International Conference of Sciences, Engineering & Environmental Technology (ICONSEET), 7(6): 46– 57, 2022

ISSN 0794-9650

www.repcomseet.org

Pros and Cons of Genetically Modified Mosquitoes in Malaria Transmission: A Critical Review

[1]Ponle B. AJAYI and [2]Oyinlola A. AJAYI (PhD)

[1]Cell Biology and Genetics Unit Department of Zoology, University of Ibadan

[2]Department of Biological Sciences, Federal Polytechnic, Ede

Abstract: Malaria remains a life-threatening disease despite several control and prevention methods. Malaria is caused by parasites that are transmitted to human through infected female Anopheles mosquitoes during bites and African countries such as Nigeria has highest malaria burden. Though, the conventional methods really helped to reduce malaria burden, the instances of resistance of malaria vector to insecticides and resistance of malaria parasites to antimalarial drugs are recurring problems and serious threats. Meanwhile, RTS,S/AS01 is the first and, to date, the only vaccine to show partial protection against malaria. However, Genetic Approach through genome editing is becoming more promising. The main idea of genetic approach to combat malaria is to create genetically modified mosquitoes which will be biocontrol agent against the wild type mosquitoes through population suppression and population alteration. This was made possible using Gene Drive based CRISPR/Cas9 biotechnology, which makes the desirable character/trait thrives and replace the wild type character/trait. This paper critically reviewed the benefits and the possible risks of this approach with a view to get a lasting solution to malaria. The ethics governing the use of this biotechnology and possible reconmmendation for future consideration are critically discussed.

Key words: Malaria, resistance, GM mosquitoes, biocontrol, and gene-based CRISPR/Cas 9 biotechnology.

Introduction

Malaria is still part of the most infectious and most severe life-threatening human vector-borne diseases (Phillips, *et al*., 2017; Akoniyon *et al*., 2022), recorded to have killed over three times as many people killed by armed conflicts in 2015 (Gething P.W.; et al., 2016). There were an estimated 241 million malaria cases and 627000 malaria deaths worldwide in 2020 with over 69000 more deaths compared to 2019 (WHO, 2021). In mosquito, *Plasmodium* parasites do invade two different epithelia: midgut and salivary gland (Ghosh, *et al*., 2000) and five species of *Plasmodium* (most importantly *P. falciparum* and *P. vivax*) that are transmitted by the bite of female *Anopheles* specie mosquitoes causes malaria in humans (Rosauro, *et al*., 2020).

Over the past years, efforts have been put in place to reduce malaria risk levels, and it's occurring in several fronts (Bhatt, *et al*., 2015). The control of malaria parasite transmission fall into four categories: basic vector control to reduce transmission, chemoprophylaxis which is the use of drug to kill the parasite, genetic approach to suppress or alter malaria vector population, and transmission-blocking immunological approach (Ostera, *et* al., 2011; Shengzhang *et al*., 2021). A component that is very important as strategy to control malaria and other insect-born disease is population control and some technologies such insecticide use are becoming less effective due to resistance or restricted usage due to environmental legislation, improper distribution and use of bednet (Parliament, 2009; Liu, 2015).

An emerging technology that could form part of strategy for controling insect-born diesease like malaria is to suppress or replace mosquito population by releasing genetically modified mosquitoes into the wild. The genetic control strategy depends on the introduction of inheritable element into the target population, so that the "modified mosquito become a biocontrol agent against its unmodified type" (Gilna *et al*., 2013). The most promising of all the biotechnology that can be used for genome editing especially towards the modification of mosquito to control malaria transmission is Clusterd Regularly Interspaced Short Palindromic Repeat

(CRISPR)-Cas9 technology which is a precise and facile molecular mechanism for editing cells, tissues and whole organisms (Ledford, 2015). Alongside the Cas9 system, Gene Drive technology as been designed(DiCarlo *et al*., 2015), through which rapid acquisition and propergation of a trait through a population is ensured. The gene drive technology was used in *Anopheles gambiae*, to drive a recessive female sterility genotype with transmission to progeny rate exceeding 90% and also in *Anopheles stephensis*, to create transgenic mosquitoes carries antipathogen effector genes targeting malaria parasite with assuarance of 99.5% to progeney(Gantz *et al*., 2015). Such an approach has the potential to suppress the spread of malaria (Hammond *et al.*, 2016). Amidst the mix feelings people have about genetically modified mosquitoes, in August 2018, the National Biosafety Agency of Burkina Faso authorized Target Malaria to release a strain of genetically modified sterile male mosquito, the first of its kind on the African continent.

MALARIA TRANSMISSON AND CONTROLS

World malaria burden is highest among people residing in resource-limited areas, like Africa having 92% of the cases, followed by Asia having 5% of malaria cases, Eastern Mediterranean having 2%, Central America and South America with 1% (WHO, 2018). Acute lower respiratory, malaria, TB, cholera, and AIDS are five most deadly infectious diseases; only malaria requires a vector for transmission (Ghosh et al., 2000; Wang et al., 2013).

