JMHT: Journal of Medicine and Health Technology
Vol. 01, No. 01, May, 2024, pp. 21-39
ISSN: 3046-6865
DOI: 10.12962/j30466865.v1i1.1172

CRISPR-Cas9 Genome Editing Technology for Zoonotic Disease Control in Indonesia: A Comprehensive Review

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Abstract — The increasing emergence of zoonotic diseases originating from vectors, rodents, mammals, and others has increased the potential for outbreaks of pandemics in the Indonesian territory. Although control and prevention have been implemented, these efforts have not yet revealed a bright spot; therefore, elaboration with advanced CRISPR-Cas9 technology is a way to accelerate efforts to control zoonotic diseases in Indonesia. However, there is limited literature on this topic. This review aims to comprehensively describe, identify, and summarize the application of CRISPR-Cas9 genome-editing technology in zoonotic disease control in Indonesia. Our findings show that CRISPR-Cas9 genome editing technology offers an innovative approach for zoonotic disease control by targeting disease vectors, modifying animal reservoirs, improving disease surveillance, enhancing vaccine development, and exploring traditional medicine candidates and immunotherapy. The high level of precision, efficiency, and versatility in targeting genomes capable of disrupting, damaging, and disrupting the disease transmission cycle in pathogens makes CRISPR-Cas9 highly effective. However, challenges such as off-target impacts, regulatory complexity, and ethical considerations must be overcome with inter- and multidisciplinary collaboration to promote transparency, equity, and public engagement throughout the process of implementing this technology in the field, especially in Indonesia.

Keywords — CRISPR-Cas9; Editing Technology; Zoonotic Disease; Infectious Disease; Tropical Medicine

How to cite: Adnyana IMDM, Eljatin DS, Sudaryati NLG, Adiwinoto RP, Raza SM, da Cruz SV. CRISPR-Cas9 Genome Editing Technology for Zoonotic Disease Control in Indonesia: A Comprehensive Review. Journal of Medicine and Health Technology, 2024.

Manuscript received 24 April 2024; revised 1 May 2024; accepted 7 May 2024. Date of publication 30 May 2024.

INTRODUCTION

Zoonotic diseases pose significant public health challenges in Indonesia. This is because Indonesia is located in a rich tropical region with high biodiversity, dense human and animal populations, and environmental factors conducive to zoonotic disease transmission [1]. Zoonotic diseases are transmitted from animals to humans vertically or horizontally (through the sylvatic cycle) [2]. Indonesia has faced threats, challenges, and obstacles to zoonotic disease control, resulting in a health burden in the country [3,4]. Despite efforts to control zoonotic diseases through traditional methods, such as vector control, vaccination, and public health education, challenges remain [5,6]. The emergence of drug-resistant pathogens, the spread of zoonotic diseases across countries, and the complexity of disease transmission dynamics require innovative approaches for disease control [7–9]. Under these conditions and problems, CRISPR-Cas9 genome editing technology is a promising new tool for overcoming the challenges of zoonotic disease control in Indonesia. Previous studies have revealed that CRISPR-Cas9 genome-editing technology has a high level of precision, efficiency, and versatility in the identification of samples containing disease pathogens

[10–13]. Ansori et al. [14] explained that CRISPR-Cas9 genome editing technology has many advantages and can be applied in various fields as an early detection, diagnostic, and evaluation tool. His findings showed that CRISPR-Cas9 can be applied in agriculture, medicine, environmental sciences, fisheries, nanotechnology, bioinformatics, and biotechnology. Hence, CRISPR-Cas9 technology has potential for use in disease control and prevention.

Traditional approaches to zoonotic disease control have limitations in terms of efficacy, sustainability, and scalability. Vector control efforts, such as insecticide spraying and mosquito net distribution, are often resource intensive and environmentally damaging [7,15]. Vaccination programs may be hampered by logistical challenges, and public skepticism regarding vaccine efficacy generates a new stigma that hinders the eradication of zoonotic diseases in the Indonesian region [16]. The development of drug-resistant pathogens poses a significant threat to the efficacy of antimicrobial therapy [17,18]. The necessity for alternative approaches to complement current control measures and tackle the emergence of zoonotic diseases has become apparent due to these challenges. This review aims to comprehensively explain, identify, and summarize the application of CRISPR-Cas9 genome-editing technology in zoonotic disease control in Indonesia. This review provides a comprehensive summary of the CRISPR-Cas9 system and its technology, its potential utilization in zoonotic disease control in Indonesia, the benefits and limitations of using CRISPR-Cas9, the challenges and ethical considerations of using CRISPR-Cas9 for zoonotic disease control, and recommendations. Hopefully, this review will provide a new perspective on zoonotic disease control efforts in Indonesia utilizing CRISPR-Cas9 genome editing technology.

CRISPR-Cas9 Genome Editing Technology

CRISPR-Cas9 is a groundbreaking gene-editing technology that has gained widespread application in various sectors, particularly in molecular biology, medicine, biotechnology, and environmental science. CRISPR, which stands for clustered regularly interspaced short palindromic repeats, is a specific segment of prokaryotic DNA comprised of a succession of short, repeating base pairs. Cas9, on the other hand, is a crucial enzyme in the bacterial immune system that plays a pivotal role in the editing of genes in living organisms [19–21]. The CRISPR-Cas9 system is adapted from a natural defense mechanism found in bacteria that protects against viral attacks by cutting DNA. To date, this genome editing method and system have expanded its application to other organisms, including animals, humans, and plants [22,23].

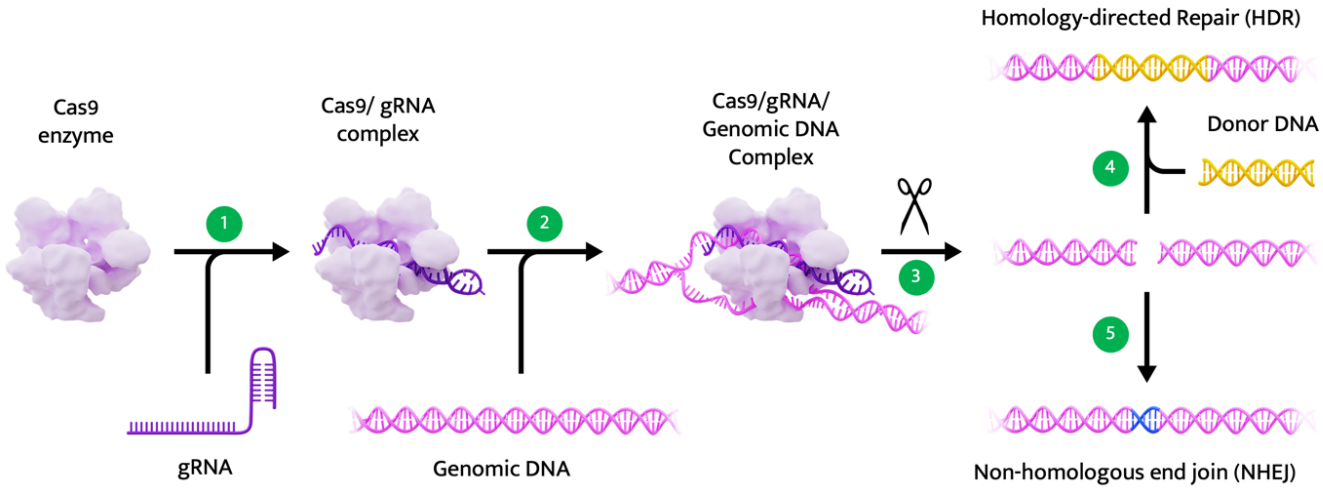

Fig. 1. Genome editing mechanism using CRISPR-Cas9 technology.

