Application of Genome Editing CRISPR \Cas9 Technology for Animal Diseases Treatment

AUTHORS DETAIL

Vajiha Sahar Khan[1*], Asma Malik[1], Rameen Nawaz[2], Farah Ahmad[3], Maryam Fatima[1], Farah Masood[1], Qamar UN Nisa[1], Muhammad Insaf[1], Aqsa Rani[1] and Rida Zahra[4]

1. Department of Biotechnology, Faculty of Environmental Sciences, Muhammad Nawaz Sharif University of Agriculture Multan, Pakistan
2. Center of Agriculture Biochemistry and Biotechnology (CABB), University of Agriculture Faisalabad, Pakistan
3. Department of Biosciences and Technology, Emerson University Multan, Pakistan
4. Department of Zoology and Animal Sciences, Faculty of Veterinary and Animal Sciences, Muhammad Nawaz Sharif University of Agriculture Multan

*Corresponding author: vajihakhan83@gmail.com

Received: 13-Oct-2024 **Revised:** 4-Nov-2024 **Accepted:** 5-Dec-2024

Cite this Article as: Khan VS, Malik A, Nawaz R, Ahmad f, Fatima m, Masood F, Nisa QU, Insaf M, Rani A and Zahra R, 2024. Application of Genome Editing CRISPR \Cas9 Technology for Animal Diseases Treatment. In: Basit A, Khan SA, Muhyuddin S and Mughal MAS (eds), Anim Health Dis Management, Pioneer Page Publishers, Beijing, China, Vol. 2: 19-28. https://doi.org/10.5281/zenodo.15456164

Abstract

Livestock is an essential living commodity that requires breeding and preservation in modern agriculture. Over the past century, farming practices have mostly changed for public health, animal welfare, sustainability, and commercial outputs. It has been suggested that altering the genetic composition of cattle is a useful technique for producing farmed animals with traits that satisfy the objectives of contemporary farming systems. The initial method of creating transgenic farm animals led to a low rate of gene transfection and random transgene insertion. In order to facilitate effective gene targeting with increased accuracy and gene stability, genome modification methods have been created. However, the CRISPR/Cas9 tool has recently emerged as the preferred technique for altering the genome of livestock species because of its effectiveness and precision.

Keywords: CRISPR/Cas, Knock-out (KO), Knock-in (KI), crRNA, Brucellosis, Tuberculosis.

1. Introduction

Livestock products are an essential source of high-quality protein. One of the main concerns for global food safety a long-standing goal for a healthy populace is the efficient production of food derived from animals. The prevalence of infectious diseases in livestock raises the risk of zoonoses in addition to having an impact on farm productivity, economics, and animal health and Welfare (Tomley and Shirley, 2009). Food safety and public health are seriously threatened by the infectious spread diseases in food animals. The main threats to the effectiveness of disease control measures include the development of antibiotic drug resistance, the lack of effective vaccinations, and the spontaneous genetic mutation of infectious microorganisms (Aslam et al., 2018).

In the 1970s, the development of DNA recombination technology cleared the way for the study of molecules. For the first time, biologists are able to directly alter DNA molecules and carry out basic genome editing. Clarifying the role of targeted genes and regulatory factors requires the use of these molecular biology tools. Animal models linked to diseases are created with the use of precise genome editing. Furthermore, a new revolution in medication development and gene therapy is being sparked by genome editing technologies (Gaj et al., 2013).

1.1. Genetic Engineering Approaches:

The main goal of biotechnology research, developing genome-modifying techniques, From last 10 years genome editing (GE) has gained prominence and interest in the due to its wide application in several life science fields. GE is an approach of genome

engineering that includes changing, replacing, deleting, or inserting DNA into a living organism's genome. GE mediates site-specific locations as opposed to traditional at random, and discovery of drugs and the production of transgenic animals, in animal breeding (Zhang et al., 2019).

In additional to having a greater DNA sequence selectivity and approach must potentially alter the genomic sequence. The improvement of a few particular molecular tools is where the concept of genome engineering first emerged. CRISPR-associated nuclease (Cas) 9) are examples of precise molecular scissors (Gaj et al., 2013)

MegNs (meganucleases)

- Zinc Finger Nucleases (ZFN)
- Translation Activator-Like Effector Nucleases (TALENs)
- CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats

CRISPR-associated nuclease (Cas 9) are examples of precise molecular scissors.

1.2. ZFN approach for disease resistant:

ZFN technology is effective at causing genetic changes in farm animals which raises the possibility of enhancing the animals' resilience to disease and productivity. For instance, mastitis is thought to be the most serious illness affecting dairy cattle and costs the sector billions of dollars every year. Exogenous DNA integration is stimulated and stretched to a specific site on the genome by the GE caused by ZFN (Klug, 2010). ZFNs were employed by Liu and his colleagues to target the human lysozyme gene to the bovine β-casein locus which results in lysozyme gene KI. They claimed that transgenic cows' milk secretions could eradicate Staphylococcus aureus, proving their resistance to mastitis (Liu et al., 2014).

