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CRISPR CAS-9 Mediated Genome Editing and One Health Related Diseases

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INTRODUCTION

One Health Concept

The concepts of One Health began in the late 19th and 20th centuries, firstly as One Medicine, then as One World and finally as One Health (Atlas 2012) with the contributions of Rodulf Virchow, known as the "Father of comparative medicine, cellular pathology and veterinary pathology" and Willian Oster, known as "Father of Modern Medicine". The most commonly used definition suggested by the One Health Global Network is: "One Health recognizes that the health of humans, animals and ecosystems are interconnected. It involves applying a coordinated, collaborative, multidisciplinary and cross-sectoral approach to address potential or existing risks at the animal-human-ecosystems interface" (Mackenzie and Jeggo 2019).

Significance of One Health

The significance and importance of one health have increased enormously over the past three decades. It has been observed for a long time that the majorities of novel and newly emerging infectious diseases were zoonotic and originated in animals, particularly wildlife. Such diseases are primarily linked to human interruption and activities related to ecosystems, land use, agriculture intensification, suburbanization, industrialization, deforestation and international trade. These factors make one health highly important to make sure that the health of people, animals and the environment is safe. These are interconnected to make one health triad (Atlas 2012) (Fig. 1).

The one health concept is based on the early detection and timely diagnosis and treatment of emerging zoonotic diseases and their prevention and control. Many diseases have a significant social impact in poor, developing or underdeveloped countries. It also leads to antimicrobial resistance (AMR), because resistance develops as a result of misuse or under usage of antimicrobials which may develop in human beings, animals and the environment and transfer from one to the other (Mackenzie and Jeggo 2019).

Emerging and Endemic Zoonotic Diseases:

Emerging and endemic zoonotic diseases have devastating consequences to human health in the past and these damages are still present (Table 1). Many emerging infectious diseases have emerged and spread worldwide like Zika, Ebola, Congo fever, Swine flu, SARS and MERS, and then the COVID-19 pandemic. In recent years, it has been seen that several zoonotic diseases have emerged and spread in both animals and humans. More than 6-70% of human infectious diseases are zoonotic and have an animal origin. Similarly, out of five newly emerging human diseases each year, three diseases have animal origin. In spite of the great deal of research, infectious diseases like tick borne diseases, anthrax, TB, brucellosis, Coronavirus infection and rabies are still threat to human health and welfare (Atlas 2012). A target based research like target oriented genome editing (discussed in detail in the later parts of the chapter) is much needed to tackle the problem of diseases related to one health as per mentioned in Table 1.

Genome editing technique and One Health

The development of genome editing technology in the 1970s marked the beginning of a new era for biology. Genome editing technology came in action from the mid-era of the 20th century. Bio-engineering technology involves knockout, knock-in, and replacement of specific section of genomic sequences resulting in controlled and

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desired alteration in a eukaryotic cell. It has revolutionized the human way of perceiving things regarding welfare and survival (Gaj et al. 2016). Pronuclear micro-injection, developed in the 1980s, was the one of the principal technique exercised for genetically modified organisms (Gordon et al. 2020). The process includes the transfer of

Table 1: Summary of various one health related diseases caused by viral, bacterial and parasitic pathogens