CRISPR-Cas9 genome editing technology operates through a series of steps that include the recognition process, which begins with the design of a guide RNA (gRNA) that complements the target DNA sequence when editing is performed. The

gRNA guides the Cas9 enzyme to a specific location in the targeted genome and forms a complex with the enzyme, wherein the Cas9 scans the DNA until it finds a sequence that matches the gRNA. This complex then cleaves the DNA at the specified location, allowing for precise editing of the genome [24]. The Cas9-gRNA complex, upon identifying the designated DNA sequence, causes the Cas9 enzyme to cleave both DNA strands at the appropriate location, resulting in a double-strand break (DSB). The natural repair mechanisms of cells are triggered when DNA is cleaved. There are two primary repair pathways involved in this process: nonhomologous end-joining (NHEJ) and homology-directed repair (HDR). NHEJ often leads to small insertions or deletions (indels) at sites of damage, which can disrupt genes, while HDR is utilized to introduce specific alterations by providing a DNA template with the desired sequence [25,26]. The mechanism of genome editing using CRISPR-Cas9 technology is shown in **Fig. 1**.

Genomic editing with CRISPR-Cas9 technology has attracted extensive interest in diverse areas of biological research due to its remarkable precision. Unlike earlier gene editing methods, CRISPR-Cas9 allows highly accurate targeting of particular DNA sequences, significantly reducing the occurrence of off-target effects. Moreover, this technology can be employed to modify genes in a wide range of organisms, such as plants, animals, and humans, making it a versatile tool for present and future scientific research and potential therapeutic use [11,27,28]. Due to its exceptional precision and versatility in handling diverse samples, CRISPR-Cas9 boasts an impressive efficiency rate. In comparison to other gene editing techniques, CRISPR-Cas9 is notably user-friendly and efficient, facilitating rapid progress in many disciplines. This technology holds immense promise for treating genetic disorders by rectifying disease-causing mutations and by revolutionizing diagnostic, therapeutic, and evaluation methods based on an individual's unique genetic makeup [29–31].

Differences in the Transfection of CRISPR-Cas9 Genome Editing Technology

CRISPR-Cas9 genome editing technology offers various methods for delivering the necessary components into cells for targeted genetic modification. These methods differ in terms of introducing the Cas9 enzyme and guiding the gRNA toward the targeted cells [19]. The advantages of the use of delivery particles for genome editing via CRISPR-Cas9 technology are presented in **Fig. 2**. In addition, three CRISPR-Cas9 transfection methods are presented in **Fig. 3.** First, CRISPR DNA (Cas9 plasmid), that is, the Cas9 gene and gRNA expression cassette, is inserted into a plasmid DNA vector [32]. This plasmid can be introduced into target cells using various transfection methods, such as electroporation or lipid-mediated transfection. Once inside the cell, the Cas9 gene is transcribed and translated, resulting in the production of the Cas9 enzyme and gRNA [33]. The Cas9 enzyme forms a complex with gRNA and initiates the gene editing process. This approach allows the stable expression of Cas9 in target cells over an extended period, allowing multiple rounds of genome editing [20,34].

Second, CRISPR RNA (Cas9 mRNA) delivers the Cas9 genome to a plasmid, which involves direct transfection of Cas9 messenger RNA (mRNA) into the target cell. gRNA can be delivered as separate RNA molecules or synthesized inside the target cell using various RNA expression systems. Cas9 mRNA is transiently expressed in cells, leading to the production of the Cas9 enzyme. gRNA guides the Cas9 enzyme to the target DNA sequence and initiates the genome-editing process as desired. This approach offers the advantage of avoiding the potential genomic integration of foreign DNA, thus reducing the risk of off-target effects and immune responses [35]. The third type is CRISPR RNP (Cas9 protein), in which the Cas9 enzyme is rearranged with gRNA to form a ribonucleoprotein (RNP) complex [11]. Cas9 protein can be purified from recombinant sources or synthesized in vitro using a cell-free protein expression system. The Cas9 RNP complex is directly delivered to the target cell via electroporation or microinjection. Once inside the cell, the Cas9 RNP complex immediately begins to edit the target DNA sequence. This approach has advantages over the previous two methods, such as rapid gene editing activity, high efficiency, and

reduced risk of off-target effects, compared to DNA-based methods. In addition, since the Cas9 RNP complex does not require transcription or translation, it can be used in nondividing cells, such as neurons and primary cells [36].

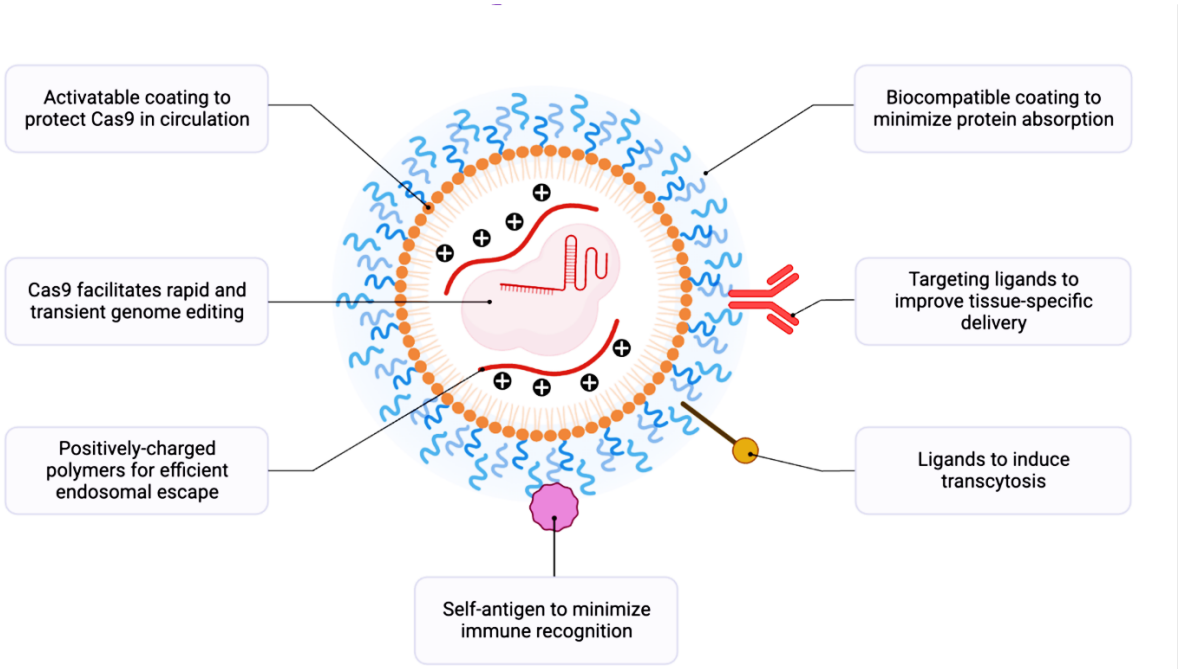

Fig. 2. Particle delivery advantages for genome editing using CRISPR-Cas9 technology.

