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Genetic Engineering of Mosquitoes Causes Malaria

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ABSTRACT

Malaria is still a health problem in the world and in Indonesia in particular. Malaria vector control is an important strategy in efforts to control and eliminate malaria because it is very effective in preventing infection and reducing disease transmission. The CRISPR/Casnuclease system is a potent new genome editing system and tool for species-specific diagnosis, drug resistance research for *Plasmodium species*, and gene driver for Anopheles population control, according to an assessment of earlier genome editimg techniques. This *Anopheles* mosquito CRISPR/Cas9 technique on plasmodium has been applied in research to detect malaria parasites by inhibiting their growth throughout the life cycle, allowing evaluation of the effectiveness of antimalarial drugs or vaccines at various stages of the parasite life cycle. In addition, CRISPR/Cas9 in Anopheles mosquitoes allows identification and double-strand breaks in target DNA, which can then be modified through genome changes. So, with the development of gene editing technology, the spread of Anopheles mosquitoes can be controlled and reduced.

Keywords: *Anopheles, Malaria, Plasmodium*

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INTRODUCTION

Malaria is transmitted through the bite of female Anopheles mosquitoes which are caused by the presence of intracellular obligate protozoa from the genus Plasmodium. (Vantaux et al., 2021) . The Anopheles mosquito theoretically acts as a vector that can transmit an infectious agent to a susceptible host. Apart from that, the main factor causing the increase in malaria incidence is unstable environmental status. Malaria was a public health problem in 2011 in 109 countries, 31 of which were listed as 'malaria-high burden countries'. Around 3.3 billion of half the world's population lives in areas at risk of

malaria. Malaria attacks around 350-500 million people every year or one death every 30 seconds (Matthews et al., 2020) .

Malaria endemic areas are mainly in tropical countries with the most cases found in Africa and several countries in Asia, Latin America, the Middle East and Europe. Anopheles gambiae is one of the most dominant malaria mosquito species found in endemic areas of Africa due to its role as a transmitter of the malaria parasite. Various Anopheles species have been found as malaria vectors in Indonesia (Nolan, 2020) . On Java, the dominant Anopheles vector is An. aconitus, An. maculatus, An. balabacensis and An. sundaicus. The existence of the Anopheles vector species is consistent with the results of the 2012 entomological survey which was confirmed in Banjarmangu District (Banjarnegara) (Keman, nd) .

The malaria control program in Indonesia has achieved the target of reducing the incidence of malaria or Annual Parasite Incidence (API) in Indonesia in the last five years (2011-2015). From 422,447 cases in 2011 to 217,025 in 2015. Thus, progress in malaria control was able to reduce cases by 5% in the last five years and from 1.75 per 1000 population in 2011 to 0.85 per 1000 population in 2015 (Keman , n.d.) .

Starting from genetic engineering, progress has been discovered in the fieled of gene engineering technology which has inspired a revolution and fresh zea, using the most recent CRISPR technology, scientists, researchers can directly change the DNA sequence of almost any organism, making it possible to elucidate genetic function at the systems level and determine the underlying genetic etology of an illnes or condition. Gene alterations, no matter how minor, have a profound impact on cell activity. CRISPR technology has provided researchers with previously unheard-of resources. (Nourani et al., 2023) .

Plasmodium parasites cause malaria, with *P. falciparum* and *P. vivax* being the most clinically significant species (Naik, 2020) . According to a WHO report published on. December 2022, it is estimated that there will be 247 million malaria cases and 619,000 deaths in 84 malaria endemic countries, in 2021 (Kilian et al., 2021) . Due to advances in defining disorders targeting *Plasmodium* and *Anopheles* over the past few decades, there has been a remarkable decline in malaria infection rates worldwide. However, in order to minimizethe reservoir of infection and prevent anti-malaria resistance, new anti-malarial medicatin and vaccines along with more potent meansure to stop the transmission of malaria to humans, must be developed in order to eradicate and control malaria. CRISPR/Cas system is one of these novel tecnologies,and it can be used for vector engineering of *plasmodium* and *anopheles*, drug-resistant strains detection, vaccine development, improved diagnosis, and vector mamagement (Bryant et al., 2017) .