Pathogen Type	Pathogens	Natural Host	Mode Of transmission	Disease caused	References
Viral Agent	Lyssaviruses	Dog, Bat, Cat, Foxes	bites or scratches of infected animal	Rabies	(León et al. 2021)
	West Nile Virus	Birds	Culex species of Mosquitoes	West Nile Fever	(Petersen et al. 2013)
	SARS-CoV-2	Bats	direct, indirect, or close contact with saliva or respiratory droplets of infected people	Respiratory Track Disease	(Yang et al. 2020)
	Monkeypox virus	monkeys, anteaters, hedgehogs, rodents	direct contact (bites, scratches, slaughtering) with infected animals	pimples or blisters throughout the body (Similar to smallpox)	(Pastula and Tyler 2022).
	Ebola virus	Bat or nonhuman primate	Direct contact with an infected animal , contagious	Ebola hemorrhagic fever	(Groseth et al. 2007)
	Zika Virus (Flaviviruses)	Nonhuman and human primates	Aedes aegypti and Aedes albopictus	Guillain-Barré syndrome, microcephaly	(Musso and Gubler 2016)
	Hanta Virus	Rodents	Direct or indirect contact with rodent's urine, droppings or saliva	Human Pulmonary Syndrome (HPS), Hemorrhagic fever renal syndrome (HFRS)	(Muranyi 2005)
	Swine Influenza (influenza virus H1N1)	Pigs	Asymptomatic carrier pigs or Pig aerosols	Respiratory disease in humans	(Olsen 2002)
	Avian Influenza Virus	Domestic Poultry or Water Bird	Direct contact between infected bird's feces, mouth or nose secretions.	Respiratory track Infection	(Lee and Saif 2009)
Bacterial Agent	Bacillus anthracis	sheep, cattle, horses and goats	Direct or indirect contact with infected animal	Anthrax	(Sidwa et al. 2020)
	Borrelia burgdorferi	mice, chipmunks, raccoons, squirrels, lizards, White Footed Mouse	ticks	Lyme Disease	(Bernard et al. 2019).
	Yersinia pestis	Rodents	Fleas	Plague	(Prentice and Rahalison 2007)
	Brucella spp.	Goats and sheep (undercooked meat or consuming unpasteurized /raw dairy products)	meningitis and endocarditis	Brucellosis	(Karponi et al. 2019)
	Salmonella sp.	Domesticated or/and wild animals (poultry, cattle, dogs, rodents, swine and cats	Food or water contaminated by feces	Gastroenteritis, diarrhea, tyohoid and enteric fever	(Ajmera and Shabbir 2022)
	Giardia duodenalis	Rodents, cows, and sheep	Direct or indirect contact with contaminated water, food, surfaces, or objects	Giardiasis	(Sprong 2009)
Parasitic Agent	Schistosoma mansoni, S. haematobium, and S. japonicum	snail	Drinking Contaminated water containing parasite eggs	Schistosomiasis	(Gryseels et al. 2006)
	Toxoplasma gondii	Cats	Eating undercooked contaminated meat, exposure from infected cat feces	Toxoplasmiosis	(Remington et al. 2004)
	Trematodes	Cats, dogs, foxes, pigs and rodents	Consumption of water and food contaminated with trematodes in the larval stages.	Trematodiasis	(Keiser and Utzinger 2005).
	Ticks (Ixodid Tick)	Cattle, sheep and goats.	Contact with infected ticks or animal blood	Crimean-Congo Hemorrhagic Fever	(Whitehouse 2004).
	Trypanosoma brucei	Domestic cattle	tsetse fly, Glossina sp.	Trypanosomiasis	(Brun et al. 2010)

foreign DNA into the nucleus of a fertilized egg which develops into a transgenic animal. The initial trial of zygote microinjection for producing transgenic animals like pigs, sheep, and rabbits was carried out in 1985

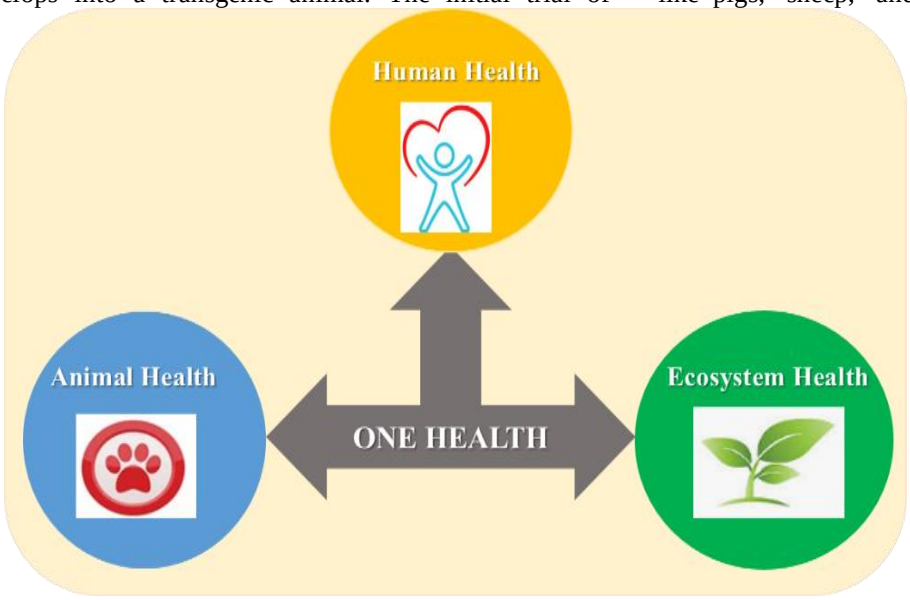


Fig. 1: The One Health Triad comprises of three components including human health, animal health and ecosystem health.