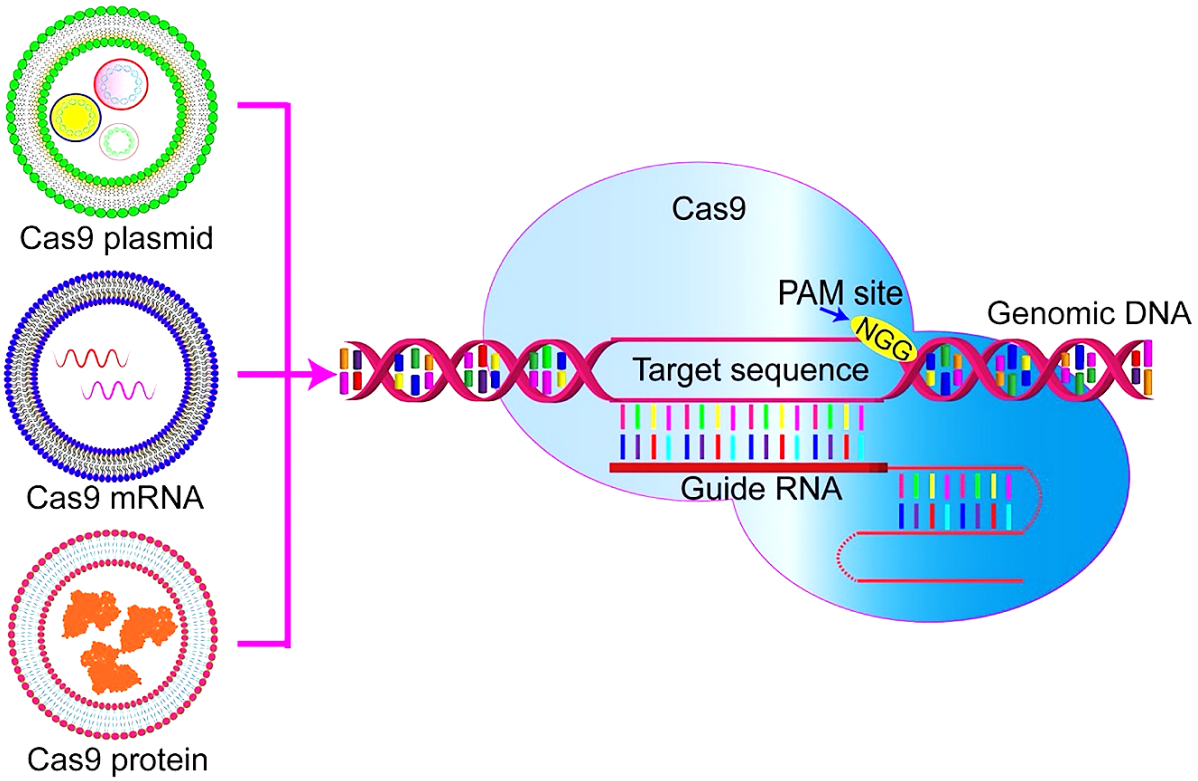

Fig. 3. Differences in the Transfection of CRISPR-Cas9 Genome Editing Technology [37].

Advanced CRISPR-Cas9 Genome Editing Technology

Babe Editing

Genome-editing technology has undergone significant development in various fields. The application of CRISPR-Cas9, which can change specific DNA sequences or codes by one letter without breaking double strands, is used for base editing [20]. This method is very promising for the repair of genetic mutations that cause various hereditary diseases (such as sickle cell anemia and cystic fibrosis). Gene editing is expected to produce new and better genetic materials. Base editing is performed by joining a Cas protein that cannot function properly with a base-converting enzyme, such as cytidine or adenine deaminase, which allows the targeted conversion of one DNA base to another, leading to a specific point mutation [14].

Base editing involves several steps, such as adding base editors, focusing on certain base pairs, chemically changing DNA bases, and fixing genetic mutations. In the introduction stage, the base editor becomes a molecular tool that can directly change specific nucleotides in the DNA sequence [19,23]. These editors have two components, namely, base-modifying enzymes such as cytidine deaminase or adenine deaminase, which change the target base pairs in DNA. Cas proteins, such as Cas9 or Cas12a, cannot perform their tasks either because they cannot recognize and bind to target DNA sequences without breaking the double strand. During the specific base pair targeting stage, the base editor creates a precise and accurate DNA target that uses guide RNA (gRNA) to guide the Cas protein to the right place in the genome. gRNA helps direct the base editor to the exact location of the genetic mutation. At this stage, the identification and recognition of the target have already been performed [13,38].

When chemical modification of DNA bases is performed by a base editor directed to the target site, the base-modifying enzymes in the complex initiate chemical changes to the DNA bases [39]. For example, if the base editor is based on cytidine deaminase, this enzyme can convert cytosine (C) to uracil (U) in the DNA sequence. When DNA replication occurs, uracil is recognized as thymine (T), which effectively converts CG base pairs into TA base pairs. In addition, when the base editor is adenine deaminase-based, this enzyme facilitates the conversion of adenine (A) to inosine (I), which is recognized as guanine (G) during DNA replication, leading to the conversion of AT to GC. In the last stage of correcting genetic mutations by inducing specific base-pair changes in the DNA sequence, a base editor can correct pathogenic mutations responsible for various genetic disorders [32,40].

Prime Editing

Prime editing in CRISPR–Cas9 is used to manipulate the genome with greater accuracy and wider utility than conventional methods. Prime editing involves the fusion of the Cas9 enzyme with reverse transcriptase engineering and a master editing guide RNA (pegRNA). The PegRNA directs the Cas9 complex to the target site and encodes the desired edit. Reverse transcriptase generates new DNA sequences based on the information carried by pegRNAs, facilitating insertions, deletions, and point mutations with high precision and efficiency. Prime editing is used to correct a wider range of genetic mutations with fewer off-target effects [19,41].

The mechanism of prime editing has been studied extensively. Two main components are utilized: Cas enzymes (e.g., modified Cas9) are utilized and engineered to retain DNA-binding ability but lack the ability to induce double-strand breaks, and engineered reverse transcriptase (RT) is utilized as the prime editing system by incorporating engineered RT enzymes that perform the important function of copying the desired genetic edits into the target DNA strand [23,42]. Furthermore, main RNA (pegRNA) editing was performed. This pegRNA plays a multifunctional role that includes targeting, characterized by pegRNA guiding the master editing complex to the exact location in the genome where the desired editing is performed. Another function is to encode edits within the desired pegRNA structure and to determine the changes to be made in the DNA sequence. When the

pegRNA has completed editing, it determines the formation of the main editing complex consisting of the Cas enzyme, engineered RT, and pegRNA, guided to the target DNA sequence by the pegRNA [43,44].