The disease is caused by protozoan pathogens of the Plasmodium species: *P. falciparum, P. ovale, P. vivax, P. malariae,* and *P. knowlesi*. Plasmodium knowlesi in Southeast Asia naturally infects macaques, and also infects humans, thereby causes malaria transmitted from animal to human 'zoonotic malaria' (CDC, 2018). Exclusive mammalian hosts, Plasmodium falciparum and Plasmodium vivax, in humans are the most common species and are responsible for the largest public health burden (Phillips et al., 2017). Plasmodium species with other pathogens (HIV, Mycobacterium tuberculosis and helminthes) do cause co-infection and people having HIV infection are at high risk of severe malaria and death (Cohen et al., 2005; Hawkes et al., 2010).

Mosquito as a malaria vector

Anopheles species that are capable of transmitting malaria are distributed all over the globe. However, the efficacy of malaria transmission depends on the vector species and, therefore, varies considerably worldwide; for example, in tropical Africa, Anopheles gambiae is a major vector (Sinka et al., 2010). The Malaria Atlas Project suggested that for a mosquito species to be effective at transmitting malaria, some characteristics are very important including: abundant in number to ensure high number of mosquitoes encounter an infectious human to pick up the malaria parasite; longevity to survive long enough after feeding on infected blood for parasite to develop and travel to the mosquito's salivary glands ready to infect the next person bitten; Contact with humans (Anopheles prefer to feed on humans rather than other animals, and be able to survive and breed in places close to homes, and be able to find people) (MAP, 2018).

Malaria Preventions and Controls

Malaria prevention and control strategies differ throughout the world depending on endemic level of the disease (Tizifa et al., 2018). The prevention and control of malaria parasite transmission will likely fall into four categories:

(a) Basic Vector Control: Vector control includes all strategies that are used to reduce or eliminate the prevalence of malaria by attacking mosquitoes in areas that are known to be faced with malaria. These vector control methods include: insecticide-treated mosquito net, indoor residual spraying, larval source management, house improvement, sugar feeding, swarm sprays, targeting livestock, spatial repellents, etc. (Gueye et al., 2016).

(b) Chemoprophylaxis: The use of drug is also known as chemoprophylaxis (YourGenome, 2016). Chemoprophylaxis have played a key role in controlling malaria in endemic areas, and it has led to significant reduction of the geographic range of malarial disease worldwide (NIAID, 2011) but yet, available anti-malarial drugs is surprisingly small and costly in low income regions (Schellenberg et al., 2006). Unfortunately, out of five malaria parasite species, three parasites (*Plasmodium falciparum, Plasmodium vivax and Plasmodium malariae*) are known to affect humans have developed resistance

to antimalarial drugs (YourGenome, 2016). Drug resistance is a major problem affecting progress on malaria control (Lambert, 2003).

(c) Vaccines: Vaccinology is the only science that has eradicated an infectious disease with great achievement of smallpox eradication in 1977 (Andre, 2003). There are three areas in which vaccine development against malaria is carried out, i.e. vaccines against: (i) the pre-erythrocytic or liver stage of the parasite, (ii) the blood stage, and (iii) the sexual stages in the mosquito vector (Peter D. *et al*., 2010). It is this third area in which transmission blocking vaccines (TBVS) are being assessed as a way to control the spread of malaria. Substantial effort has been made towards the development of a malaria vaccine for P. falciparum. In October 2021, the World Health Organization endorsed the first-ever malaria vaccine, the protein-based RTS,S/AS01 (Sullivan, 2021).The major obstacle hindering the development of effective malaria vaccines is parasite antigenic diversity. On the hand, there are concerns regarding the transmission blocking vaccine approach, such as the potential loss of natural immunity in the population (Carter, 2001). In transmission blocking vaccine approach, the antibodies generated do not directly benefit the vaccinated person, but they instead prevent the development of the parasite in the mosquito, thereby benefitting the community (Ramirez et al., 2009).

1.

(d) Genetic Approach: Contrast to vaccines, drugs, and basic vector control methods, genetic approach to malaria control is wide and can potentially provide new, species-specific, environmentally friendly methods for mosquito control (Alphey, 2014). Genome editing can be achieved using engineered nucleases such as Cluster Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated Cas9 (CRISPR/Cas9) Nucleases, Transcription Activator-like Effector Nucleases (TALENs) or Zinc Finger Nucleases (ZFNs), Meganucleases (MGNs). Viral systems such as Recombinant Adeno-associated Virus (rAAV), and transposons can also be used in genome editing (Chen et al., 2016; Horizon, 2018). Genetic approaches, fuelled by advances in the CRISPR-Cas9 gene editing technology, represent an exciting area of development for novel insect control strategies (Gantz et al., 2015; Doudna et al., 2014). In the control and eradication of malaria, genetic engineering is currently using two main approaches which are: population suppression and population alteration (Burt, 2014; Akoniyon *et al*., 2022). Successful development of this technology permitted the creation of genetically modified mosquitoes impaired in their ability to transmit the malaria parasite. An example was the creation of an Ae. aegypti expressing defensin in the Haemolymph (Kokoza et al. 2000). One of the works done on mosquitoes using Anopheles stephensis confirmed that transgenic *Anopheles stenphesis* mosquitoes co-expressing single-chain antibodies resist Plasmodium falciparum development (Isaacs et al., 2012). Another is the evidence for a highly efficient gene-drive system that can spread antimalarial genes into a target vector population (Gantz et al., 2015).