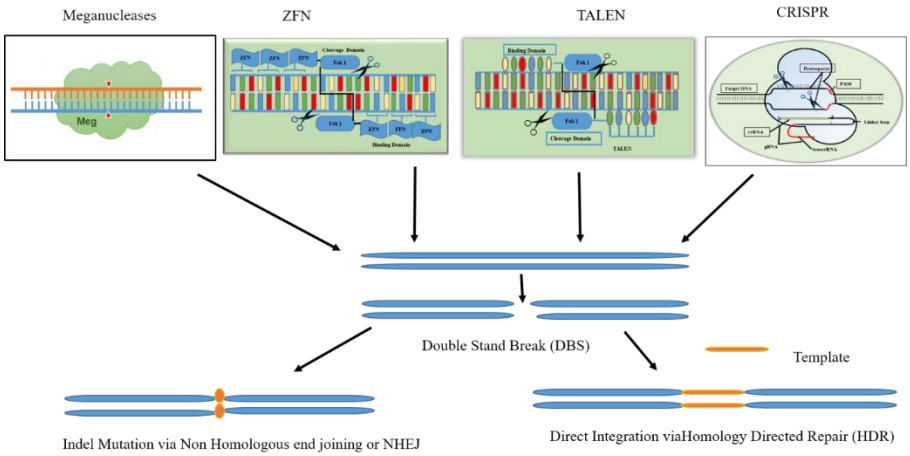


Fig. 2: Repair mechanisms of Gene Editing Techniques including meganucleases, zinc finger (ZF) nucleases, transcription activator-like effectors nucleases (TALENs) and CRISPR-Cas9 system.

(Navarro-Serna et al. 2020). In the late 1990s, Somatic Cell Nuclear Transfer (SCNT) further modifies genome editing technique by facilitating the transfer of the nucleus from a somatic cell into the enucleated fertilized egg cell. The transfer results in the production of an organism genetically identical to the enucleate nucleus (Gouveia et al. 2020).

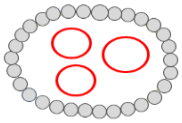
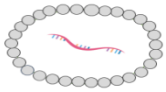
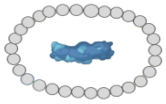
DNA double strand breaks (DSBs) is considered the most effective way to do site specific genome editing Four major classes of DNA-binding proteins have been engineered so far to achieve target site based genome editing which includes meganucleases, zinc finger (ZF) nucleases, transcription activator-like effectors (TALEs) and most recently the RNA-guided DNA endonuclease Cas9 from the type II bacterial adaptive immune system CRISPR. These molecules are also referred as molecular scissors and follow more or less the same type of mechanism of genome editing (Khalil 2020).

Initially, these molecules identify the targeted sequence, bind with it and induce a DSB at that particular position where editing is required (West and Gill 2016). Endonucleases go either through non homologous end joining (NHEJ) or homology directed repair (HDR) mechanisms to restore DSB (Fig. 2). Ninety percent of restoration occurs through NHEJ because it can perform its activity during all phases of the cell cycle except in the M phase. Without a repair template, NHEJ undergoes linkages of disconnected ends straightway, resulting in insertion or deletion (indel) at the DSB. Whenever a mutation occurs at that particular site and causes knockout/in of gene and loss/gain of function, it is error prone. While HDR, along with a repair template, will join similar sequences containing dsDNA breaks and result in targeted modifications at the desired locus (Petersen et al. 2013). Furthermore, NHEJ is active during cell cycle (G1 – S–G2/M), whereas HDR pathway is only active during the S and G2/M phases (S>G2/M) (Ranjha 2018) as mentioned in Table 2.

Meganucleases

The technique is based on identifying target site, and then cutting it using endonucleases activity. The benefit of using the Meganucleases technique is its less toxicity towards cells because it is naturally present in cell. In contrast, drawback of this method is less or accurate interaction between meganucleas-protein and target DNA sequence specificity which lessens its use as genome editing technique (Zaslavskiy et al. 2014).