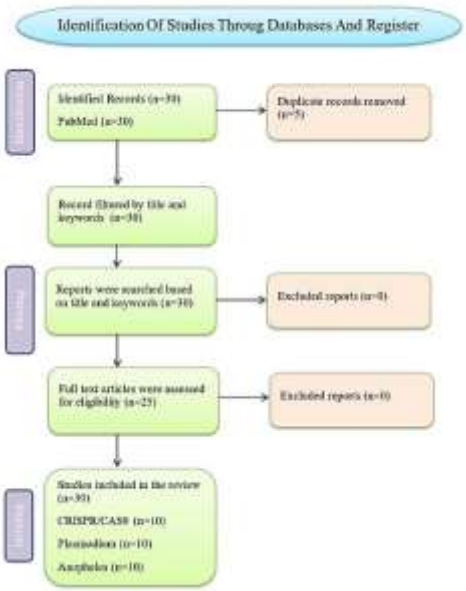
2012 saw a revolutionary development in gene editing techniques within the biological sciences and clinical fields, thanks to the regular clustering of short palindromic repeats-associated protein-9 nuclease (CRISPR/Cas9) of Streptococcus pyogenes. Because of its route, CRISPR/Cas9 can be efficiently understood as a three-component system. Improvements focused on Plasmodium species homology. The use of short RNA help as a specificity defining factor for double-strand breaks is the primary distinction amongst

CRISPR, ZFP, and TALEN. Furthermore, this innovative method eliminates the requirement for costly site-specific nucleases and just needs a single guide RNA (gRNA) (Crawford et al., 2017) . In this intance, The CRISPR nuclease Cas9 proved that it could detect gRNAs that resembled phage sequences in a distinctive way and subsequently build natural chains. Immune apparatus for CRISPR antiviral defense. Target DNA is recognized and cleaved by the Cas9/gRNA complex using CRISPR/Cas9. Watson-Crick base pairing is then used to carry out genome alteration modifications. Regardless of the method, the parasite has to receive the Cas9 nuclease, guide RNA, and donor template (Hammond et al., 2016) .

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) began to appear with its first publication in 2013. CRISPR is a genome editing innovation adapted from the bacterial defense system against bacteriophages (viruses that attack bacteria). This idea is stated to be able to contribute to world health (*A total of 94,610 cases of malaria occurred in Indonesia in 2021* , nd) . What researchers often say about genetic engineering is that its application requires a fast time, low cost and a high level of precision compared to genetic engineering in previous years which took years and of course was very expensive. Apart from that, CRISPR can surprisingly cure HIV/AIDS, genetic diseases, malaria and cancer (Gantz et al., 2015) .

RESEARCH METHODOLOGY

Extensive search for published research before July 31 2024 is carried out using the PubMed database. Interesting keywords are “CRISPR/Cas9”, “ Malaria”, “anopheles”, and “Plasmodium”. Literature research is completed with Pubmed. Through the Indonesian journal site, several articles authored in Indonesian were located. The suitability of the other references was evaluated using the complete text, abstract, and title. The criteria for inclusion and removal were decided upon in advance. All document related to Plasmodium which causes malaria, genetic engineering, and associated incidences of malaria. The following exclusion criteria did not apply to any records: Research on CRISPR research not in mosquitoes unrelated to genetic engineering, research written in languages other than English and Bahasa Indonesia.



RESULT AND DISCUSSION

During the rainy season like this, the increasing number of malaria cases is a serious concern for residents in various tropical regions. High rainfall creates a very suitable environment for Anopheles mosquitoes, which carry malaria, to breed rapidly. Rainwater that stagnates in several areas becomes a fertile breeding ground for mosquitoes. Increased air humidity also contributes to increasing the life span and breeding cycle of mosquitoes. As a result, there has been a significant increase in the number of reported malaria cases, which threatens public health (Crawford et al., 2017) . To overcome this, preventive measures such as the use of mosquito nets, insecticides and environmental control efforts need to be increased . Close collaboration between the government, community and health institutions is the key to reducing the negative impacts caused by the increase in malaria cases during the rainy season. Even though global efforts have been made to overcome malaria, the number of cases remains high throughout the world (*Geography and Social Distribution of Malaria in Indonesian Papua: A Cross-Sectional Study - PMC* , nd) .

According to data from the World Health Organization (WHO), millions of cases of malaria occur each year, especially in Sub-Saharan Africa, where the majority of cases and deaths are reported (Keman, n.d.) . Various factors such as political instability, lack of access to health services, drug resistance, and climate change contribute to the high number of cases. Despite this, efforts continue to be made by governments, health institutions, and non-profit organizations to reduce the burden of this disease through the distribution of insecticide-treated bed nets, case monitoring, and increasing access to treatment (Li et al., nd) . Wider global collaboration is urgently needed to accelerate the reduction in the number of malaria cases and achieve the target of eliminating this disease globally. Meanwhile, Indonesia also faces similar challenges with the high incidence of malaria. With the characteristics of a large and geographically diverse region and a

tropical climate that supports the breeding of Anopheles mosquitoes as disease vectors, the malaria population in Indonesia continues to be a major concern. Lack of access to health services, habitats that support mosquito breeding, and a lack of understanding of how to prevent and treat malaria further worsen this situation (Wamaket et al., 2021) . Collaboration between the government, health institutions and the community is very necessary to deal with this problem effectively and reduce the number of malaria cases in Indonesia. In 2023, the most cases will be in Papua. The following is a map of the distribution of malaria cases in Indonesia.