Once the main editing complex is bound to the target site, the engineered RT within the complex performs the dual function of nick generation, which allows the RT to generate nicks on the DNA strand to expose the region for editing, creating single-stranded DNA breaks and editing the DNA strand using PegRNA, which serves as a template for the RT to copy the desired genetic editing into the cut DNA strand. RT uses pegRNA as a guide to precisely insert, delete, or replace genetic information in the target DNA strand. In the last stage, DNA repair and finalization are performed by sealing the DNA pieces, resulting in the incorporation of edited genetic information into the genome [28,45].

CRISPR–Interference (CRISPRi)

CRISPR interference (CRISPRi) is a gene regulation technique that uses modified Cas proteins (such as dCas9) fused to repressive domains or effector molecules to enable the repression of targeted genes without altering the DNA sequence. This method involves guiding the dCas9 complex to specific gene promoters or regulatory regions using a guide RNA, thereby inhibiting gene expression by blocking transcription or recruiting repressive chromatin-modifying complexes [22,24,46]. CRISPRi can be used to study gene function, elucidate disease mechanisms, and potentially modulate gene expression in therapeutic contexts.

The mechanism involved in CRISPR interference is that CRISPRi uses a modified form of Cas protein (such as dCas9 or dCas12a) that lacks the endonuclease activity needed for DNA cleavage, rendering it catalytically inactive (dCas). Next, a guide RNA (gRNA) was designed to direct the catalytically inactive Cas protein to a specific region of the genome, such as the gene promoter or DNA regulatory sequence. CRISPRi targets gene expression by binding to the target DNA, where the designed gRNA guides the catalytically inactive Cas protein to the target DNA sequence, precisely positioning it within the desired gene. Once bound to the target site, dCas blocks the transcriptional machinery from initiating gene expression.

This is achieved by physically blocking the binding of RNA polymerase or recruiting a repressive chromatin-modifying complex to the gene promoter, thereby repressing gene transcription. In some CRISPR systems, catalytically inactive Cas proteins are fused to effector domains or repressive elements. These effector molecules enable more precise and robust gene regulation by modulating chromatin structure or interacting with the transcription machinery [14,47,48]. By enabling the selective and reversible induction or downregulation of particular genes, CRISPRi facilitates a more exhaustive examination of gene function and the repercussions of alterations in gene expression. In addition to its capacity to specifically inhibit the expression of disease-associated genes, CRISPRi aids in clarifying the molecular pathways that underlie a multitude of disorders, perhaps leading to the identification of therapeutic targets [49].

Epigenome Editing

Epigenome editing refers to the manipulation of epigenetic marks or modifications in the genome without directly changing the genetic code. Epigenetic modifications such as DNA methylation, histone modification, and chromatin remodeling play important roles in regulating gene expression and cellular function without altering the underlying DNA sequence. Epigenome editing technologies enable precise control over epigenetic marks, allowing researchers to modulate gene expression patterns and investigate the functional consequences of epigenetic changes in various biological processes and diseases [50,51].

The mechanism and technique of epigenome editing were determined using the CRISPR–Cas system (**Fig. 4**). A modified version of the Cas protein is fused to the domain of an epigenetic effector or protein responsible for inducing a specific epigenetic modification, and a catalytically inactive Cas protein (dCas9 or dCas12a) is fused to various epigenetic modifiers,

such as methyltransferases, demethylases, acetyltransferases, and histone deacetylases, enabling precise targeting of specific epigenetic marks at desired genomic locations. Epigenome editing is performed in two main stages: guiding the editing complex using gRNA to deliver the epigenome-editing complex to a specific genomic region. The gRNA directed the modified Cas protein toward the target DNA sequence with high precision. Once targeted to the desired genomic location, the fused epigenetic effector domain alters the epigenetic landscape; this process is known as epigenetic mark modulation [22,45,52].

Epigenome editing was used to probe genetic regulation. Epigenome editing facilitates the investigation of how specific epigenetic modifications regulate gene expression, cell differentiation, development, and disease progression. In addition, this method has been used for disease modeling and therapy. Understanding the role of epigenetic modifications in cellular diseases will contribute to the identification of new therapeutic targets for conditions affected by epigenetic dysregulation [51,52].

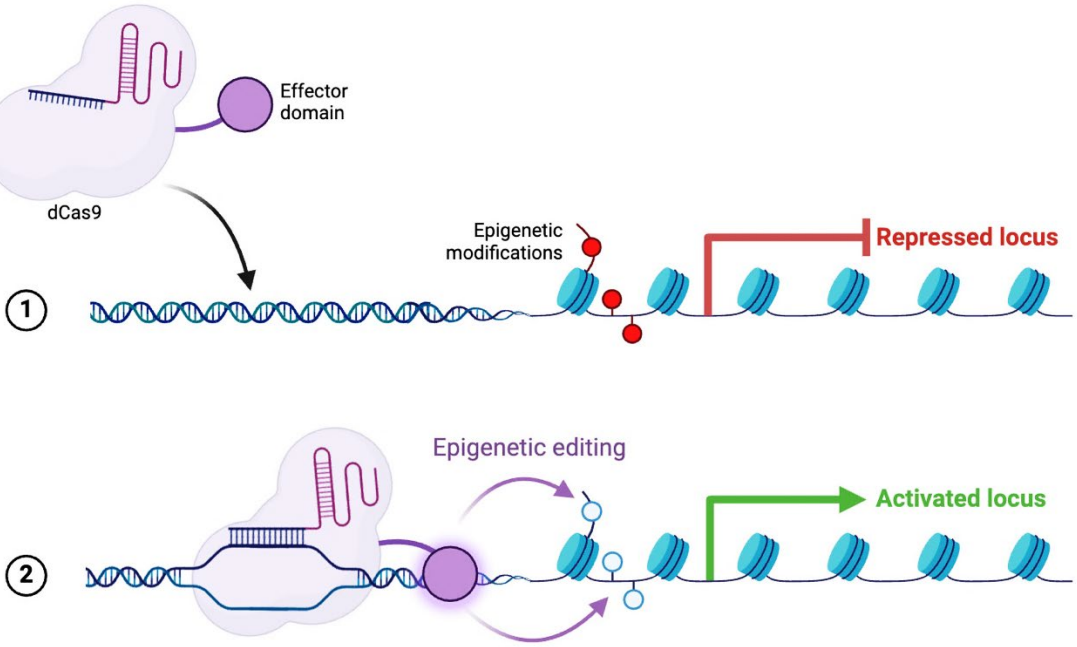

Fig. 4. Mechanism and technique of epigenome editing using CRISPR–Cas9

Overview of zoonotic diseases in Indonesia

Indonesia is one of the countries in the world that is home to a wide range of zoonotic diseases due to its rich biodiversity, dense human and animal populations, and environmental factors conducive to disease transmission and proliferation [53]. This has resulted in significant problems affecting human health, animal welfare, and socioeconomic development. It is estimated that more than 200 zoonotic diseases (animal-to-human disease infections) and 25 new strategic infectious animal diseases are considered threats to public health in Indonesia. These include rabies, avian influenza, anthrax, leptospirosis, toxoplasmosis, dengue fever, Zika, chikungunya, and malaria [54–56].