Table 2: Delivery Strategies for CRIPR-CAS9 system

Delivery method	Characteristics	Limitations	References
Delivery of CRISPR-Cas9 via plasmid 	-Simplest and Cost effective way. -Delivery of Cas9 and gRNA via two plasmid or integration of gRNA expression on the same Cas9 plasmid.	-Greater chances of off target effect due to longer expression time. -Time consuming due to transcription and translation. Contains the potential for insertional mutagenesis.	(Chen et al. 2020)
Delivery of CRISPR-Cas9 via mRNA 	-Faster and easier than plasmid DNA. -Transcription not needed. -Short lived Cas9 expression so minimum chances of off target effect.	-It can only be used to express Cas9 for a short period of time due to mRNA instability and susceptibility to RNase degeneration.	(Humphrey and Kasinski 2015)
Delivery of CRISPR-Cas9 via protein 	-Gene editing efficiency is higher than that of DNA and mRNA. -Lesser chances of off target effect due to transient Cas9 expression.	-Costly as compare to DNA and mRNA. -Illicit immunologic responses due to Delivery via bacterial origin protein.	(Liang et al. 2015)

Zinc Finger Nucleases (ZFNs)

ZFNs are not naturally occurring nucleases and can be generated by joining restriction endonucleases with ZF protein providing a very specific target site cutting. This technique has the advantage over meganucleases as it is simple and specific (Ochiai and Yamamoto 2017). However, assembling zinc finger domains to bind an extended array of nucleotides with high affinity has not proven easy. Despite improving this technique with advancements in research, ZFPs binding specificity with desired targets remains a significant challenge (Ely et al. 2021).

Transcription activator-like effector nucleases (TALEN)

TALENs resemble ZFNs in terms of formation of DNA binding protein and endonucleases complex and mode of action. Instead of using ZF, another DNA-binding protein domain called TAL effector is used to bind with endonucleases (Hensel and Kumlehn 2019). TALENs are a little more specific because these can target even a single nucleotide unlike ZFNs where at least three nucleotides can be targeted (Chandrasegaran and Carroll 2016). However, the limitations of both techniques are almost the same, which are mentioned above in ZFNs (Juillerat et al. 2014).

Clustered Regularly interspaced short palindromic repeats (CRISPR)

The first hint of CRISPR manifestation was reported in 1987 from a unique repeating DNA sequence found in *Escherichia coli* while studying phosphate metabolism genes. Clustered regularly interspaced short palindromic repeats (CRISPRs) were identified in archaea in 1993, notably in *Haloferax mediterranei* (Ishino et al. 2018). The CRISPR/Cas-system complex an ancient immune (acquired) system of some bacteria and archaea species actively protecting against bacteriophage DNA. The initial application of CRISPR on the mammalian genome was started in 2013 (Cong et al. 2013). The short time line of CRISPR CAS9 development is shown in Fig. 3 (Khurshid et al. 2018).

Structure of CRISPR-Cas9 system

CRISPR I made up o two RNAs; CRISPR RNA (crRNA) and tracer RNA (trans-activating RNA). Both crRNA (spacer + palindromic repeats) and tracrRNA are distinct entities. The crRNA gives Cas9 target specificity, but it cannot attach to Cas9 directly. These two portions (crRNA + tracrRNA) are joined by a linker to produce a single molecule known as single-guided RNA (sgRNA), which can be synthesized in the laboratory. sgRNA binds to the Cas protein, resulting in the creation of the Cas Complex. CRISPR also contains spacer DNA, 26 to 72 bp short sequence segments, homologous to phage or plasmid DNA. Each spacer is separated from another spacer through repeat sequences of similar size (Jiang and Doudna 2015).

Cas9 can be directed to break dsDNA at any location on the genome specified by the guide RNA sequence and a Protospacer Adjacent Motif (PAM). The PAM is a critical targeting component to protect the CRISPR domain from attack. A 5′-NGG-3′ PAM is a widely used sequence,