In another study, single strand breaks in DNA that result in HDR were also induced using zinc finger nickases, a programmable nuclease made from ZFNs. According to a study by Liu and his colleagues, HDR increases the addition of the lysostaphin gene. They discovered that somatic cell nuclear transfer (SCNT) using ZFNickase-treated cells can be profitable and produce gene-targeted cows in utero. Additionally, by silencing the myostatin (MSTN) gene in sheep, and cows, ZFN technology has been utilized to enhance muscle growth and raise the albumin content in chicken egg whites. The mRNA microinjection has historically been used to accomplish ZFN-mediated genome editing in fish. ZFNs have been utilized to damage germ line and somatic cell genes in Danio rerio (zebrafish) by causing targeted mutagenesis that results in DSBs (Doyon et al., 2008).

1.3. TALENs for disease resistant:

Technical limitations like as primary cell culture, the need to produce reliable reagents at a cheap cost, and embryo injection have slowed the application of ZFNs to cattle genome engineering while allowing for a wide range of genetic modifications. On the other hand, TALENs might be produced more effectively and with more primary cell activity. Gene KO is successfully induced by injecting TALEN mRNAs into bovine zygotes' cytoplasm. Ossabaw swine foetal cells altered by TALENs have been shown to function as efficient nuclear donors, resulting in tiny animlas. TALENs have been used in aquaculture for both applied research and basic functioning studies. It has been applied to fish and frog genome alterations to investigate the functional genomics (Wang et al., 2021).

Site-specific genetic engineering is a useful technique for faster breeding, biomedical model development, and pharmacological research. . Knockout and knock-in genes in mammalian cells are constituted a complicated problem when applied to large domestic species. Recent developments in engineered nucleases have made it possible to precisely alter the genomes of various species by introducing site-specific double-strand breaks. These breaks can be fixed using either homology-directed repair or non-homologous end joining machinery when a homologous template is available (Bevacqua et al., 2016).

The application of developed nuclease to create a fundamental tenet of genetic engineering. The two main DNA repair processes, homology directed repair (HDR) and non-homologous end joining (NHEJ), are then activated. While HDR uses homologous sequences as templates to regenerate missing DNA after DSB and NHEJ uses a number of enzymes to directly join the ends of the DNA. This aids in implementing the intended modification at the DSB site. Every GET has unique properties that allow for the on-target editing of particular genomic sequences (Li et al., 2020).

2. Timeline of gene alteration techniques:

The use of meat and milk is rapidly increasing globally as a result of urbanization, overpopulation, and changes in lifestyle in this developing globe. Developing nations have the opportunity to use these technical advancements to enhance the livestock industry. In developing nations, biotechnological advancements pertaining to the welfare, nutrition, upbringing, and reproduction of animals are progressing reasonably. Some transgenic plant vaccines are examples of cutting-edge discoveries that are inevitably going to explore various fields. In 1983, WHO developed the Polymerase Chain Reaction (PCR) which is a method of modifying the genome.

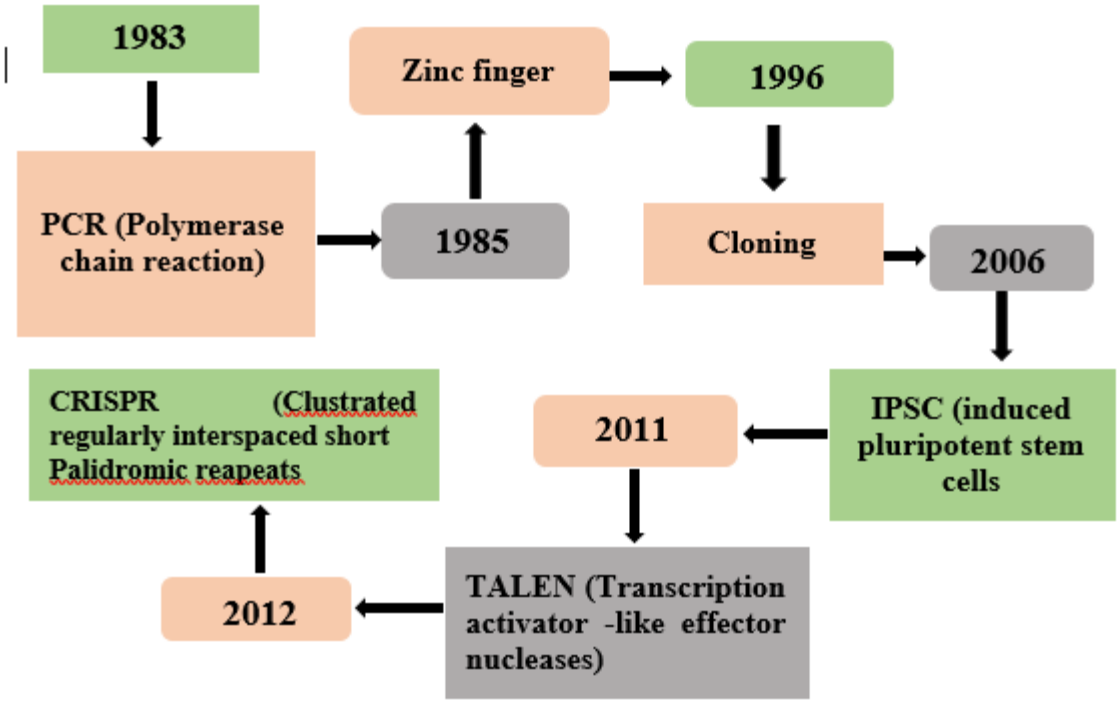

Fig: 1 Timeline of gene altering techniques

Kary B. Mullis's invention of PCR for gene amplification in the nineteenth century marked the origins of genetic manipulation. It established a basis for the advancement of gene modification through the use of Zinc Fingers, cloning, iPSC, and the most cutting-edge methods like TALEN and CRISPR.