Figure 1, https://malaria.kemkes.go.id/case

WHO shows that malaria cases in Indonesia are still quite high. As of 2021, 811,636 estimated new malaria cases were reported in Indonesia. Estimated deaths due to malaria reached 1,412 cases. And 89% of malaria cases in Indonesia occur in Papua province (Sukardi, 2023). Despite global efforts to reduce the burden of malaria, Nigeria continued to face significant challenges in 2020 with case rates remaining high. The country remains one of the most affected by malaria, with millions of cases reported each year. Some of the factors contributing to the high incidence of malaria in Nigeria include limited access to quality health services, lack of distribution of insecticide-treated bed nets, and complex socioeconomic and infrastructure problems. To overcome this problem, it is necessary to make more intensive efforts in prevention, treatment and vector control, as well as increase cooperation between government, health institutions and civil society to achieve the target of eliminating malaria in Nigeria (Keman, nd) .

Figure 2, https://ourworldindata.org/malaria

Scientists continue to strive to find effective solutions to overcome the increasing cases of malaria through research and experiments. With collaboration between the scientific community and health institutions, they are developing innovative measures such as new vaccines, more advanced vector control technologies, and more focused prevention strategies. It is hoped that these discoveries will provide more effective solutions in dealing with the global health challenges posed by malaria. Efforts that can be made to reduce the population of mosquitoes that cause malaria can be done by:

a. CRISPR genetic engineering – Cas9 of the anopheles mosquito

CRISPR-Cas strategies and previous gene editing methods have changed the landscape in clinical and biological fields with great potential to address malaria-related challenges (North et al., 2020) . Plasmodium parasites, especially P. falciparum and P. vivax, cause significant clinical impact, with millions of cases and thousands of deaths each year (Kotepui et al., 2020) . Despite a global decline in malaria infections in recent decades, the development of new antimalarial drugs, vaccines, and more effective vector control strategies remains essential to combat this disease. CRISPR/Cas9, a new gene editing system developed in 2012, has revolutionized the way we view gene editing. This technology enables specific and efficient genome editing, using short RNAs as a determining factor for specificity. CRISPR/Cas9 allows identification and double-strand breaks in target DNA, which can then be modified through genome changes. This technique is more efficient and cheaper than previous gene editing methods such as ZFP and TALEN (Miller et al., 2011) .

Before the advent of CRISPR/Cas9, genome editing methods relied on site-specific nucleases such as meganucleases, zinc finger proteins (ZFP), and TALENs obtained from xanthomonas bacteria (Miller et al., 2011) . Although these methods have their uses, they have limitations in terms of specificity and efficiency. The development of CRISPR/Cas9 has expanded the possibilities of genome editing by enabling the use of short RNAs as guides, leading to improvements in specificity and efficiency (Arisandi et al., 2016) .

Although there are technical challenges in developing gene editing methods for malaria parasites, mainly related to transfection efficiency and the difficulty of inserting plasmid vectors, CRISPR/Cas9 technology provides new hope in efforts to overcome these problems. The use of CRISPR/Cas9 for Plasmodium and Anopheles vector engineering, detection of drug-resistant strains, vaccine development, and vector control can bring significant changes in the fight against malaria (Kotepui et al., 2020) .

Using CRISPR/Cas9 technology, research on Anopheles mosquitoes has enabled the creation of mutant strains that can help in controlling malaria transmission. This method has opened the door to producing mosquito populations consisting only of males, wingless mosquitoes, and other mutants that can stop disease transmission without affecting the balance of the ecosystem (Domingos et al., 2017) . The use of CRISPR/Cas9 as a powerful genome editing tool enables direct targeting of mosquito vectors, which provides great potential in controlling the spread of malaria (Nolan, 2020).