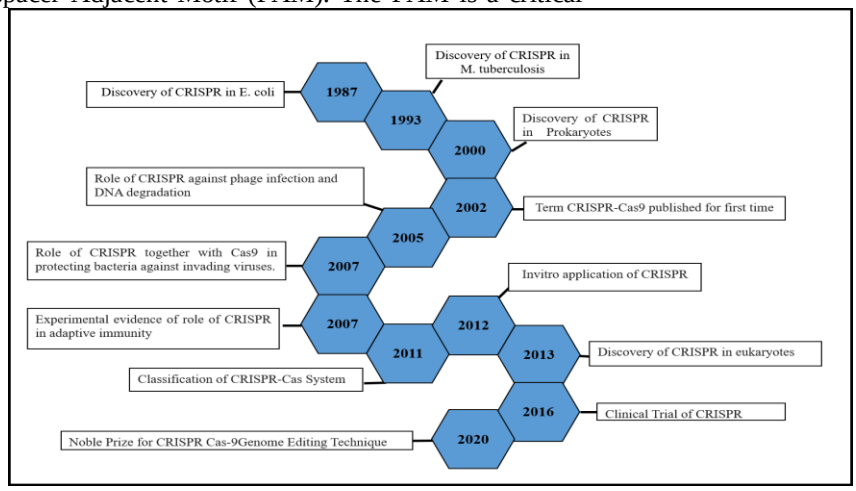


Fig. 3: A brief timeline of CRISPR technique development

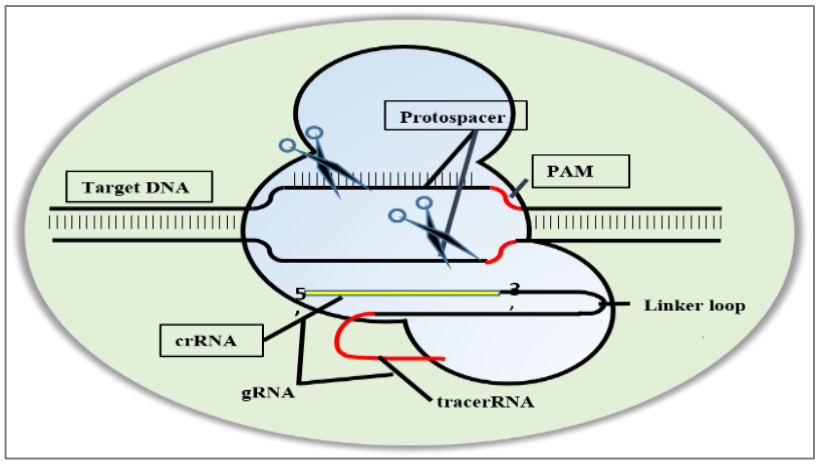


Fig. 4: Schematic diagram of Structural Framework of CRIPR-Cas9 system

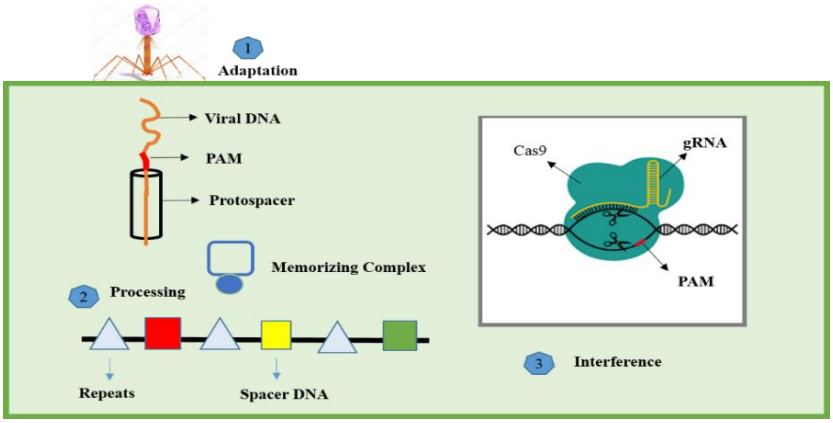


Fig. 5: Mechanism of CRISPR Working

derived from *Streptococcus pyogenes* (SpCas9) (Cong et al. 2013). Basic structural framework of CRISPR is shown in Fig. 4.

How does CRISPR-Cas system work?

The immune response to CRISPR-Cas consists of three phases; adaptation, expression and interference as shown in Fig. 5.

Adaptation phase: After detecting a discrete, short pattern PAM, a separate complex of Cas proteins attaches to a target DNA. The adaptation complex inserts the protospacer

DNA into the array after duplicating the repeat at the 5' end of the CRISPR array and converting it to a spacer.

Expression phase: The pre-CRISPR RNA (pre-crRNA) is processed into mature crRNA, containing the spacer sequence and parts of the flanking repeats.

Interference phase: A sequence of amino acids is inserted as a guide for identifying the protospacer in a virus or plasmid's invading genome, cleaved and down regulated by a Cas nuclease/s (Lander et al. 2016).