The development of the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated enzyme 9 (Cas9) system is one of the most successful recent developments in genome editing technologies based on programmable nucleases. Only a few years ago, transcription activator-like effector nucleases (TALEN), zinc fingers nucleases (ZFN), and mega nucleases were discovered. Despite the fact that ZFN and TALEN have been shown to be effective gene editing methods and their use is severely limited by a number of issues, such as their high time requirements, poor efficiency, and low specificity. CRISPR/Cas9 has shown itself to be a strong and adaptable technology for genome editing in a variety of cell types and organisms. Thus far, CRISPR/Cas9 has been effectively used in mice, yeast, zebrafish, bacteria, and monkeys (Shen et al., 2013).

3. CRISPR-Cas9 approach for disease resistant:

The revolutionary genome editing technique known as CRISPR-Cas9 technology which makes it possible to fix harmful mutations with little harm. The greatest evolution in our lives has been brought about by modern advanced technologies. The main difficulties in cattle breeding are the rate constraint brought on by the lengthy generation period and the obstacles posed through hereditary assets. But these days, genome-editing methods like CRISPR provide the finest answers to the issues at hand at a reasonable price. Consequently, it is predictable that these developments will result in the production of a sizable number of genomes that modify domesticated animals in the near future (Ruan et al., 2017).

In this technology, a nuclease and an enzyme (Cas9) work together to cut DNA which identify the region of the genome where RNA contacts. Enzyme of Cas9 that is attached splits both strands of DNA at that spot. By using the Cas-9 DNA sequence can be altered, deleted or added. CRISPR less expensive and evolving more quickly. However, the problem of intellectual property rights is one of CRISPR's disadvantages. There are numerous issues related to the CRISPR-Cas9 patent environment but the key one at the moment is figuring out how to maintain a balance between the appropriate intellectual property rights and the present genetic engineering domains so that CRISPR-Cas9 applications can advance (Xie, et al., 2014).

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated nuclease (Cas) 9 became the preferred technique mostly because of its straightforward manufacturing and design. Then, utilizing CRISPR/Cas9 altered cells as donors, gene-edited goats and pigs were successfully created by somatic cell nuclear transfer. More recently, a simpler method that involved injecting one-cell embryos into the cytoplasm produced genome-edited mice, rats, sheep, monkeys, pigs, goats, and rabbits. Up until now, efficiency rates have varied, ranging from 15% to 21% in goats to 63% in pigs. Furthermore, mosaicism may arise via the injection of CRISPR/Cas9 RNA into zygotes (Broeders et al., 2020).

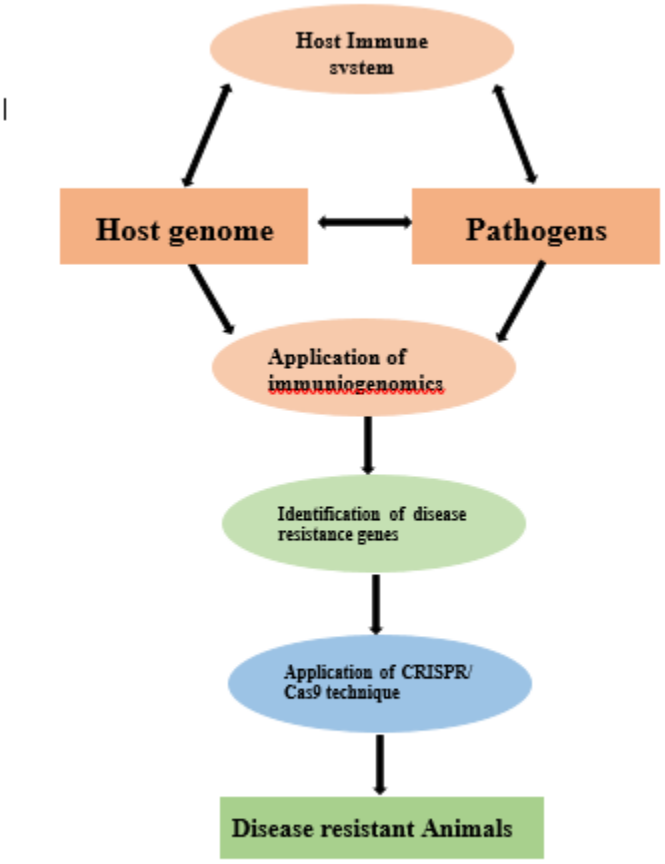

Fig: 1 Diagrammatic representation of how genome editing and immunogenomics are used resistant to disease. Severity determined by the pathogens and host immune system. Bioinformatics used to determined candidate gene. The host genome modified by using the CRISPR/Cas9 method.