Indonesia faces a high burden of zoonotic diseases, including vector-borne diseases as well as diseases transmitted through direct contact with animals. The country's tropical climate and diverse ecosystems provide ideal conditions for the proliferation of disease vectors and reservoirs, thus contributing to endemicity and seasonal outbreaks of zoonotic diseases [57–59]. Mosquito-borne diseases, particularly dengue fever, are endemic in Indonesia, with millions of cases reported each year [60]. According to information from the Ministry of Health of the Republic of Indonesia, until the 14th week of 2024 or April,

there were 60,296 cases of dengue fever in Indonesia, with a mortality rate of 455, which is expected to increase due to climate change and environmental anomalies that increase vector productivity [61].

In addition to vector-borne diseases, Indonesia is still haunted by emerging zoonotic diseases that have the potential to become pandemics, such as avian influenza (H5N1 and H7N9) in humans, avian influenza A (H5N6) in poultry, Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Monkeypox (MPX), Nipah virus disease, Lassa fever, Legionellosis, meningococcal meningitis, listeriosis, and Crimean-Congo Hemorrhagic fever, which have increased in recent months. Wild and domestic animals are reservoirs for zoonotic pathogens in Indonesia [62,63]. The wildlife trade and habitat destruction contribute to the spread of pathogens from wildlife to humans, leading to outbreaks of diseases such as rabies and leptospirosis [64–66]. Additionally, close contact with livestock, poultry, and companion animals increases the risk of zoonotic transmission. Thus, massive eradication, prevention, and control efforts must be carried out.

Potential Applications of CRISPR-Cas9 in Zoonotic Disease Control

Advances in CRISPR-Cas9 genome editing technology include the early detection of complex diseases, prevention efforts involving precision diagnostic results, and control efforts involving surveillance systems. Here, we summarize the role of CRISPR-Cas9 genome-editing technology in zoonotic disease control by targeting disease vectors, modifying animal reservoirs, enhancing disease surveillance, and developing vaccines, traditional drug candidates, and immunotherapies, as presented in **Fig. 5** and described as follows.

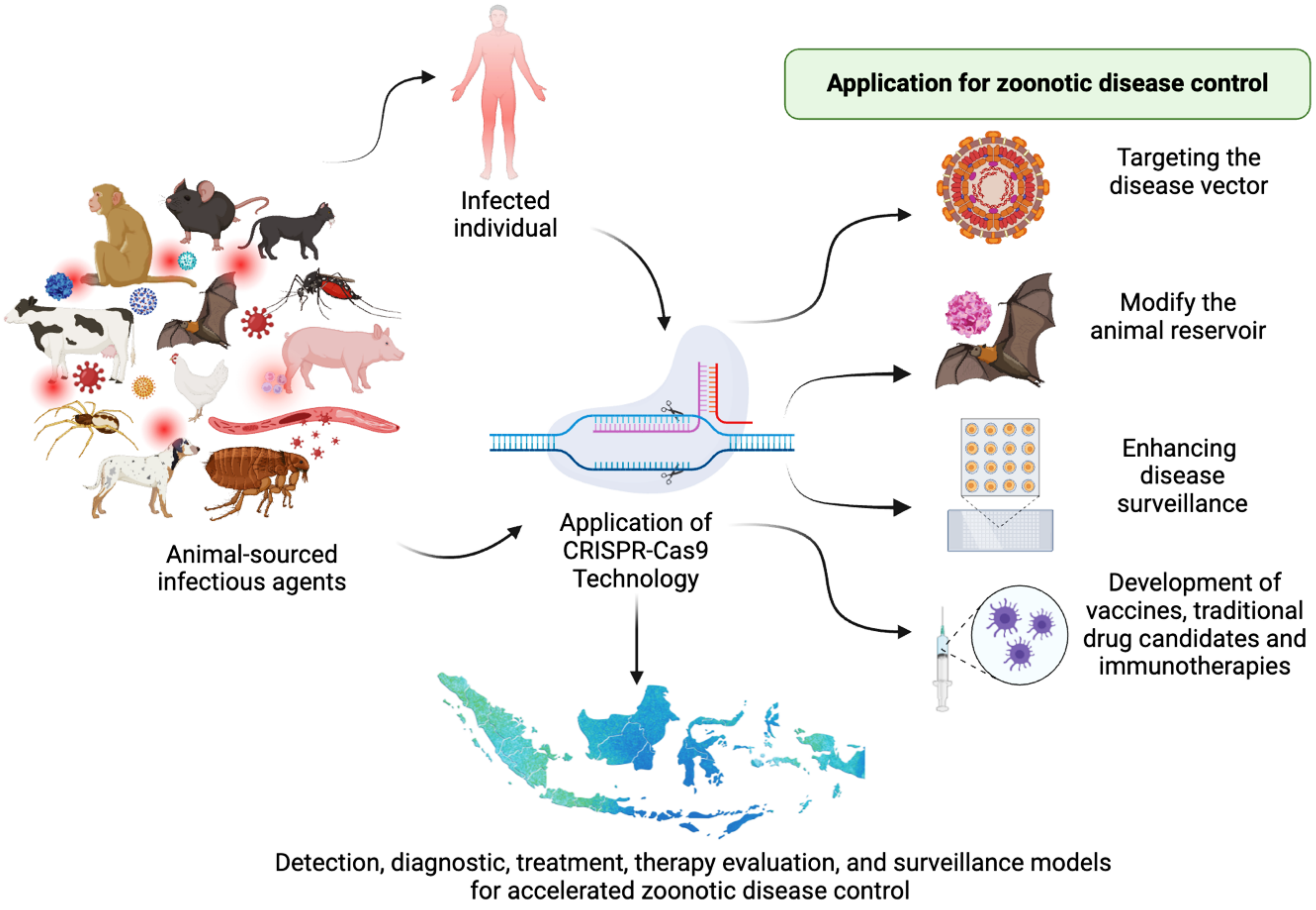

Fig. 5. Potential Applications of CRISPR-Cas9 in Zoonotic Disease Control in Indonesia.

CRISPR-Cas9 in targeting disease vectors

Due to its ability to target disease vectors such as mosquitoes, ticks, mites, and other arthropods that transmit pathogens to humans and animals and/or vice versa (sylvatic cycle), CRISPR-Cas9 genome editing technology plays a major role in modifying the genetic makeup of these vectors with precision with the aim of reducing or eliminating the ability of these vectors to transmit diseases, with implications for improving the quality of public health [67]. The role and mechanism of targeting disease vectors using CRISPR-Cas9 involves various stages, namely, the identification of target genes that are important for vector competence, reproduction, or survival, especially those that act as pathogen recognition genes, transmission, or vector-specific traits, such as regulating vector bionomics and reproduction [68,69].

Second, construction of the target genome was carried out, and a specific guide RNA (gRNA) was designed to guide the Cas9 enzyme to the desired genomic locus. This gRNA recognizes and binds to a unique sequence in the target gene, leading to the formation of a Cas9-gRNA complex [34,70,71]. Third, the CRISPR-Cas9 component is delivered to the target vector population via transgenesis methods through CRISPR induction into the vector genome or somatic cell editing. Fourth, gene editing and transmission blocking are performed by the Cas9 enzyme, which cleaves the target gene at a specific location and causes damage to the DNA. This can result in gene disruption, knockout, or modification depending on the repair mechanism used by the vector organism [45,72]. Fifth, the edited vector population can be propagated and released into the environment to promote the desired genetic modifications throughout the population. Over time, altered traits may become prevalent in vector populations present in the environment, thereby reducing disease transmission in a sustainable manner.