Gene modification strategies to curb the ability of mosquitoes to transmit malaria involve inserting genetic elements into mosquito populations. This can include inactivation of transgenic host factor genes, manipulation of miRNAs and lncRNAs, as well as expression of anti-parasitic effector genes in target populations (Kilian et al., 2021) . These genetically engineered mosquitoes can influence the development of Plasmodium without harming their hosts, with the potential to suppress or replace existing mosquito populations and/or disrupt the transmission of malaria parasites (Gantz et al., 2015) . The application of CRISPR/Cas9 in Anopheles mosquito research has accelerated the development of various insect gene drive strategies. These findings result in the great possibility of developing more effective methods in controlling malaria transmission, both through suppressing mosquito populations and through more specific approaches in influencing the disease ecosystem (Crawford et al., 2017) . Thus, CRISPR/Cas9 technology promises a potentially revolutionary solution in global efforts to address the malaria problem (Nolan, 2020) .

The use of CRISPR/Cas9 has opened the door to sophisticated genetic manipulation of malaria vectors, such as the Anopheles mosquito. Control of this vector is very important in efforts to eliminate malaria. Traditional methods such as the use of insecticides have shown progress, but face challenges such as mosquito resistance to insecticides (Oringanje et al., 2021) . CRISPR/Cas9 provides a powerful tool for altering the mosquito genome, allowing the creation of mutant strains with characteristics that can stop malaria transmission, such as mosquito populations consisting of only males, wingless mosquitoes, and other mutants (Zhang et al., 2014) .

The strategy for using CRISPR/Cas9 involves the insertion of genetic elements into mosquito populations, including inactivation of transgenic host factor genes, manipulation of miRNAs and lncRNAs, and expression of anti-

parasitic effector genes in the target population. These genetically modified mosquitoes can influence the life cycle of Plasmodium without harming the mosquito itself, with the potential to reduce existing mosquito populations or disrupt the transmission of malaria parasites (Crawford et al., 2017) . The application of CRISPR/Cas9 in Anopheles mosquito research has accelerated the development of various insect gene drive strategies. Various studies have shown success in creating transgenic mosquitoes with anti-malarial effector genes, manipulating mosquito sex, and producing sterile mosquitoes (Kotepui et al., 2020) Scientists have obtained CRISPR/Cas9 to produce transgenic lines with a sterile female phenotype with the gene disconnects or inactivates some parts of the genome. The Anopheles gambiae genome, for instance, was altered using this technique to introduce three disrupted genes (AGAP005958, AGAP011377, and AGAP007280) that confer a recessive female sterility phenotype. This was achieved by inserting a CRISPR/Cas9 gene drive construct in the malaria mosquito vector, which was intended to target and edit each gene. The findings point to the necessity of deeper population modeling and a strong focus on the molecular level in cage studies. (Hammond et al., 2016).

b. CRISPR genetic engineering – Cas9 plasmodium causes Malaria

Plasmodium transgenic lines have become a major focus in research aimed at understanding gene function and supporting creation of malaria vaccines and medications (Harding & Meissner, 2014) . Several species of human malaria parasites, such as P. falciparum and P. knowlesi, can be cultured in vitro, allowing for genome editing and the production of transgenic strains (Miller et al., 2011). In vivo studies are frequently carried out employing malaria parasites in rodents, especially P. yoelii and P. Berghei, due to the limitations of animal models for research malaria parasites in humans. However, the proteins produced by Plasmodium genes in rodents differ in sequence and structure from proteins in humans. Therefore, the generation of Plasmodium transgenic lines in rodents allows the expression of human malaria target proteins, which is important for accurate in vivo experimental design (Kotepui et al., 2020).

Traditionally, the generation of Plasmodium transgenic lines is carried out via single or double crossover recombination without targeted double-strand breaks (Harding & Meissner, 2014) . However, the capacity to locate and cut particular locations on DNA has evolved thanks to technical advancements like zinc finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN) (Garrido-Cardenas et al., 2019). Nevertheless, there are certain drawbacks to this strategy, namely the restricted selection of zinc finger target locations and the modifications needed to distinguish the target regions using the TALEN method. Subsequently, the CRISPR/Cas9 technology made it possible to modify the Plasmodium parasite genome more quickly, affordably, and effectively than with earlier methods.

As a result, a multitude of novel Plasmodium transgenic lines have been generated through the utilization of the CRISPR/Cas9 system. These lines include those that express the reporter gene, Cas9, chimeric Plasmodium, knockdown and knockout parasites, as well as parasites that express alternative alleles. This opens up new avenues for investigation into the mechanisms behind malaria and the development of more effective control strategies.

Development of Plasmodium transgenic lines using CRISPR technology allows the expression of reporter genes such as GFP or luciferase. This technique has been applied in research to detect malaria parasites by inhibiting their growth throughout the life cycle, allowing evaluation of the effectiveness of antimalarial drugs or vaccines at different stages of the parasite life cycle.