3.1. History of CRISPR\Cas9:

The discovery of the CRISPR/Cas9 system has been short and speedy. It started with the discovery of a set of 29-nucleotide repeats in E. coli that were separated by non-repetitive sequences that are now known as spacer. Researchers have suggested that CRISPR might work as defenders of immunity by using a mechanism similar to eukaryotic RNA interference (RNAi) (Makarova et al., 2006).

The discovery that CRISPR-induced immunity was used to defend bacteria from phages in 2007 a turning point (Barrangou et al., 2007). The pathway mechanism of the CRISPR/Cas system has been cleared by scientists. The development of CRISPR/Cas has advanced significantly showed that Cas9 was guided to cleave target DNA by spacer sequences. The tracrRNA and crRNA formed a duplex structure that was linked to Cas9. It proved that Cas9 was an endonuclease guided RNA. It was first genome editing in eukaryotic cells by using the CRISPR-CAS9 (Ousterout et al., 2015).

3.2. CRISPR\Cas9 Structure:

Many bacteria and archaea have been shown that have three different types of CRISPR systems (1-3) as far. The three elements of its formation are similar: transactivating CRISPR RNA (tracrRNA), CRISPR RNA (crRNA), and a group of CRISPR-associated (Cas) genes (Garneau et al., 2010).
The most widely applied Cas9 and SpCas9 was obtained from S. pyogenes. It is generated in the host to break down the invasive plasmids or virus under the guidance of crRNA and tracrRNA (Chylinski et al., 2014).
Researchers discovered that the Cas9 protein contained two functional domains, RuvC and HNH, based on the structure of CRISPR-Cas9. Together, they generate a DSB after each nicks a single strand of the target DNA (Gasiunas, et al., 2012). The matured crRNA targets complementary DNA flanked by a brief protospacer adjacent motif (PAM) and determines the specificity of cleavage. Furthermore, it is necessary to identify and cleave target genes. An engineered chimeric single guide RNA (sgRNA) can be produced by structurally fusing the native crRNA and tracrRNA duplex when tracrRNA forms a loop with crRNA. A typical sgRNA has a 20 nt sequence that uses Watson-Crick base pairing to identify the target DNA (Upadhyay et al., 2013). By altering the sgRNA sequence, CRISPR/Cas9 can be guided to any target sequence that is next to PAM. The PAM located close to the target sequence's 3' terminal serves as a crucial basis for Cas9 recognition and cleavage. PAM sequences differ amongst Cas9 orthologs. For instance, the popular spCas9 has a low frequency of 5'-NAG-3' PAM or a common 5'-NGG-3' PAM. Researchers used X-ray crystallography to thoroughly examine the Cas9 protein's crystal structure in order to ascertain the role of Cas9 nuclease. The two primary lobes nuclease (NUC) lobe and the Cas9 recognition (REC) domain make the core of cas9. Both of these lobes are necessary for site-specific gene editing (Anders et al., 2014).

3.3. CRISPR\Cas9 mechanism:

In nature, the CRISPR/Cas9 system recognize and breaks down invasive genetic sequences to protect bacteria or archaea. Researchers make use of this characteristic to reconstitute certain programmable designed Cas9 nucleases from archaea or bacteria. For instance, the genomes of animal cells and organisms are frequently edited using human codon-optimized Cas9 and the necessary sgRNA (Garneau et al., 2010).
Three steps involved in the the mechanism of crisper cas9.

- Recognition
- Cleavage
- Repair

i. Recognition:

Precision cleavage depends on accurate target sequence recognition. The interactions between sgRNA and Cas9 were significantly influenced by the REC domain of Cas9. The Cas9 nuclease identifies and attaches itself to the target sequence following the creation of the sgRNA-Cas9 complex. Through RNA-DNA base pairing, spCas9 could be directed to any desired target upstream of a necessary 5'-NGG PAM. PAM is crucial for sgRNA-Cas9 to bind to the correct region of the target gene. It was discovered that target DNA unwinding and Cas9 recognition are PAM-dependent (Mekler et al., 2017). When Cas9 detects PAM, sgRNA-Cas9 uses complementary base pairing processes to read out and capture the DNA of interest, achieve site-specific binding, and prevent unanticipated selfmutilation. To create a sgRNA-target DNA heterodupex and initiate R-loop formation, the CRISPR-Cas9 nuclease unzips DNA complementary to the sgRNA seed sequence and selectively binds a target DNA having a conventional 5'-NGG-3' PAM (Cong et al., 2013).

ii. Cleavage:

The synthesis of DSB and the subsequent cellular DNA repair process are prerequisites for CRISPR/Cas9-mediated genome editing. The target dsDNA unstable at the PAM motif once the target sequence was identified and formed an RNA-DNA heteroduplex. The two functional nickase domains of Cas9 (HNH and RuvC) are catalytically activated by these events. The PAM motif is also necessary for Cas9's double-stranded endonuclease activity (Allemailem et al., 2024). In fact, when the PAM motif is absent then Cas9-sgRNA ignores even completely complementary sequences. Cas9 functions like a pair of scissors when cleaving target DNA. The HNH nuclease domain nicks the DNA strand complementary to the guide RNA while the RuvC domain cuts the displaced strand, resulting in a site-specific DSB (Nishimasu et al., 2014).

iii. Repair:

Two primary repair mechanisms are the non-homologous end joining (NHEJ) pathway and the homology-directed repair route that are activated when nuclease-induced DSBs are present in DNA. Furthermore, DSB start HDR-mediated DNA repair which needs a donor dsDNA sequence or ssDNA as a repair template that contains homology. High fidelity and accurate editing are made possible via the HDR route. Furthermore, HDR makes it simple to accomplish long target sequence insertion or single-base replacement alteration (Song et al., 2021).