CRISPR/Cas9 GENE DRIVE TECHNOLOGY

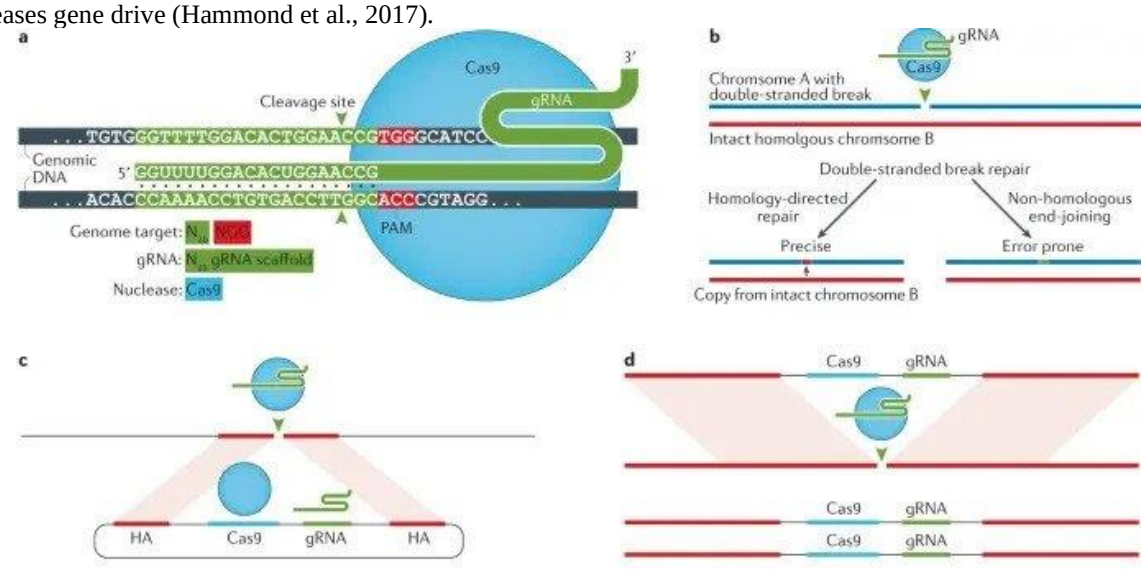
There is an urgent need to develop new tools to assist existing measures to combat the burden of malaria in endemic regions (Bhatt, *et al*., 2015). Gene drive concept originated from nature of naturally occurring "selfish genes" found spreading within natural populations through copying from one chromosome to its homologue in a non-Mendelian fashion. 'Gene drives technology' allows the spread of engineered genes through insect generations without the need to use a large population of insects (Burt A., 2003). The mechanism can be harnessed to increase the frequency of particular genes in a wild species' targeted population in other to reduce disease-vector population (Pare Toe, *et al.*, 2022).

There are two gene-drive designs: (i) "**Population suppression** (reduction) gene drive design" aims reducing the number of females through release of male insects bearing a gene drive construct that will increase their frequency in populations over successive generations and (ii) "**population modification** (alteration) gene drive approach" where insects are genetically engineered so that transmission of the parasites in their offspring is blocked (Pennisi, 2013; Ledford, 2015).

Mutagenic Chain Reaction (MCR)

This is a more advanced gene drive system of genetic engineering trough CRISPR technology (Burt et al., 2018). Gene drive is a phenomenon where one or more genetic elements bias their inheritance above and beyond what is predicted by Mendelian genetics which is 50%, therefore increasing their frequency within a population over generations (Gantz et al., 2015). The modification that is spread through the population is therefore self-sustaining and ideally suited for vector control as the short generation time of insects allows them to spread to fixation very quickly even if released from a low initial frequency (Burt, 2003). A gene drive could be engineered to reduce the potential of an insect vector population to transmit disease (replacement) or its potential to reproduce (suppression/elimination), thereby reducing or eliminating vector-borne diseases such as malaria. Among these genes drive methods are: transposable elements, heritable microorganisms, genetic underdominance, Maternal Effect

Dominant Embryonic Arrest (MEDEA), meiotic drives and the natural 'genetic scissors' homing endonucleases gene drive (Hammond et al., 2017).



2.

3. Figure 1: Synthetic CRISPR system

4. (**a**). A guide RNA (gRNA; green) binds Cas9 (cyan) directing it to bind and cleave DNA at complementary sites 20 nucleotides in length. The protospacer-adjacent motif (PAM) site (NGG; red) is required for Cas9 binding to genomic targets. In eukaryotic cells, double-stranded breaks are repaired either by the error-prone non-homologous end-joining or by homology-directed repair (HDR), the pathway acting in the germline

5. **(b**). Insertion of a cassette encoding Cas9 (cyan) and a gRNA (green) flanked by homology arms (HAs) results in HDR-mediated copying of the cassette from the plasmid into the genomic cut site

6. (**c**). The HAs directly flank the gRNA-directed cleavage site. Once inserted into the genome, the Cas9 + gRNA cassette directs cleavage of the homologous chromosome in the germline

7. (**d**) and is copied into the DNA break by HDR resulting in nearly all progeny (99%) inheriting the 'gene-drive' cassette.