Delivery System for CRISPER Cas9

In the type II CRISPR Cas system, the crRNA effector complex is a single multi-domain protein called Cas9. Cas9 signature gene alone is insufficient for categorizing it as a specific CRISPR gene hence Cas1 and Cas2 genes are used as additional genes for type II system identification. Cas9 requires two accessory nuclease domains to cleave the target DNA, HNH, and Ruv C-like, and each nuclease only cleaves one strand of target DNA (Krzysztof 2014).

CRISPR CAS9 protein has a molecular weight of 160 kDa, and the long phosphate backbone of the sgRNA provides a net negative charge to the complex when it forms a ribonucleoprotein (RNP) complex which makes CRISPR delivery challenges Both the vehicle (CRISPR-Cas9) and the cargo (CAS nucleus and guide RNA) are involved in the CRISPR-Cas9 delivery. Delivery can be performed via DNA, mRNA, or proteins as shown in Table 2 (Jinek et al. 2014).

CRISPR Cas-9 and its application in One Health related zoonotic diseases

CRISPR Cas system has huge number of applications because it is simple to use and works in a precise manner. It has multiple applications in diagnosing and treating of diseases including one health-related diseases. Some of those are discussed as follows:

Bacterial Diseases:

Anthrax

Anthrax, caused by *Bacillus anthracis (B. anthracis*) is a lethal zoonotic disease with a very high mortality rate. *B anthracis* spreads by spores which have been recognized as a bioweapon (Sidwa et al. 2020). Various studies have shown that CRIPR Cas technique can reduce this organism's virulence and pathogenicity (Inglesby et al. 2002; Wang et al. 2021; Choate et al. 2021).

B. anthracis possesses two plasmids responsible for virulence including pXO1 and pXO2. The pXO1 encodes anthrax toxin proteins; protective antigen (PA), the lethal factor (LF) and the edema factor (EF). The pXO2 plasmid encodes for the capsule. Researchers constructed a CRISPR system consisting of scissors plasmids which deliver via a shuttle plasmid and target virulent toxin protein genes. A 20nt long sequence guiding the Cas9 nuclease is used as scissors to break the target DNA. The study has shown that these two plasmids had a key role in virulence of *B. anthracis*, and the loss of any one plasmid due to CRISPR-Cas system resulted in a great increase in the elimination rate of the organism (Wang et al. 2021).

Anthrax toxin receptor 2 (ANTXR2) is a membrane receptor of *B. anthracis*. In a study it has been seen that changes in the levels of expression of ANTXR2 were observed between human and non-human primates using CRISPR activation (CRISPRa). The study's findings revealed a complex evolutionary relation between human and non-human primates in ANTXR2 expression. In human immune cells, ANTXR2 was 8-fold down-regulated compared to non-human primates, indicating regulatory changes early in the evolution of modern humans. This study provided a model which showed the wadaptation of humans to anthrax disease. Along with other applications using the CRISPR-Cas system can also help in identifying the early ecological changes (Choate et al. 2021)

Brucellosis

Member of genus Brucella is.the causative agent of the Brucellosis. Most common species of *Brucella is B. melitensis*, and it mainly affects ruminants, causing reproductive track-related conditions like abortions and infertility. In humans, it causes severe clinical conditions like arthritis, endocarditis, meningitis, infertility and abortion in women. The treatment against brucellosis is usually not very effective, and currently no effective vaccine is available (Karponi et al. 2019).

In a study, *B. melitensis* infected ovine macrophages were used to simulate the host cell and pathogen's interaction *in vitro*. Lentivirus vectors with CRISPR-Cas9 system to target Brucella RNA pol A gene was transduced into the infected cells. By targeting the RNA pol A gene bacterial load per cell was reduced significantly (Karponi et al. 2019).

Xu et al (2022) have developed a system to detect different Brucella species in infected blood and milk using the CRISPR-Cas12a system with recombinase polymerase amplification (RPA). This platform uses CRISPR-Cas12a-RPA fluorescent biosensor and CRISPR-Cas12a-RPA electrochemical biosensor which could detect 2-3 copies of plasmid DNA and can help in the early diagnosis or detection of brucellosis.

Lyme Disease

Lyme disease is a tick-borne infection caused by the bacterial spirochete *Borrelia burgdorferi* and is transmitted by the bite of an *Ixodes* genus tick (Bernard et al. 2019).

White-footed mice are the main reservoir of the *Borrelia* bacteria that causes the disease. It is a zoonotic disease and can be transmitted from mice to humans through the bites of black-legged ticks. If untreated, disease progression may lead to early arthritis, neurologic manifestations and cardiac involvement problems (Norris 2018).