CRISPR system:

Rapid progress was made of the RNA-guided CRISPR/Cas9 system is a new and promising genome editing technology. Cas9 and sgRNA expression cassettes are the two crucial parts of the RNA-guided CRISPR/Cas9 system that are constructed according to its basic principles. Usually, a nuclear localization signal is attached to the Cas9 gene which has been codon optimized for expression in a range of cell types and species. More than 300 different types of Cas9 plasmids have been added to the Add gene database thus far. The majority of these plasmids are members of the well-researched spCas9 family. At first, scientists used the sgRNA and Cas9 expression vectors independently. A simple all-in-one expression system was used. In actuality, both approaches have been effectively used in a wide variety of species and cell types. Numerous biological firms sell the Cas9 and sgRNA all-in-one expression plasmids. All users have to do is create the right sgRNA based on the PAM sequence's location. Online CRISPR design tools, like the CRISPR Design Tool and the ZiFiT Targeter software, were created and made available by a number of groups. Cas9-mediated genome editing may be impacted by the promoter that starts sgRNA expression. It is important to note that the commonly employed U6 promoter likes a guanine (G) nucleotide as the first base of its transcript; if the 20-nt guide sequence does not start with G, an additional G is attached at the 5' of the sgRNA. For a target locus, more than two sgRNAs should be designed at the same time (Ran et al., 2013).

4. CRISPR\Cas9 applications for welfare of animals against diseases:

The enhancement of reproductive characteristics, and the creation of animals that serve as models for scientific studies. As more reports of the widespread application of the CRISPR technique surfaced. Cas9 systems can cleave DNA in a specific pattern and have a large, detectable site. Devices that alter genetic material are made to stick to any known DNA sequence and have a cutting zone and a DNA sticking zone (Wells & Prather, 2017).
Goats and sheep are practical domestic animals that are raised for their meat, milk, fibre and other byproducts. Sheep and goats are genetically engineered on a massive scale and due to their suitable size, short gestation period, and higher milk secretions. Numerous assisted procedures have been proposed to improve the efficiency of gene modifications and facilitate the creation of genetically modified initiators (Zhu et al., 2020).

4.1. Diseases linked genes identification:

CRISPR-Cas9 improves the detection of genetic variants linked to animal illnesses. Scientists can introduce mutations or alterations to examine the phenotypic changes that arise by identifying particular genes or genomic regions of interest. Researchers can determine the genetic components causing a disease's susceptibility, progression, or responsiveness to therapy by linking these alterations to specific illnesses or characteristics.

4.2. Application of CRISPR\Cas9 in Tuberculosis disease:

The CRISPR system has emerged as a dependable technique for granting animal's disease resistance. Recently, exogenous knock-in of NRAMP1 in BFFs (bovine foetal fibroblasts) using CRISPR/Cas9 nickase was done to create genetically engineered cattle that are resistant to tuberculosis. Nine cows that demonstrated resistance to TB were produced by them (Gao YuanPeng et a.., 2017).
PrPSc is an infectious protein that causes fatal conditions in humans and cattle like bovine spongiform encephalopathy, Creutzfeldt-Jakob disease, and chronic wasting disease (in cervids), is encoded by PRNP gene. By precisely editing the bovine PRNP coding exon 3, CRISPR/Cas9 has been used to produce knockouts in both cow foetal fibroblasts and early embryos. Cattle that have the NRAMP1 gene inserted in their genomes are resistant to M. bovis-caused bovine tuberculosis. More than 5 million cattle worldwide have contracted M. bovis. Bovine tuberculosis has become much more common and are limiting the movement of 10% of cattle and costing £91 million to slaughter (Bevacqua et al., 2016).

4.3. Application of CRISPR\Cas9 in IARS (Isoleucyl-tRNA synthetase) syndrome:

Japanese black cattle were affected by a disorder known as IARS (Isoleucyl-tRNA synthetase) syndrome is a recessive disorder. This syndrome was caused by the single nucleotide substitution mutation. Modern technique CRISPR ccas9 was used for the treatment of this syndrome by a donor DNA-containing synonymous codon (Aequorea coerulescens Green Fluorescent Protein) incorporate to a donor DNA to correct the amino acid arrangement or sequence by applying the CRISPR technique (Singh and Ali, 2021).

4.4. Application of CRISPR\Cas9 in Chronic enteritis and Johne's diseases:

The microorganism Mycobacterium avium subspecies paratuberculosis (MAP) is the cause of chronic enteritis and Johne's diseases (JD) in cattle. In dairy cattle, SNPs in the interleukin-10 receptor alpha gene (IL10RA) are linked to mastitis and MAP

infection. This gene produces the IL-10 receptor's alpha chain whose ligand is IL-10, is a crucial modulator of inflammation and has been connected to the aetiology of MAP disease. Furthermore, an IL10RA knock-out was produced in MAC-T cells, which were derived from bovine mammary epithelial cells are using the CRISPR/Cas9 gene editing technique. Additionally, the results show the wide-ranging and crucial effects of a knock-out of the IL10RA gene in altering pro-inflammatory cytokine expression and aiding the immunoregulatory component of IL10RA in inducing an anti-inflammatory response as well as the possible functional interaction affiliation among immune responses linked to JD (Mallikarjunappa et al., 2020).