Construction of MCR

Taking advantage of the CRISPR/Cas9 genome editing method described above, a strategy to convert a heterozygous recessive mutation into a homozygous condition manifesting a mutant phenotype has been developed (Gantz et al., 2015). Autocatalytic insertional mutants were generated with a construct having three components:

(1) a Cas9 gene (expressed in both somatic and germline cells)
(2) a guide-RNA (gRNA) targeted to a genomic sequence of interest
(3) homology arms flanking the Cas9/gRNA cassettes that match the two genomic sequences immediately adjacent to either side of the target cut site.

With such a tripartite construct, Cas9 should cleave the genomic target at the site determined by the gRNA and then insert the Cas9/gRNA cassette into that locus via homology-directed repair (HDR). Cas9 and the gRNA produced from the insertion allele should then cleave the opposing allele, followed by HDR-driven propagation of the Cas9/gRNA cassette to the companion chromosome. This trans-acting mutagenesis scheme is referred to as a mutagenic chain reaction (MCR). Cas9-mediated gene drive based on a system adapted from MCR works well in a malaria mosquito (Gantz et al., 2015).

Research on mechanisms for introducing antipathogen effector genes into target populations supports a number of approaches, including inundative releases and those based on gene-drive systems (Robert et al., 2014).

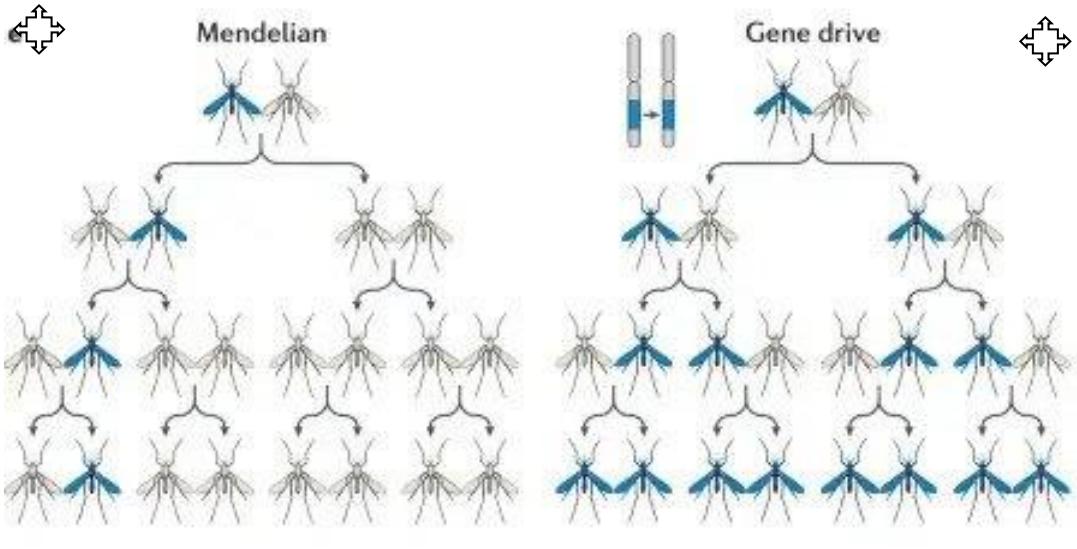


Figure 2: Mendelian versus gene-drive inheritance patterns. In each case, a transgenic individuals (blue) is introduced to wild-type (white)

Inundative approaches rely on releases of engineered mosquitoes in numbers substantially exceeding those of the local population to drive gene frequencies high enough to have an epidemiological impact. Inundative releases of chemically or radiation treated insects were successful in population suppression of mosquitoes using sterile insect technologies (Klassen *et al*., 2005). However, modeling of gene-drive systems, which exceed rates of Mendelian inheritance, shows a more rapid population-level transformation with fewer releases than inundative approaches (Robert, et al., 2014), and this would result in sustainable local malaria elimination at much reduced costs (Macias et al., 2015).

POSSIBLE RISKS OF GENE DRIVE TECNOLOGY

There are many possible risks in the use of genetic approach to create genetically modified mosquitoes to combat malaria. But in this study, we have carefully put the possible risks under six points. Some of these risks are discussed as follows:

1. **Risk of Hybridization**: It is the interbreeding or mating of individuals from two distinct populations or groups of populations distinguishable on the basis of one or more heritable characters (Harrison *et al*., 2014). Genetically modified mosquitoes may disrupt the ecosystem by interbreeding with related species (Virginie *et al*., 2020) and can lead to extinction of either parent species through demographic swamping or genetic swamping (Todesco et al., 2016). The hybrids and the modified mosquitoes can affect public health by becoming host for other pathogens and they can also promote the evolution of pathogens with increased virulence (Barclay et al., 2012) and thereby bring about emergence of new disease(s).

2. **Risk of Resistance**: This is another possible risk of genetically modified mosquitoes. A large number of studies have shown that both the mosquitoes and the malaria parasites have built resistances to chemicals and drugs designed to eliminate malaria (Liu 2015; Antony 2016). All control systems are subject to evolution and the potential for resistance (Alphey, 2014), therefore, genetic control method might also come to a point of

system decay in terms of mosquitoes building resistance (Deredec et al., 2008). Genetically modified mosquitoes if released into the wild may behave as planned but like it happened to other approaches over the years, there might also be emergence and spread of resistance to genetic modification (Tom *et al*., 2020) either by the malaria parasite or vector and this could reverse the malaria control program and success achieved so far worldwide.

