., and Qureshi, A. S. (2023). *Continental Veterinary Journal*.

Rao, N. V., Rho, J. G., Um, W., Ek, P. K., Nguyen, V. Q., Oh, B. H., Kim, W. and Park, J. H. J. P. (2020). *Hyaluronic Acid Nanoparticles as Nanomedicine for Treatment of Inflammatory Diseases*. 12(10), 931.

Rose, P. W., Bi, C., Bluhm, W. F., Christie, C. H., Dimitropoulos, D., Dutta, S., Green, R. K., Goodsell, D. S., Prlić, A. and Quesada, M. (2012). The Rcsb Protein Data Bank: New Resources for Research and Education. *Nucleic Acids Research*, 41(D1), D475-D482.

Safavi, A., Kefayat, A., Abiri, A., Mahdevar, E., Behnia, A. H. and Ghahremani, F. (2019). In Silico Analysis of Transmembrane Protein 31 (Tmem31) Antigen to Design Novel Multiepitope Peptide and DNA Cancer Vaccines against Melanoma. *Molecular Immunology*, 112, 93-102.

Saleh, A., Qamar, S., Tekin, A., Singh, R. and Kashyap, R. (2021). Vaccine Development Throughout History. *Cureus*, 13(7).

Sander, V. A., Sánchez López, E. F., Mendoza Morales, L., Ramos Duarte, V. A., Corigliano, M. G. and Clemente, M. (2020). Use of Veterinary Vaccines for Livestock as a Strategy to Control Foodborne Parasitic Diseases. *Frontiers in Cellular and Infection Microbiology*, 10, 288.

Shams, H. (2005). Recent Developments in Veterinary Vaccinology. *The Veterinary Journal*, 170(3), 289-299.

Shao, D., Lu, M. M., Zhao, Y. W., Zhang, F., Tan, Y. F., Zheng, X., Pan, Y., Xiao, X. A., Wang, Z., Dong, W. F., Li, J. and Chen, L. (2017). The Shape Effect of Magnetic Mesoporous Silica Nanoparticles on Endocytosis, *Biocompatibility and Biodistribution. Acta Biomater*, 49, 531-540. https://doi.org/10.1016/j.actbio.2016.11.007

Shuja, A., Qureshi, J. A. and Shuja, N. (2022). Traditional and Recent Approaches for the Development of Animal Vaccines. A Review. *Pakistan Journal of Medical and Health Sciences*, 16(12), 460-460.

Smith, T. R., Patel, A., Ramos, S., Elwood, D., Zhu, X., Yan, J., Gary, E. N., Walker, S. N., Schultheis, K. and Purwar, M. (2020). Immunogenicity of a DNA Vaccine Candidate for Covid-19. *Nature Communications*, 11(1), 2601.

Soria-Guerra, R. E., Nieto-Gomez, R., Govea-Alonso, D. O. and Rosales-Mendoza, S. (2015). An Overview of Bioinformatics Tools for Epitope Prediction: Implications on Vaccine Development. *Journal of Biomedical Informatics*, 53, 405-414. https://doi.org/10.1016/j.jbi.2014.11.003

Tan, X., Letendre, J. H., Collins, J. J. and Wong, W. W. (2021). Synthetic Biology in the Clinic: Engineering Vaccines,

Diagnostics, and Therapeutics. Cell, 184(4), 881-898.

Tandrup Schmidt, S., Foged, C., Smith Korsholm, K., Rades, T. and Christensen, D. (2016). Liposome-Based Adjuvants for Subunit Vaccines: Formulation Strategies for Subunit Antigens and Immunostimulators. *Pharmaceutics*, 8(1), 7.

Tebas, P., Kraynyak, K. A., Patel, A., Maslow, J. N., Morrow, M. P., Sylvester, A. J., Knoblock, D., Gillespie, E., Amante, D. and Racine, T. (2019). Intradermal Syncon® Ebola Gp DNA Vaccine Is Temperature Stable and Safely Demonstrates Cellular and Humoral Immunogenicity Advantages in Healthy Volunteers. *The Journal of Infectious Diseases*, 220(3), 400-410.

Weiner, D. B. and Nabel, G. (2018). *Development of Gene-Based Vectors for Immunization*. 1305.

Wollner, C. J., Richner, M., Hassert, M. A., Pinto, A. K., Brien, J. D. and Richner, J. M. (2021). A Dengue Virus Serotype 1 Mrna-Lnp Vaccine Elicits Protective Immune Responses. *Journal of Virology*, 95(12), 10.1128/jvi. 02482-02420.

Yan, X., Liu, X., Zhao, C. and Chen, G.-Q. (2023). Applications of Synthetic Biology in Medical and Pharmaceutical Fields. *Signal Transduction and Targeted Therapy*, 8(1), 199.

Yenkoidiok-Douti, L. and Jewell, C. M. (2020). Integrating Biomaterials and Immunology to Improve Vaccines against Infectious Diseases. *ACS Biomaterials Science and Engineering*, 6(2), 759-778.

Yurina, V. and Adianingsih, O. R. (2022). Predicting Epitopes for Vaccine Development Using Bioinformatics Tools. *Therapeutic Advances in Vaccines and Immunotherapy*, 10, 25151355221100218. https://doi.org/10.1177/25151355221100218

Zhang, N. and Nandakumar, K. S. (2018). Recent Advances in the Development of Vaccines for Chronic Inflammatory Autoimmune Diseases. *Vaccine*, 36(23), 3208-3220.

Zhang, Y., Lin, S., Wang, X. Y. and Zhu, G. (2019). Nanovaccines for Cancer Immunotherapy. Wiley Interdisciplinary Reviews: *Nanomedicine and Nanobiotechnology*, 11(5), e1559.

Zuo, K., Gao, W., Wu, Z., Zhang, L., Wang, J., Yuan, X., Li, C., Xiang, Q., Lu, L. and Liu, H. (2024). Evolution of Virology: Science History through Milestones and Technological Advancements. *Viruses*, 16(3), 374.