4.5. Application of CRISPR\Cas9 in Brucellosis Disease:

One of the most serious zoonotic diseases in the world is brucellosis. In order to deactivate a gene involved in Brucella replication within host cells, specifically the virulence-associated gene virB10 or RpolA (RNA polymerase subunit A) that are recently transduced infected cells with lentiviral vectors harbouring the CRISPR/Cas9 gene editing system. They found that the quantity of internalized brucellae/cells is significantly decreased on the first and fourth days following transduction with the CRISPR/Cas9 vector against bacterial RpolA at a multiplicity rate infection (MOI) of 60 (Karponi et al., 2019).

4.6. Application of CRISPR\Cas9 in Porcine Reproductive and Respiratory Syndrome Virus (PRRSV):

In the pig industry, it was discovered that farmed pigs were frequently infected with the Porcine Reproductive and Respiratory Syndrome (PRRS) virus, which causes symptoms that impact reproductive health, respiratory tract illnesses, and can even be fatal. Currently there is no treatment of PRRS that's why the development of PRRS resistance is crucial. Since genome editing seems to be the only method of protecting animals from this virus, Genus and Roslin Institute are actively working to build disease resistance to PRRS. Using the CRISPR-Cas9 technology, genetically modified animals are created by knocking down CD163 receptors. All animals with knocked-out CD163 receptors are immune to PRRSV and have never displayed any signs of infection (Chae ChanHee, 2016).

4.7. Application of CRISPR\Cas9 in Avian flu virus:

Poultry are particularly susceptible to viruses that are known to spread quickly and cause significant death rates, such as the highly deadly ALV-J (avian leucosis virus J), MDV (Marek's disease virus), and AIV (avian influenza virus). The chNHE1-KO (Chicken Na+/H + exchange type-knockout) homozygous mutant chickens immune to ALV infection were successfully bred using CRISPR-Cas9. Another study used CRISPR-Cas9 to alter the chANP32A gene residues in order to reduce AIV replication. By altering the NHE1 (Na+-H+-1) gene using CRISPR/Cas9 the hens can be made resistant to the avian flu virus and the leucosis virus (Chojnacka-Puchta and Sawicka, 2020).

4.8. Application of CRISPR\Cas9 in Lung adenocarcinoma syndrome:

Lung adenocarcinoma syndrome was induced in lambs by eliminating the hyaluronidase 2 (HYAL2) gene is confirming CRISPR-Cas9's capacity to produce antiviral animal models. They have made a major contribution to the area by being the first to use sheep deficient in the otoferlin (OOF) gene as an effective model for deafness research (Lu et al., 2023).

4.9. Application of CRISPR\Cas9 in Skin diseases:

Using CRISPR-Cas9 through homologous recombination is a single nucleotide mutation in the PPIB gene that results in a skin disease in horses and a detrimental mutation in the GBE1 gene that causes an autosomal recessive disorder known as glycogen branching enzyme deficiency were fixed in horse fibroblasts (Pinzon-Arteaga et al., 2020).

4.10. Application of CRISPR in Coat Color of sheep:

For any class is crucial characteristic for both productivity and economics. It is expected that the local coat color design will be used to deal with the biological and atmospheric conditions. Numerous earlier studies suggest that sheep coat color may be connected to energy maintenance, body weight, and reproduction. Numerous studies have attempted to control and alter coat colour during generation and elucidate the process of coat colour development since the colour of domestic sheep's coat is a crucial characteristic for production. This theory suggested that Cas-9-based modifications, mutations, and replications were primarily responsible for variations in the physical makeup of coat colours (Xie et al., 2017).

5. Bioethical issues of CRISPR-Cas9:

There are certain drawbacks to genome editing technology that should not be neglected in addition to its benefits. As everyone is aware, the study of molecular biology has undergone significant transformation as a result of genome editing techniques. Compared to prior technologies like ZFN and TALEN, CRISPR/Cas9 has become the most widely used gene editing approach because of its many benefits, including low cost, high precision, and ease of use. Any molecular biology lab can readily

implement CRISPR/Cas9 technology due to these benefits. However, the quick development of CRISPR-Cas9 has brought about new legal, bio ethical, and societal issues in the fields of medicine, agriculture, livestock, and the environment (Ayanoğlu, et al., 2020).

The risk for off-target effects—unintentional alterations in the genome that take place outside of the intended target site whichis a significant ethical concern. Unintended consequences like new mutations or abnormalities in normal gene processes may arise from these actions. Reducing off-target effects requires precise target site selection, rigorous experimental design, and extensive confirmation of editing results (Piergentili et al., 2021).