The implications of applying CRISPR-Cas9 genome-editing technology for targeting disease vectors are vast and profound, particularly in the realm of public health. This technology has the potential to be utilized for the control of vector-borne diseases [73,74]. These diseases, such as malaria, dengue fever, Zika virus, Lyme disease, Japanese B. encephalitis, and Q fever, pose significant health threats in Indonesia. This is because diseases are endemic to several Indonesian regions each year. CRISPR-Cas9-based interventions have the potential to modify and disrupt the disease transmission cycle by reducing vector populations or the ability of vectors to transmit disease pathogens, such as viruses, bacteria, and other fungi and parasites [16,69].

In terms of identifying and targeting disease vectors, a number of studies have reported modifications to genome structures that regulate the dengue virus replication cycle in *Aedes aegypti* mosquitoes, including lethal(2)-essential-for-life (l(2)efl) [75], capsid-coding region 1 (CCR1) [76], AAEL001123, TALENs, ZFNs, and homing endonucleases [77], nanos, β2-tubulin, or zpg [78] and sgRNA-expressing elements (*kmo*^{sgRNAs}) [79], which produce different effects, including the inability of vectors to transmit dengue virus more widely, produce stable and highly effective germline truncation at the kynurenine 3-monooxygenase (kmo) locus and act as a gene activator. In addition, its application results in diverse mutations through different repair mechanisms, which contribute to reducing viral infection, leading to a decrease in dengue virus in the mosquito population.

Research by Ranian et al. [80] has provided a specific picture of CRISPR-Cas9-mediated genome modification. In his study, he modified the doublesex gene (*Aedsx*), which plays an important role in controlling sex differentiation in mosquitoes. Using CRISPR/Cas9 technology, researchers have specifically targeted and disrupted female-specific genes known as *Aedsx*^{F1} and *Aedsx*^{F2}, which are expressed exclusively in female mosquitoes. A previous study reported that modifying the Aedsx gene contributes to abnormalities in adult female mosquitoes, including differences in wing size, proboscis length, ovary size, and body size. These abnormalities can result in reduced fertility, which is characterized by a 23-31% reduction in fecundity. Compared with those of wild-type mosquitoes, offspring characterized by this abnormality showed a 28-36% hatching inhibition rate. Thus, disrupted *Aedsx*^{F} contributes significantly to the ability of mosquitoes to suck blood, reducing the fertility rate of the vector in developing dengue virus in the body and allowing it to spread to humans. CRISPR-Cas9 genome-editing technology is effective at decreasing the sex ratio in mosquito populations.

Another study by Zulhusnain et al. [81] targeted and attenuated two genes found in *Aedes aegypti* mosquitoes, namely, doublesex (Aaedsx) and sexlethal (AaeSxl). Inoculation of *Aedes aeypti* eggs with the Cas9 protein (333 ng μl^{-1}) and gRNAs (100 ng μl^{-1}) resulted in the generation of the Aaedsx and AaeSxl knockout (KO) strains. As a result, there was a decrease in survival rate, mortality rate, and egg hatching failure of up to 38.33% compared to those of the control. A total of 11.66% of the Aaedsx female isoform-knockout larvae died, whereas only 3.33% of the AaeSxl-knockout eggs survived. These results indicate that CRISPR-Cas9 has the potential to reduce the population of dengue-causing vectors, which leads to a decrease in the level of infection in the environment [82]. Although CRISPR-Cas9 genome editing technology has high potential for application in various fields, especially vector control, to date, CRISPR-Cas9 technology has not been fully recognized and used as a companion to traditional vector control in Indonesia. The implementation of this technology could help reduce the spread of dengue fever virus by mosquitoes and make a significant contribution to zoonotic disease control efforts in Indonesia.

In addition, CRISPR technology helps reduce the dependence on insecticides, larvicides, pesticides, and other vector life-disrupting substances [74]. Traditional vector control methods, such as insecticides, larvicides, and pesticides, can have negative impacts on the environment and contribute to the development of resistance in broad-spectrum vectors that have harmful effects on nontarget organisms and ecosystems [83,84]. CRISPR-based interventions offer a more targeted and sustainable approach to vector control by identifying genes that have developed resistance, thereby reducing the reliance on these chemicals. Finally, this technology helps to prevent emerging disease outbreaks [33,53,85]. As bioclimatological changes, urbanization, and globalization continue to drive the emergence and spread of vector-borne diseases, CRISPR-Cas9 technology-assisted disease control approaches have sought to target source-based disease vectors to mitigate the incidence and risk of future outbreaks.

CRISPR-Cas9 in modifying animal reservoirs

Modifying animal reservoirs using CRISPR-Cas9 genome editing technology is a promising approach for controlling source-based zoonotic diseases in Indonesia. Animal reservoirs play a role in the transmission of zoonotic pathogens to humans, serving as hosts in which pathogens can replicate and potentially evolve to become more infectious or deadly. Targeting reservoir species with a high level of precision and accuracy has the potential to reduce their ability to transmit diseases to humans, thereby reducing the risk of zoonotic disease outbreaks, especially in Indonesia [29].

CRISPR-Cas9 technology precisely modifies the genome of animal reservoirs to confer resistance to specific pathogens or to reduce their ability to infect host pathogens. For example, CRISPR can be used to target genomes involved in the immune response of reservoir species, thereby increasing their ability to resist infection by zoonotic pathogens. Alternatively, genes important for pathogen replication or transmission in reservoir species could be targeted, thereby reducing the capacity of the reservoir population to sustain a more robust transmission cycle [4,8,71].

One example of the potential application of CRISPR-Cas9 in modifying animal reservoirs is to treat rodent-borne diseases, such as leptospirosis, hantavirus, salmonellosis, and Lassa fever, by targeting the genomes involved in the replication or transmission of these viruses and bacteria in rodent populations, potentially reducing the ability of reservoir species to spread disease to humans and cause infection [86,87]. Wolbachia technology has also been developed and used in Indonesia. Wolbachia, a naturally occurring symbiotic system, exhibits extensive transmission dynamics and is primarily inherited from mothers to offspring. It strategically enhances its spread by modulating host reproduction mechanisms, such as feminization, parthenogenesis, male killing, and/or cytoplasmic incompatibility (CI). CI arises when males carry Wolbachia mates with uninfected females, yielding offspring that are not viable. Notably, Wolbachia can impede or prevent infections caused by diverse pathogens, including DENV, yellow fever, ZIKV, various arboviruses, and malaria parasites [32,88,89].