Gene editing progress in ticks has been slow, mainly because ticks coat their eggs in large amounts of wax, preventing the successful injection of CRISPR components into embryos at the correct developmental stage without destroying the egg (Sharma et al. 2022). Therefore, instead of targeting the disease vector, researchers have used CRISPR to prevent tick-borne disease by using CRISPR-based genome editing to heritably immunize the white-footed mice responsible for infecting many different types of ticks. Introducing antibody-encoding resistance alleles into the local mouse population was anticipated to disrupt the disease transmission cycle for decades. To achieve this, engineered mice were released in the natural population to breed with natural mouse populations. In this way, the off springs can be immunized without gene drive (Buchthal et al. 2019).

Viral Diseaes

Rabies

Rabies is acute, progressive encephalitis caused by a lyssavirus. These viruses are bullet-shaped, single-stranded, negative-sense RNA viruses. The reservoir hosts are known to be bats. Most mammalian species, including human beings, are susceptible to this disease. The virus transfers to the susceptible host via direct transmission through infected animal bites and transdermal inoculation of virus-laden saliva (León et al. 2021).

Currently rabies treatments primarily focus on mass vaccination and *post-exposure prophylaxis (*PEP). However, tools like gene therapy or gene editing are needed to treat or control rabies in the future. A CRISPR-Cas9 system in combination with (Induced pluripotent stem cells) iPSCs can make corrections *in vitro* in genes. Gene delivery tools can be used later to deliver the corrected/edited genes to the target organs. In the same way gene therapy based on the CRISPR-Cas9 system in combination with the Microhomology-mediated end-joining method can also stop/eliminate rabies virus in the infected cells (neurons). This technique can also be used to treatment rabies even after signs appear (Nelwan 2018).

Coronavirus Disease 19 (COVID-19)

Coronaviruses (SARS-CoV-2) belong to the Coronaviridae family in the Nidovirales order. The Respiratory system is the primary target of SARS-CoV-2; however, it also can infect other organs/systems. Due to COVID19 pandemic a large number of human causalities occurred. This disease is also zoonotic and known to be transmitted from bats (Yang et al. 2020).

Scientists have developed a host-pathogen protein-protein interaction map for SARS-CoV-2 and other coronaviruses. This interaction network identified hundreds of relevant proteins and then used high-throughput CRISPR gene knockout to functionally validate each of these. CRISPR has massively accelerated both the search for novel drug targets for treating infectious diseases and the repurposing of existing drugs to treat emerging pathogens. For example, CRISPR knockout of a key enzyme in the fatty acid metabolism pathway, fatty acid synthase (FASN), demonstrated its role in SARS-CoV-2 infection and replication and suggested existing inhibitors of FASN could prevent infection (Gordon et al. 2020).

Human Papillomavirus (HPV)

Human papillomavirus (HPV) is a small DNA virus known to cause cervical and other cancers. Researchers have identified the key genes of HPV responsible for oncogenesis; E6 and E7, leading to these genes being targeted with CRISPR as a potential therapeutic tool for HPV. For example, several studies have confirmed that targeting the E6 and E7 genes with CRISPR-Cas9 leads to decreased levels of the proteins, apoptosis of infected cells, and growth inhibition (Zhen and Li 2017).

Pre-clinical studies used stealth liposomes to deliver CRISPR-Cas9 editing components to target the E7 gene in HPV16 tumors in mice. The treatment effectively cleared the tumors without causing toxicity to the liver or spleen of the mice. Positive results like these led to clinical trials of this therapy being tested in human patients (Jubair et al. 2021).

Human Immunodeficiency Virus (HIV)

Acquired immune Deficiency Syndrome (AIDS) is still a major global health problem. It is caused by Human Immunodeficiency Virus (HIV) infection and destroys host's immune system (Bowers et al. 2014). Due to the latent nature, it is challenging to eliminate this virus even after highly active antiretroviral therapy (HAART). To overcome this challenge, the CRIPR-Cas9 trial has been initiated which, up until now, are providing satisfactory results regarding HIV treatment (Xiao et al. 2019). Experiments have been conducted to target the HIV-1 genome using the CRISPR-Cas9 guide RNA to target conserved sites in the LTR-U3 region. The results demonstrated inactivation of viral gene expression and restriction of virus replication in different HIV-1 latently infected cell lines including the T cell line, pro-monocytic cell line, and microglial cell line. It has also been observed that genotoxicity, and off-target effects were very little (Hu et al. 2014; Lebbink et al. 2017). Recently, the employment of CRISPR-Cas9 and gRNA of Staphylococcus aureus in lentiviral vector have been shown

to excise latent HIV-1 provirus and suppress proviral reactivation (Wang et al. 2008).