6. Conclusion

The CRISPR/Cas9 tool is more effective than other genetic engineering methods and can modify DNA more effectively than existing technologies like ZFN and TALEN thanks to its relative simplicity of use. Its primary goal is to enhance the health, traits, in animals as well as their relationship to environmental. In genetic engineering is CRISPR-Cas9, because of its ease of use and efficiency, it is used in a variety of animals (cattle, sheep, cows, goats, and pigs). As a result, CRISPR has significantly advanced genetic engineering and is currently regarded as the most popular molecular biology method for genome alteration.

References

1. Allemailem, K. S., Almatroudi, A., Rahmani, A. H., Alrumaihi, F., Alradhi, A. E., Alsubaiyel, A. M., . . . Sindi, A. A. (2024). Recent Updates of the CRISPR/Cas9 Genome Editing System: Novel Approaches to Regulate Its Spatiotemporal Control by Genetic and Physicochemical Strategies. International Journal of Nanomedicine, 3, 5335-5363.
2. Anders, C., Niewoehner, O., Duerst, A., & Jinek, M. (2014). Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. Nature, 513(7519), 569-573.
3. Aslam, B., W. Wang, M.I. Arshad, M. Khurshid, S. Muzammil, M.H. Rasool, M.A. Nisar, R.F. Alvi, M.A. Aslam and M.U. Qamar. (2018). Antibiotic resistance: a rundown of a global crisis. Infection and drug resistance, 11,1645-1658.
4. Ayanoğlu, F. B., Elçin, A. E., & Elçin, Y. M. (2020). Bioethical issues in genome editing by CRISPR-Cas9 technology. Turkish Journal of Biology, 44(2), 110-120.
5. Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., . . . Horvath, P. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. Science, 315(5819), 1709-1712.
6. Bevacqua, R. J., Fernandez-Martín, R., Savy, V., Canel, N. G., Gismondi, M. I., Kues, W. A., . . . Taboga, O. A. (2016). Efficient edition of the bovine PRNP prion gene in somatic cells and IVF embryos using the CRISPR/Cas9 system. Theriogenology, 86(8), 1886-1896.
7. Broeders, M., Herrero-Hernandez, P., Ernst, M. P., van der Ploeg, A. T., & Pijnappel, W. P. (2020). Sharpening the molecular scissors: advances in gene-editing technology. Iscience, 23(1).
8. Chae ChanHee, C. C. (2016). Porcine respiratory disease complex: interaction of vaccination and porcine circovirus type 2, porcine reproductive and respiratory syndrome virus, and Mycoplasma hyopneumoniae. The veterinary journal , 212,1-6.
9. Chojnacka-Puchta, L., & Sawicka, D. (2020). CRISPR/Cas9 gene editing in a chicken model: current approaches and applications. Journal of Applied Genetics, 61(2), 221-229.
10. Chylinski, K., Makarova, K. S., Charpentier, E., & Koonin, E. V. (2014). Classification and evolution of type II CRISPR-Cas systems. Nucleic acids research, 42(10), 6091-6105.
11. Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., . . . Marraffini, L. A. (2013). Multiplex genome engineering using CRISPR/Cas systems. Science, 339(6121), 819-823.
12. Doyon, Y., J.M. Mccammon, J.C. Miller, F. Faraji, C. Ngo, G.E. Katibah, R. Amora, T.D. Hocking, L. Zhang and E.J. Rebar.(2008). Heritable targeted gene disruption in zebrafish using designed zinc-finger nucleases. Nature biotechnology, 26,702-708.
13. Gaj, T., C.A. Gersbach and C.F. Barbas. (2013). ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends in biotechnology, 31,397-405.
14. Gao YuanPeng, G. Y., Wu HaiBo, W. H., Wang YongSheng, W. Y., Liu Xin, L. X., Chen LinLin, C. L., Li Qian, L. Q., . . . Zhang Yong, Z. Y. (2017). Single Cas9 nickase induced generation of NRAMP1 knockin cattle with reduced off-target effects.
15. Garneau, J. E., Dupuis, M.-È., Villion, M., Romero, D. A., Barrangou, R., Boyaval, P., . . . Moineau, S. (2010). The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature, 468(7320), 67-71.