3. **Risk of Loss of biodiversity:** Every living thing plays a role in the food chain and the ecosystem at large and the extinction of certain species whether prey or predators can leave behind significant impacts. If one species in the food web ceases to exist, one or more members in the rest of the chain could cease to exist too (Cardenas et al., 2017). Loss of biodiversity directly disrupts the functioning of the ecosystem and directly or indirectly affects human well-being (Diaz et al., 2006). The species of mosquitoes that are modified to suppress the vector population will eventually affect the food chain and pose threat to non-targeted predators such as amphibians, bats, insects, reptiles that feeds on mosquitoes and some aquatic organisms that feeds on their larva (Maxmen, 2012). Not also far from possibility is the fact the genes inserted or knocked-in into the genomes of the mosquitoes designed to achieve population alteration might produce toxins that will be lethal to their predators.

4. **Risk of Unrecoverable Gene Drive**: Although, Gantz et al., (2015) claimed to have designed constructs that can clean the genetic modification and restore the population to normal if the biotechnology failed to work as planned. It is not certain that the restoration will be total because the vectors are migrating insect and some would have escaped beyond the location they were released to unpredictable far distances. Recently, a novel approach to clean the wild is making gene drive biodegradable (Josef *et al*., 2021) but yet, among the questions that begs for response is; "before the gene become degraded, how sure can we be that the parasite would have gone to extinction or, after the gene drive degradation, malaria parasite will invade again"?

5. **Risk of Misuse**: The production and use of genetically modified mosquitoes is yet to have strong bodies that can regulate it and if the use of this biotechnology is allowed, some human can misuse it by creating genetically modified mosquitoes to serve as bioweapon against fellow human.

6. **Risk of the Unthinkable**: On the use of genetically modified mosquitoes to fight malaria, there might be emerging risk(s) which will only be known or well understood after the engineered mosquitoes are released into the wild and this cannot be overmphasized.

POSSIBLE BENEFITS OF GENE DRIVE TECHNOLOGY

Here are some of the common benefits cited by proponents of genetically modified mosquitoes:

1. **Malaria reduction or total eradication:** The main benefit of genetically modified mosquitoes is that it will lower and possibly eliminate the population of malaria vectors over time.

2. **Cost effectiveness:** The result of genetically modified mosquitoes on malaria reduction and elimination will worth the cost compared to other approaches that requires continuous spending to maintain them. The gene drive technology allow resources to be directed to new sites while providing confidence that treated areas will remain malaria-free (Macias et al., 2015)

3. **Environmental Friendly:** No chemical is added to the environment when using genetically modified mosquitoes. Therefore, genetically modified mosquitoes will help in keeping our environments safe unlike other approaches.

4. **Easy Application:** Genetically modified mosquitoes' method is relatively easy to use because they take care of themselves in the wild and low-manpower activity is involved after they are produced and released. The introduction of genetically modified mosquitoes can therefore supplement existing mechanical efforts to control mosquito populations.

5. **Alternative Designs:** the gene drive technology directed towards anti-parasite effectors' gene to achieve population alteration and not necessarily suppress the vectors population, the mosquitoes' population will remain, and only the malaria parasite will be eliminated.

USING GENETICALLY MODIFIED MOSQUITOES

Scientists have developed genetically modified mosquitoes, and for about a decade, scientists have debated how and when to carry out the first test release of transgenic mosquitoes designed to fight human disease (Enserink, 2010) knowing full well that ethical and regulatory issues should be considered when it comes to using modified organism in human population. Field trials of genetically modified mosquitoes have raised various ethical issues (Macer, 2003) and some people have religious or philosophical objections to all forms of genetically engineered life
(Macer, 2003).

In 2009 and 2010, researchers funded by Oxitec, a private company, released male mosquitoes of the species Aedes aegypti, which carries dengue, into the wild on an island near Grand Cayman, in the Caribbean. The genetically modified mosquitoes were considered 'infertile' because they have a gene that causes 96% of offspring to die before reaching maturity. The trial resulted in an 80% reduction of the local Aedes aegypti population, according to the company (Harris et al., 2011). The trial angered some researchers, because they felt that Oxitec had kept its work secret and that more research was needed on the public health and environmental impacts of genetically modified mosquitoes before release into the wild should occur (Enserink, 2011). Luke Alphey, the chief scientific officer of Oxitec reacted to this allegation and he said "I will completely reject any notion that this was done secretively." He notes that the trial was well-known within the island's population of 50,000, "but just not picked up internationally" (Enserink, 2010). Others were concerned about the public backlash of releasing genetically modified mosquitoes into the wild without appropriate community engagement and regulatory oversight (Pollack, 2011). A proposed field trial of Oxitec's genetically modified mosquitoes in Key West, Florida scheduled for January, 2012, was postponed indefinitely by the Florida Keys Mosquito Control District, due to protests from local residents. 100,000 people signed a petition to stop the release of these insects (Maxmen, 2012). Although dengue has reemerged in Key West after a 65-year absence, many were concerned about the public health and environmental risks of the proposed trial.
Some protesters speculated that drastically reducing Aedes mosquitoes in the area could lead to a decline in bats, which feed on the mosquitoes (Maxmen, 2012). Other communities have taken a different stance toward genetically modified mosquito field trials. In 2012, residents of Juazeiro, Brazil expressed a mixed reaction to Oxitec's genetically modified mosquitoes. Though some welcomed the trial, others did not (Panjwani et al., 2016). One reason why some Brazilians had a more positive attitude toward the trial than the Floridians did is that dengue is a much worse problem in Brazil than it is in Florida (Resnik, 2014). Government agencies and scientists from France, Guatemala, India, Malaysia, Mexico, Panama, Philippines, Singapore, Thailand, the USA, and Vietnam have also been evaluating the release of genetically modified mosquitoes (Revees et al., 2012).