West Nile Virus

West Nile virus (WNV) is a member of the Flaviviridae family. It is an enveloped single-stranded RNA virus. Mosquitos are mainly responsible for transmitting this pathogen to humans (Petersen et al. 2013). WNV causes a neurological infection which leads to massive neuronal cell death. Seven genes were found to be involved in WNV-induced cell death. These genes, including *EMC2*, *EMC3*, *SEL1L*, *DERL2*, *UBE2G2*, *UBE2J1* and *HRD1* were identified using CRISPR-Cas9 based identification method. By disrupting these genes protection against WNV-induced cell death in three cell lines was conferred. Interestingly replication of WNV was not blocked even after knocking out of these genes which proved that these genes were essential for the pathways involved host cell death (Ma et al. 2015).

Parasitic Diseases

Malaria

Malaria is caused by a *Plasmodium* parasite and is transmitted by *Anopheles gambiae* mosquitoes. Several gene drive systems have been developed to control mosquito -borne diseases using genome/gene-edited mosquitoes. CRISPR system was also used to make the male gene dominant over the female one by editing or modifying the sex-determining gene. This modification was then spread using a gene drive, which later helped to eliminate infected female mosquitoes from the population. It was observed that within 7-11 generations, the modified gene became 100% prevalent. egg production reduced gradually and the population totally collapsed within the laboratory environment (Hammond et al. 2016).

Similarly, the researchers studied the inactivation of the fibrinogen-related protein 1 (*FREP1*) gene. CRISPR-*FREP1* knockout mosquitoes exhibited a slower development in their pre adult life stage and were less likely to feed on blood meals. Those knock-out mosquitoes also laid fewer eggs with less viability (Dong et al. 2018).

Toxoplasmosis and Chagas Disease

Toxoplasma gondii, is an intracellular protozoan and is the etiological agent of Toxoplasmosis. It is also a zoonotic disease and most infections in humans are asymptomatic. However, in newborns, children and immune- suppressed individuals a significant disease can be produced through primary infection/ reactivation. Vertical transmission, organ transplantation, and ingesting of infected tissue cysts are main transmission routes. Improperly cooked/raw meat and contaminated food and water may have tissue cysts or oocysts, which can cause infection (Saadatnia and Golkar 2012).

Similarly, Chagas disease is also a vector borne disease caused by the parasite *Trypanosoma cruzi* and the vector for this disease is a reduviid bug. (Brun et al. 2010). Genome editing techniques were also employed for both of these parasitic pathogens. CRISPR-Cas9 high-throughput analysis was used to simultaneously knock out/knock down all nuclear protein-coding genes in *Toxoplasma gondii* (Nødvig et al. 2015). To achieve this guide RNA library constitutively expressing Cas9 was transfected into parasites. CRISPR-Cas9 was also used to silence the gene encoding GP72 (responsible for flagellar attachment) of *T. cruzi* (Lander et al. 2017). Similarly, in another study knockout of P21 gene of *T. cruzi* was performed using CRISPR-Cas9. As a result loss of expression of P21 was observed. It has also been observed that this process has a direct impact on the cell cycle. It caused the cessation of the cell cycle at the G1 phase which eventually slowed cell division and growth rate of epimastigotes (Teixeira et al. 2022).

Conclusion

Genome editing especially the CRISPR-Cas system has endless applications. Much work still needs to be done to eliminate zoonotic diseases and diseases related to one health. CRISPR based diagnosis and treatments need time. Nowadays focus of CRISPR based genome editing is on prevention and treatment of infectious diseases caused by bacteria and viruses of zoonotic importance. Safe, effective and less off-target effects are desired in CRISPR-based therapies. The most important lesson learnt in the COVID 19 pandemic, is to act proactively against zoonotic, newly emerging or re-emerging viral infections. However, due to higher mutability in viruses, conventional methods become less effective, hence focus should be diverted on the techniques like CRISPR to combat these challenges. New drugs, vaccines diagnostic techniques and products should be developed using CRISPR technology to reduce the damages caused by zoonotic infectious diseases.

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