16. Gasiunas, G., Barrangou, R., Horvath, P., & Siksnys, V. (2012). Cas9–crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proceedings of the National Academy of Sciences, 109(39), E2579-E2586.
17. Karponi, G., Kritas, S. K., Papadopoulou, G., Akrioti, E.-K., Papanikolaou, E., & Petridou, E. (2019). Development of a CRISPR/Cas9 system against ruminant animal brucellosis. BMC veterinary research, 15, 1-10.
18. Klug, A. (2010). The discovery of zinc fingers and their applications in gene regulation and genome manipulation. Annual review of biochemistry, 79(1), 213-231.
19. Li, H., Yang, Y., Hong, W., Huang, M., Wu, M., & Zhao, X. (2020). Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. Signal transduction and targeted therapy, 5(1), 1-10.
20. Liu, X., Y. Wang, Y. Tian, Y. Yu, M. Gao, G. Hu, F. Su, S. Pan, Y. Luo and Z. Guo. (2014). Generation of mastitis resistance in cows by targeting human lysozyme gene to β-casein locus using zinc-finger nucleases. Proceedings of the Royal Society B: Biological Sciences, 281,2013-3368.
21. Lu, Z., Zhang, L., Mu, Q., Liu, J., Chen, Y., Wang, H., . . . Wang, Z. (2023). The Prospect and Application Research Progress of Precision Gene Editing Technology Based on CRISPR/Cas9 in Livestock Genetics and Breeding.
22. Makarova, K. S., Grishin, N. V., Shabalina, S. A., Wolf, Y. I., & Koonin, E. V. (2006). A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action. Biology direct, 1, 1-26.
23. Mallikarjunappa, S., Shandilya, U. K., Sharma, A., Lamers, K., Bissonnette, N., Karrow, N. A., & Meade, K. G. (2020). Functional analysis of bovine interleukin-10 receptor alpha in response to Mycobacterium avium subsp. paratuberculosis lysate using CRISPR/Cas9. BMC genetics, 21, 1-11.
24. Mekler, V., Minakhin, L., & Severinov, K. (2017). Mechanism of duplex DNA destabilization by RNA-guided Cas9 nuclease during target interrogation. Proceedings of the National Academy of Sciences, 114(21), 5443-5448.
25. Nishimasu, H., Ran, F. A., Hsu, P. D., Konermann, S., Shehata, S. I., Dohmae, N., . . . Nureki, O. (2014). Crystal structure of Cas9 in complex with guide RNA and target DNA. Cell, 156(5), 935-949.
26. Ousterout, D. G., Kabadi, A. M., Thakore, P. I., Majoros, W. H., Reddy, T. E., & Gersbach, C. A. (2015). Multiplex CRISPR/Cas9-based genome editing for correction of dystrophin mutations that cause Duchenne muscular dystrophy. Nature communications, 6(1), 6244.
27. Piergentili, R., Del Rio, A., Signore, F., Umani Ronchi, F., Marinelli, E., & Zaami, S. (2021). CRISPR-Cas and its wide-ranging applications: From human genome editing to environmental implications, technical limitations, hazards and bioethical issues. Cells, 10(5), 969.
28. Pinzon-Arteaga, C., Snyder, M. D., Lazzarotto, C. R., Moreno, N. F., Juras, R., Raudsepp, T., . . . Long, C. R. (2020). Efficient correction of a deleterious point mutation in primary horse fibroblasts with CRISPR-Cas9. Scientific Reports, 10(1), 7411.
29. Ran, F., Hsu, P. D., Wright, J., Agarwala, V., Scott, D. A., & Zhang, F. (2013). Genome engineering using the CRISPR-Cas9 system. Nature protocols, 8(11), 2281-2308.
30. Ruan, J., Xu, J., Chen-Tsai, R. Y., & Li, K. (2017). Genome editing in livestock: Are we ready for a revolution in animal breeding industry? Transgenic research, 26, 715-726.
31. Shen, B., Zhang, J., Wu, H., Wang, J., Ma, K., Li, Z., . . . Huang, X. (2013). Generation of gene-modified mice via Cas9/RNA-mediated gene targeting. Cell research, 23(5), 720-723.
32. Singh, P., & Ali, S. A. (2021). Impact of CRISPR-Cas9-based genome engineering in farm animals. Veterinary Sciences, 8(7), 122.
33. Song, B., S. Yang, G.-H. Hwang, J. Yu and S. Bae. (2021). Analysis of NHEJ-based DNA repair after CRISPR-mediated DNA cleavage. International journal of molecular sciences, 22(2),6397-6420.
34. Tomley, F. M., & Shirley, M. W. (2009). Livestock infectious diseases and zoonoses : The Royal Society, 364,2637-2642.
35. Upadhyay, S. K., Kumar, J., Alok, A., & Tuli, R. (2013). RNA-guided genome editing for target gene mutations in wheat. G3: Genes, Genomes, Genetics, 3(12), 2233-2238.
36. Wang, M., Z. Sun, F. Ding, H. Wang, L. Li, X. Li, X. Zheng, N. Li, Y. Dai and C. Wu. (2021). Efficient TALEN-mediated gene knockin at the bovine Y chromosome and generation of a sex-reversal bovine. Cellular and Molecular Life Sciences, 78,5415-5425.
37. Wells, K. D., & Prather, R. S. (2017). Genome-editing technologies to improve research, reproduction, and production in pigs. Molecular Reproduction and Development, 84(9), 1012-1017.

38. Xie, K., Zhang, J., & Yang, Y. (2014). Genome-wide prediction of highly specific guide RNA spacers for CRISPR–Cas9-mediated genome editing in model plants and major crops. Molecular plant, 7(5), 923-926.
39. Zhang XueMei, Z. X., Li WenRong, L. W., Liu ChenXi, L. C., Peng XinRong, P. X., Lin JiaPeng, L. J., He SanGang, H. S., . . . Wu YangSheng, W. Y. (2017). Alteration of sheep coat color pattern by disruption of ASIP gene via CRISPR Cas9. Molecular Therapy, 27,735-746.
40. Zhu, X. X., Zhan, Q. M., Wei, Y. Y., Yan, A. F., Feng, J., Liu, L., . . . Tang, D. S. (2020). CRISPR/Cas9-mediated MSTN disruption accelerates the growth of Chinese Bama pigs. Reproduction in Domestic Animals, 55(10), 1314-1327.