CRISPR-Cas9 intervention attempts to orchestrate a phenomenon akin to CI within the Aedes mosquito, facilitated by genetically engineered hens. In a study conducted by Wang et al. [88], the combination of mosquito technology and Wolbachia revealed the pivotal roles of the cif^{A} and cif^{B} genes. These genes orchestrate induction and rescue by expressing bacterial elements in the host germline, mirroring the CI phenotype and impeding viral replication. Symbiont applications in vector control include introducing natural symbionts into mosquitoes to perturb mosquito physiology, thereby diminishing vector competence or eliciting antipathogenic effects. Alternatively, symbionts can be genetically modified to yield antipathogenic effector molecules, which are subsequently introduced into mosquitoes to confer resistance against pathogens or to attenuate vector competence. The tangible outcomes of Wolbachia technology have been pronounced, with a remarkable reduction of up to 77.1% in dengue virus infections and an 86.2% decrease in hospitalization rates over a decade-long study in Yogyakarta [90]. High dengue infection rates in Brazil mirrored these outcomes, with a 69% decline in dengue fever, a 56% reduction in chikungunya incidence, and a 37% decrease in Zika cases post-Wolbachia deployment and ongoing surveillance [91].

Another potential application is to modify livestock species that serve as reservoirs for zoonotic pathogens [92]. For example, the use of CRISPR-Cas9 genome editing technology to enhance the immune response of livestock species to pathogens such as avian influenza, avian influenza or bovine tuberculosis reduces the possibility of transmission to humans through the consumption of infected meat. Thus, the potential use of CRISPR-Cas9 technology in this field is very high and appropriate for minimizing the potential negative impacts on ecosystems and animal populations.

CRISPR-Cas9-mediated enhancement of disease surveillance

CRISPR-Cas9 technology has the potential to improve the surveillance of vector-borne diseases, ranging from early detection to early prevention efforts, the evaluation of disease progression, and decision-making for disease control, especially for zoonotic diseases. Disease surveillance is required to monitor the spread of pathogens, identify outbreaks early, and implement timely interventions to prevent or reduce the impact of infectious diseases on human and animal populations. CRISPR technology holds significant potential for disease surveillance through the creation of highly sensitive, specific, and rapid diagnostic tools for detecting zoonotic pathogens [93].

One approach involves the use of CRISPR-based nucleic acid detection platforms such as Specific High-sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK) and DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR)), which utilize the ability of the Cas enzyme to cleave a target nucleic acid sequence in response to the presence of a specific pathogen. This platform can detect low concentrations of pathogenic DNA or RNA with high specificity, enabling the early detection of zoonotic diseases in human, animal, and environmental populations. For instance, CRISPR-Cas9 was used to detect zoonotic diseases, especially African swine fever (ASF), quickly and precisely using the DNA endonuclease-targeted CRISPR trans reporter (DETECTR) assay. Integrating CRISPR with the DETECTR method demonstrated exceptional sensitivity, detecting as few as eight copies of ASF viral DNA without interference from other swine viruses. When applied to clinical blood samples, the DETECTR assay showed 100% concordance (30/30) with real-time polymerase chain reaction analysis. Rapid and accurate identification of ASF viruses could lead to rapid eradication strategies and strict sanitation protocols to effectively manage and prevent ASF transmission [94]. Another study was also used to detect SARS-CoV-2 (COVID-19) disease. The CRISPR-based DETECTR approach simultaneously eliminated negative strains due to N gene mutations, and the ability of this approach reached 100% in the identification of lateral flow strips in the sample. DETECTR is 100% more specific for SARS-CoV-2 than for other human coronaviruses [95,96]. Thus, this technology is potentially and appropriately applied for disease surveillance, especially in Indonesia, for zoonotic diseases.

In addition, CRISPR-based surveillance methods have advantages over traditional diagnostic techniques, including rapid turnaround time, high sensitivity and specificity, and potential for multiplex detection of multiple pathogens in a single assay [14]. These capabilities are particularly valuable in regions where many zoonotic diseases circulate and resources for laboratory diagnostics are limited. CRISPR-based surveillance systems can be applied in various settings, including point-of-care facilities, field laboratories, public health laboratories, and clinics, even in remote areas, enabling timely detection and response to zoonotic disease outbreaks in various environments and under various conditions [97]. This technology can be adapted for use in continuous human, animal, and environmental health surveillance, facilitating the One Health approach for zoonotic disease monitoring and control in Indonesia [2]. Despite their high potential, CRISPR-based surveillance methods also face challenges and limitations, such as the need for optimization and validation of testing protocols, ensuring the robustness and reliability of test results, and overcoming logistical and infrastructure constraints in implementing surveillance systems in resource-limited settings.

The use of CRISPR-Cas9 in the development of vaccines, traditional drug candidates and immunotherapies

The use of CRISPR-Cas9 technology in vaccine development, herbal drug discovery, and immunotherapy has great potential to advance the prevention, treatment, and control of zoonotic diseases, particularly in Indonesia. Given Indonesia's rich wildlife and population, zoonotic diseases are endemic in this region. Every year, zoonotic disease cases in Indonesia continue to increase, including dengue, Zika, malaria, chikungunya, avian influenza, rabies, anthrax, brucellosis, leptospirosis, Japanese B. Encephalitis, bovine tuberculosis, salmonellosis, schistosomiasis, Q fever, campylobacteriosis, trichinellosis, paratuberculosis, toxoplasmosis, and cysticercosis/taeniasis, which are fifteen priority zoonoses to be controlled and mitigated. Efforts can be made to explore herbal medicine candidates based on community beliefs (ethnobotany, ethnopharmacology, ethnozoology, and other studies), develop vaccines derived from disease-carrying animals or others, and develop immunotherapies derived from natural materials, animals and plants [72,98].

For instance, Siegrist et al. [99] sought to target three genes from Orthopoxvirus, namely, A17L, E3L, and I2L, for monkey pox vaccine development. This study demonstrated the efficacy of CRISPR targeting in human embryonic kidney (HEK293) cells with plasmids encoding SaCas9 and individual sgRNAs, resulting in a 93.19% reduction in the VACV titer per target. After the verification of CRISPR targeting, targeted delivery of VACV CRISPR antivirals was tested using adenovirus packaging vectors (AAVs) for SaCas9 and sgRNA, leading to viral titer reductions of up to 92.97% for individual targets. Thus, CRISPR is used not only for targeting viruses but also for detecting genes that can be attenuated for therapeutic candidates. In addition, vaccines, immunotherapy agents, and traditional medicine candidates have been developed using this technology [100]. These approaches offer new strategies to combat infectious diseases, especially zoonotic diseases, and address global health challenges comprehensively and sustainably. Continued research and development efforts are essential to realize the full therapeutic potential of CRISPR-based interventions against zoonotic diseases.

Traditional herbal medicine has long been used in many cultures for the treatment and prevention of various diseases, including infectious, noncommunicable, and zoonotic diseases [101]. CRISPR technology facilitates the identification and characterization of bioactive compounds found in medicinal plants (leaves, fruits, seeds, flowers, stems, roots, and juice) that have potential anti-zoonotic properties, such as anti-inflammatory, antibacterial, antiviral, antifungal, insecticidal, larvicidal, and other capabilities [102]. By targeting the genes involved in the biosynthesis of bioactive compounds, researchers can increase the production of therapeutic herbal extracts or engineer plants to produce novel compounds with anti-zoonotic activity. This approach offers the potential to discover new traditional medicine candidates with improved efficacy, safety, and sustainability for the treatment of zoonotic diseases in Indonesia [35,103].