The use of genetically modified mosquitoes for the control of malaria and other mosquito-borne diseases has been proposed in malaria-endemic countries, such as Nigeria, which has the largest burden in Africa (Federal Ministry of Health 2012; WHO 2021). Okorie, et al., (2014) carried out a survey to know the perceptions and recommendations of scientist on releasing genetically modified mosquitoes in Nigeria (Okorie et al., 2014). The main concerns expressed by the scientists were that genetically modified mosquitoes can spread in an uncontrolled way beyond their release sites and will mate with other mosquito species to produce hybrids with unknown consequences. Most scientists that participated agreed that there had to be evidence of contingency measures available to remove the genetically modified mosquitoes should a hazard become evident during the course of the release before approving the release of these mosquitoes in Nigeria. In general, a majority of about 83.5% of scientists who participated in the study were skeptical about a potential release in Nigeria, while few numbers of the scientist of about 16.5% of the participants were in support.

Ethical Issues

So far, there are many ethical issues concerning the use of genetically modified mosquitoes. Some of these issues include: protecting the public and the environment, balancing benefits and risks, collaborating with the local community, avoiding exploitation, safeguarding the rights and welfare of research subjects and one more critical issue is on protecting the welfare of community members who will be impacted by the release of mosquitoes but who are not enrolled in the study as research subjects or did not give informed consent.

Conclusion

As good as this technology is, the possible risks it could bring should not be overlooked. Although, laboratory studies have assessed the effectiveness of the genetic modifications of mosquitoes in controlling malaria transmission, but until they are released into the wild, it is impossible to know precisely or exactly how genetically modified mosquitoes will interact with the wild type mosquitoes and what the outcome(s) will be on ecosystem and public health.

Recommendations

Based on the known possible benefits and risks of genetically modified mosquitoes, as reviewed, we will recommend that more proper risk assessments should be done and maximizing the potential benefits of genetically modified mosquitoes should be considered. There should be strict agencies or bodies that will regulate the production and use of genetically modified mosquitoes or any other organism to avoid misuse of this biotechnology and this will at least give some level of confidence to the public. The approach should not be implemented unless research indicates that overall public health benefits will be significantly greater than public health risks. More field trials should be done in isolated locations with different environmental conditions and trials should be subjected to the test of time: this is really important. Finally, the concerned bodies should give great weight to the ability of science to ameliorate serious risks to human health.

REFERENCES

Akoniyon, O.P.; Adewumi, T.S.; Maharaj, L.; Oyegoke, O.O.; Roux, A.; Adeleke, M.A.; Maharaj, R.; Okpeku, M. (2022). Whole Genome Sequencing Contributions and Challenges in Disease Reduction Focused on Malaria. *Biology* , 1-28.

Alphey, L. (2014). Genetic Control of Mosquitoes. Annu. Rev. Entomol., 59, 205-224.

Alphey, L., Benedict, M.; Bellini, R., Clark, G.G., Dame, D.A., et al. (2010). Sterile-insect methods for control of mosquito-borne diseases: an analysis. Vector Borne Zoonotic Dis, 10, 295-311.

Andre, F. (2003). Vaccinology: past achievements, present roadblocks and future promises. Vaccine, 21, 593-595.

Antony, H.A. and Parija, S.C. (2016). Antimalarial drug resistance: An overview. Tropical Parasitology, 30-41.

Barclay, V.C.; Sim, D.; Chan, B.H.K.; Nell, L.A.; Rabaa, M.A.; Bell, A.S.; et al. (2012). The Evolutionary Consequences of Blood-Stage Vaccination on the Rodent Malaria Plasmodium chabaudi. PLoS Biol, 10(7), e1001368.

Bhatt, S.; Weiss, D.J.; Cameron, E.; Bisanzio, D.; Mappin, B.; Dalrymple, U.; Battle, K.E.; Moyes, C.L.; Henry, A.; et al. . (2015). The effect of Malaria Control on Plasmodium falciparum in Africa between 2000 and 2015. *Nature* , 1-9.

Burt, A. (2003). Site-specific selfish genes as tools for the control and genetic engineering of natural populations. Proc R Soc B Biol Sci, 270, 921-928.

Burt, A. (2014). Heritable strategies for controlling insect vectors of disease. Phil. Trans. R. Soc., 20130432.

Burt, A.B.; Coulibaly, M.; Crisanti, A.; Diabate, A. and Kayondo, J.K. (2018). Gene drive to reduce malaria transmission in sub-Saharan Africa. Journal Of Responsible Innovation, 566-580.