The use of chimeric parasites expressing orthologous genes from other species has also provided valuable insight into the characteristics of CSP species for vaccine development. CRISPR/Cas9 genome editing in Plasmodium knowlesi has also shown potential for generating transgenic lines expressing P. vivax Duffy binding protein (PvDBP), which can inhibit the proliferation of Plasmodium strains (Garrido-Cardenas et al., 2019) .

Overall, the development of Plasmodium transgenic lines with CRISPR technology opens up opportunities to better understand malaria disease mechanisms and develop more effective control strategies. This technology has also been used to investigate parasite genome function and generate transgenic lines expressing alternative alleles, opening the potential for functional evaluation of gene diversity and single nucleotide polymorphisms in vaccine development and malaria control strategies (North et al., 2020) .

c. Comparison of genetic engineering of CRISPR – Cas9 of Anopheles mosquitoes and genetic engineering of CRISPR – Cas9 of plasmodium which causes malaria

1. Research purposes
   a. CRISPR genetic engineering – Cas9 of the anopheles mosquito : The main goal of genetic engineering in Anopheles mosquitoes is to produce a mosquito population that is unable to transmit malaria parasites, by specifically changing the mosquito genome using CRISPR/Cas9 technology.
   b. CRISPR genetic engineering – Cas9 plasmodium causes Malaria : The main goal of genetic engineering in Plasmodium is to understand the mechanisms of malaria and develop more effective control strategies, including the production of transgenic strains for research into vaccines and antimalarial drugs.
2. Gene editing methods
   a. CRISPR genetic engineering – Cas9 of the anopheles mosquito : In Anopheles mosquitoes, gene editing is carried out using CRISPR/Cas9 technology to specifically change the mosquito genome, by inactivating or modifying the genes responsible for transmitting the malaria parasite.

b. CRISPR genetic engineering – Cas9 plasmodium causes Malaria : In Plasmodium, gene editing also uses CRISPR/Cas9 technology, but the goal is to produce transgenic strains that express the reporter gene, Cas9, chimeric Plasmodium, knockdown, knockout parasites, or express alternative alleles.

3. Implications in disease control
   a. CRISPR genetic engineering – Cas9 of the anopheles mosquito : Gene engineering in Anopheles mosquitoes has direct implications in controlling malaria by producing mosquito populations that are unable to transmit the disease.
   b. CRISPR genetic engineering – Cas9 plasmodium causes Malaria : Gene engineering in Plasmodium is also important for malaria control, but the focus is more on understanding disease mechanisms and developing vaccine and drug strategies..
4. Test and evaluation approach
   a. CRISPR genetic engineering – Cas9 of the anopheles mosquito : Testing on Anopheles mosquitoes involved creating mutant strains, assessing their effectiveness in inhibiting malaria transmission, and monitoring their impact on the ecosystem.
   b. CRISPR genetic engineering – Cas9 plasmodium causes Malaria : Testing on Plasmodium involves producing transgenic strains and evaluating their effectiveness in vaccine research, antimalarial drugs, and understanding disease mechanisms.

So in the table above it can be observed that in genetic engineering the CRISPR – Cas9 genetic engineering of the Anopheles mosquito is more about controlling disease vectors. Meanwhile, genetic engineering of CRISPR - Cas9 plasmodium that causes malaria is more about understanding and developing direct control strategies for the parasite that causes it. Thus, although both use CRISPR/Cas9 technology, the gene engineering approach to Anopheles mosquitoes and the genetic engineering of Plasmodium which causes malaria have different goals, methods and implications in the context of malaria control.

CONCLUSION

Malaria vector control is an important strategy in efforts to control and eliminate malaria because it is very effective in preventing infection and reducing disease transmission. Lack of access to health services, habitats that support mosquito breeding, and a lack of understanding of how to prevent and treat malaria further exacerbate this situation. The tremendous development of CRISPR/Cas-oriented profiling for the identification of certain species and drug resistance indicators will hasten the detection process using single responses and liwer parasitemia. Anopheles strains driven by genes have the ability to transmit antimalaria genes and prevent the emergence of wild popolations. However, there have been no large-scale experiments that have proven

successful in eliminating malaria. For large-scale malaria control, CRISPR-based gene-edited organisms will also offen an economical, efficient, and sustainable system, even when paired with other approaches. Generation of Plasmodium transgenic lines is carried out via Recombination by single or double crossing without intended double-strand breaks. The use of CRISPR technology in developing Plasmodium transgenic lines allows the use of reporting genes such as GFP or luciferase. This approach has been used in studies to identify malaria parasites by disrupting their growth throughout the life cycle, allowing assessment of the effectiveness of antimalarial drugs or vaccines at different stages of the parasite life cycle

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