CRISPR-Cas9 technology can be used for vaccine development against zoonotic diseases [16]. CRISPR technology can be used to precisely engineer vaccine candidates by modifying the pathogen genome to increase immunogenicity or attenuate virulence. CRISPR enables the development of new vaccine platforms, such as viral vector vaccines, DNA vaccines, or RNA vaccines, that can generate strong and long-lasting immune responses against zoonotic pathogens [104]. These advances promise to accelerate vaccine development timelines, improve vaccine efficacy, and overcome the challenges related to vaccine production and distribution. Inactivated virus replicates in vaccines can be mass-produced, resulting in adequate vaccine availability [105–107].

In addition, advances in immunotherapy models have emerged as a promising approach to combat zoonotic diseases by utilizing the immune system to target and eliminate pathogens [32]. CRISPR technology has enhanced the development of immunotherapies such as monoclonal antibodies, adaptive T-cell therapy, and stem cell therapy to target zoonotic pathogens [68]. CRISPR can be used to engineer immune cells to express chimeric antigen receptors (CARs) or enhance antipathogen effector functions, thereby increasing the efficacy of immunotherapeutic approaches against zoonotic diseases [108–110]. CRISPR-mediated genome editing in animal models can facilitate the preclinical evaluation of immunotherapies, accelerate their application in clinical practice, and provide reliable efficacy information.

Challenges and ethical considerations related to the use of CRISPR-Cas9 in zoonotic disease control

While CRISPR-Cas9 technology has great potential for transforming the control of zoonotic diseases, its implementation also presents a series of challenges and ethical considerations that must be carefully addressed to ensure responsible and effective utilization. Among the concerns with CRISPR-Cas9 technology is the potential for off-target effects, in which the Cas9 enzyme inadvertently alters unintended genetic sequences. Off-target effects can have unintended consequences on infectious pathogens, animals, and hosts, such as the generation of harmful mutations or disruption of important genes, posing risks to both target and non-target organisms [111,112]. Minimizing off-target effects requires the development of more precise and efficient CRISPR systems as well as robust methods to assess and minimize off-target activity with very little bias [113]. Additionally, zoonotic pathogens and their vectors often exhibit high levels of genetic diversity, which may facilitate the emergence of resistance to CRISPR-mediated interventions. Pathogens or vectors may develop mechanisms to evade CRISPR targeting, rendering interventions ineffective. In addition, genetic diversity within the host population may affect the effectiveness of CRISPR interventions, as certain individuals may be less susceptible to genetic modifications. Strategies to mitigate the risk of resistance include targeting multiple genetic loci simultaneously, optimizing transmission methods, and monitoring the emergence of strains resistant to resulting modifications [114,115].

This is in line with the moral and societal consequences of employing this technology to alter genetic makeup. The application of CRISPR-Cas9 technology raises questions about the equitable distribution of advantages and hazards, potential unforeseen consequences, and enduring consequences for ecosystems and biodiversity. Moreover, ethical issues may arise concerning the alteration of animal genomes, the discharge of genetically modified organisms into the environment, and the possibility of unintentional harm to non-target species [112,116]. An example is Wolbachia technology, which is used to combat and control dengue fever in Indonesia. Engaging stakeholders, including researchers, academics, policymakers, ethicists, epidemiologists, tropical medicine providers, public health providers, virologists, environmentalists, and community members, in open and inclusive discussions is essential for addressing these complex ethical and social issues.

The regulatory framework surrounding the utilization of CRISPR-Cas9 technology in zoonotic disease management is intricate and varies among different countries. Regulatory authorities must address issues such as risk assessment, environmental impact assessment, and public involvement in the decision-making process. Establishing a clear and transparent regulatory

framework that effectively balances innovation with safety and ethical considerations is crucial for facilitating the implementation of CRISPR-based interventions [114,115]. In Indonesia, regulations related to priority zoonoses for control and mitigation can be modified by the addition of CRISPR-Cas9, One Health, Ecological Triad, and other technological approaches to accelerate zoonotic disease control. Ensuring equitable access to CRISPR-based interventions is another challenge for zoonotic disease control. The high costs associated with research, development, and implementation may limit access to CRISPR technologies, particularly in low- and middle-income countries, where zoonotic diseases pose a significant public health burden. Efforts to promote global equity and access must address barriers such as intellectual property rights, technology transfer, capacity building, and the affordability of interventions, and publications must be improved and focused on the impact of the findings. By addressing these issues, we are confident that zoonotic diseases can be controlled in Indonesia.

Future Directions and Recommendations

The future direction of CRISPR-Cas9 technology for zoonotic disease control requires a holistic approach. Policymakers can utilize the full potential of CRISPR-Cas9 genome editing technology interventions to control zoonotic diseases and improve public health and welfare in Indonesia. Continued investments in research and development to advance CRISPR interventions for zoonotic disease control include the optimization of CRISPR systems, the development of new delivery methods, the exploration of innovative applications of gene drives, and the development of advanced synthetic biology approaches. In addition, collaboration between researchers, government agencies, nongovernmental organizations, industry partners, universities, and affected communities is essential for advancing CRISPR interventions. Multidisciplinary collaboration facilitates knowledge exchange, resource mobilization, and capacity building to accelerate the translation of research findings into practical solutions for the benefit of society.

The development of a clear and transparent regulatory framework is needed to ensure the safe and responsible use of CRISPR-Cas9 technology in zoonotic disease control, including risk assessment, environmental impact assessment, and surveillance of CRISPR interventions, while encouraging innovation and facilitating technology transfer [115,116]. Capacity-building efforts should focus on training researchers, technical zoonosis personnel, health professionals, policymakers, and community members in the responsible use of CRISPR-Cas9. Education and outreach programs can increase awareness of the potential benefits and risks of CRISPR interventions, encourage community engagement, and promote informed decision-making by relevant stakeholders. The capacity building of governments, research institutions, and universities should be built under one umbrella. International partnerships, technology transfer initiatives, and funding mechanisms can also facilitate the equitable distribution of resources and encourage shared responsibility for addressing zoonotic diseases on a broader scale.

CONCLUSION

CRISPR-Cas9 technology offers an unparalleled opportunity to revolutionize zoonotic disease control in Indonesia. Recent outbreaks of zoonotic diseases pose significant threats to human, animal, and environmental health, necessitating the development of effective control strategies. The precision and versatility of CRISPR-Cas9 in targeting specific genetic elements in zoonotic pathogens, vectors, and animal reservoirs has the potential to disrupt the disease transmission cycle. By improving disease surveillance systems, developing new vaccines, exploring traditional medicine candidates, immunotherapy, and modifying disease vectors and reservoirs, CRISPR-Cas9 holds great promise for reducing the burden of zoonotic diseases and preventing outbreaks in Indonesia. Its precise genome-editing capabilities make this technology a helpful complement to existing control measures and overcome the challenges that have arisen in zoonotic disease control in Indonesia. Further research is

needed in the future to achieve zoonotic disease control in Indonesia through the comprehensive application of CRISPR-Cas9 genome editing technology.

ACKNOWLEDGEMENT

The authors would like to thank the entire team that helped in writing this article as well as PT. Mega Science Indonesia for facilitating the translation and proofreading of the authors so that they are appropriate for publication.

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