Carter, R. (2001). Transmission blocking malaria vaccines. Vaccine, 2309-2314.

Cardenas, R.E., Donoso, D.A., Argoti, A., and Dangles, O. (2017). Functional consequences of realistic extinction scenarios in Amazonian soil food webs. Ecosphere, 8(2).

Chen, X. and Goncalves, M.A. (2016). Engineered Viruses as Genome Editing Devices. Molecular Therapy, 24(3), 447-457.

Cohen, C.; Karstaedt, A.; Frean, J.; Thomas, J.; Govender, N.; Prentice, E.; Dini, L.; Galpin, J. and Crewe-Brown H. (2005). Increased prevalence of severe malaria in HIV-infected adults in South Africa. Clin Infect Dis., 41(11), 1-7.

Deredec, A.; Burt, A. and Godfray, H. (2008). Population genetics of using homing endonuclease genes in vector and pest management. Genetics, 179, 2013-2026.

Diaz, S., Fargione, J., Chapin, F.S. and Tilman, D. (2006). Biodiversity loss threatens human well-being. PLoS Biology, 4.

DiCarlo, J.E.; Chavez, A.; Dietz, S.L.; Esvelt, K.M. and Church, G.M. (2015). Safeguarding CRISPR-Cas9 gene drives in yeast. Nat. Biotechnol., 33, 1250-1255.

Doudna, J.A. and Charpentier, E. (2014). The new frontier of genome engineering with CRISPR- Cas9. Science, 346, 1258096(1-9).

Enserink, M. (2011). GM Mosquito Trial Alarms Opponents, Strains Ties in Gates-Funded Project. Science, 330, 1030-1031.

Federal Ministry of Health, F. (2012). National malaria control programme 2012 1st & 2nd quarter report. Abuja, Nigeria: National Malaria Control Programme.

Gantz, V.M. and Bier, E. (2015, March 19). The mutagenic chain reaction: A method for converting heterozygous to homozygous mutations. Sciencexpress, 1-5.

Gantz, V.M., Jasinskiene, N.; Tetarenkova, O.; Fazekas, A.; Macias, V.M. and Bier, E. et al. (2015, Oct). Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi. Proc. Natl. Acad. Sci. USA, 112, 1-8.

Gething, P. W.; et al. (2016). Mapping Plasmodium falciparum mortality in Africa between 1990 and 2015. N. Engl. J. Med., 375, 2435-2445.

Ghosh, A.; Edwards. M.J. and Jacobs-Lorena, M. (2000). The Journey of the Malaria Parasite in the Mosquito: Hopes for the New Century. Parasitology Today, 16(5), 196-201.

Gilna, B.; Kuzma, J. and Otts, S.S. (2013). Governance of genetic biocontrol technologies for invasive fish. Biol. Invas.

Gueye, C.S.; Newby, G.; Gosling, R.D.; Whittaker, M.A.; Chandramohan, D.; Slutsker, L.; et al. (2016). Strategies and approaches to vector control in nine malaria eliminating countries: a cross-case study analysis. Malaria Journal, 1-14.

Hammond, A. et al. (2016). A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae. Nat. Biotechnol., 34, 78-83.

Hammond, A.M. and Galizi, R. (2017). Gene drives to fight malaria: current state and future directions. Pathogens and Global Health, 111(8), 412-423.

Harrison, R.G. and Larson, E.R. (2014). Hybridization, Introgression, and the Nature of Species Boundaries. Journal of Heredity(105), 795-809.

Hawkes, M.; Li, X.; Crockett, M.; Diassiti, A.; Liles, W.C.; Liu, J. and Kain K.C. (2010). Malaria exacerbates experimental mycobacterial infection in vitro and in vivo. Microbes Infect., 12(11), 864-874.

Horizon. (2018). Gene Editing. Retrieved June 2018, from Horizon Discovery Group plc: https://www.horizondiscovery.com/gene-editing

Josef Zapletal, Neda Najmitabrizi, Madhav Erraguntla, Mark A. Lawley, Kevin M. Myles and Zach N. Adelman. (2021). Making gene drive biodegradable. *Biological Sciences , 376* (1818).

Kokoza, V.; Ahmed, A.; Cho, W.L.; et al. (2000). Engineering blood meal-activated systemic immunity in the yellow fever mosquito, Aedes aegypti. Proceedings of the National Academy of Sciences of the United States of America, 97, pp. 9144-9149.

Krafts, K.; Hempelmann, E. and Skórska-Stania, A. (2012). From methylene blue to chloroquine: a brief review of the development of an antimalarial therapy. Parasitol Res, 111, 1-6.

Lambert, P. (2003). Malaria: Past and Present. Retrieved May 2018, from Noble Prize: https://www.nobelprize.org/educational/medicine/malaria/readmore/treatment.html

Lea Pare Toe, Bakara Dicko, Richard Linga, Nourou Barry, Mouhamed Drabo, Naima Sykes and Delphine Thizy. (2022). Operationalizing stakeholder engagement for gene drive research in malaria elimination in Africa—translating guidance into practice. *Malaria Journal* , 1-16.

Li, F., Patra, K.P. and Vinetz, J.M. (2005). An anti-Chitinase malaria transmission-blocking single-chain antibody as an effector molecule for creating a Plasmodium falciparum refractory mosquito. J Infect Dis, 192(5), 878-887.

Liu, N. (2015). Insecticide Resistance in Mosquitoes: Impact, Mechanisms, and Research Directions. Annual Review of Entomology, 60, 537-559.

Macer, D. (2003). Ethical, legal and social issues of genetically modified disease vectors in public health. World Health Organization.

Macias, V.M. and James, A.A. (2015). Impact of genetic modification of vector populations on the malaria eradication agenda. Genetic Control of Malaria and Dengue, ed Adelman ZN, 423-444.

MAP (2018). Mosquito Malaria Vectors. Retrieved June 16, 2018, from MAP: https://map.ox.ac.uk/mosquito-malaria-vectors/

Maxmen, A. (2012). Florida Abuzz over Mosquito Plan. Nature, 487(7407).

NIAID. (2011, March 8). Malaria Prevention, Treatment, and Control Strategies. Retrieved April and Infectious Diseases: from National Institute of Allergy 2018, https://www.niaid.nih.gov/diseases-conditions/malaria-strategies

Ostera, G.R. and Gostin, L.O. (2011). Biosafety Concerns Involving Genetically Modified Mosquitoes to Combat Malaria and Dengue in Developing Countries. American Medical Association, 930-931.

Panjwani, A. and Wilson, A. (2016). What Is Stopping the Use of Genetically Modified Insect for Disease Control? (U. o. Carolyn B. Coyne, Ed.) PLoS Pathog, 12(10), e1005830.

Parliament, E. (2009). Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, 1107/2009.

Pennisi, E. (2013). The CRISPR craze. Science, 341, 833-836.

Peter D. Crompton; Susan K. Pierce; and Louis H. Miller. (2010). Advances and challenges in malaria vaccine development. *J Clin Invest.*

Phillips, M.A.; Burrows, J.N.; Manyando, C.; Van Huijsduijnen, R.C.; Van Voorhis, W.C. and Wells, T.N.C. (2017). Malaria. Nature Reviews/Disease Primers, 3, 1-24.

Pollack, A. (2011). Concerns are Raised about Genetically Engineered Mosquitoes. New York Times, Bl.

Ramirez, J.L.; Garver, L.S and Dimopoulos, G. (2009). Challenges and Approaches for Mosquito Targeted Malaria Control. Current Molecular Medicine, 9, 116-130.

Resnik, D. (2014). Ethical Issues In Field Trials Of Genetically Modified Disease-Resistant Mosquitoes. Bioethics, 14, 37-46.

Revees, R.G.; Denton, J.A.; Santucci, F.; Bryk, J. and Reed, F.A. (2012). Scientific Standards and the Regulation of Genetically Modified Insects. PLoS Negl Trop Dis, 6(1), e1502.

Robert, M.A.; Okamoto, K.W.; Gould, F. and Lloyd, A.L. (2014). Antipathogen genes and the replacement of disease-vectoring mosquito populations: A model-based evaluation. Evol. Appl, 7(10), 1238-1251.

Rosauro Varo; Carlos Chaccour and Quique Bassat. (2020). Update on malaria. *Med. Clin. , 155* (9), 395-402.

Schellenberg, D.; Abdulla, S. and Roper, C. (2006). Current Issues for Anti-Malarial Drugs to Control P. falciparum Malaria. Current Molecular Medicine, 253-260.

Shengzhang Dong; Yuemei Dong; Maria L. Simões and George Dimopoulos. (2021). Mosquito transgenesis for malaria control. *Trends in Parasitology* , 13.

Sinka, M,E,; Bangs, M.J.; Manguin, S.; Coetzee, M.; Mbogo, C.M.; Hemingway, J.; et al.,. (2010). The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. Parasites & Vectors, 3, 1-34.

Sullivan, K. (2021). *First Malaria Vaccine a Major Milestone Despite Hurdles Ahead.* WebMD.

Tizifa, T.A.; Kabaghe, A.N.; McCann, R.S.; van den Berg, H.; Van Vugt, M. and Phiri, K.S. (2018). Prevention Efforts for Malaria. Current Tropical Medicine Reports, 5, 41-50.

Todesco, M., Pascual, M.A., Owens, G.L., Ostevik, K.L., Moyers, B.T., Hubner, S. et al., (2016). Hybridization and extinction. Evol Appl., 892-908.

Tom A. R. Price, Nikolai Windbichler, Robert L. Unckless, Andreas Sutter, Jan-Niklas Runge, Perran A. Ross, Andrew Pomiankowski, Nicole L. Nuckolls, Catherine Montchamp-Moreau, Nicole Mideo, Oliver Y. Martin, Andri Manser, Mathieu Legros, et al. (2020). Resistance to natural and synthetic gene drive systems. *Journal of Evolutionary Biology , 33* (10), 1345-1360.

Virginie Courtier-Orgogozo, Antoine Danchin, Pierre-Henri Gouyon, Christophe Boëte. (2020). Evaluating the probability of CRISPR-based gene drive contaminating another species. *Evolutionary Applications , 13* (8), 1888-1905.

Wang, S. and Jacobs-Lorena, M. (2013). Genetic approaches to interfere with malaria transmission by vector mosquitoes. Trends Biotechnol., 31(3), 185-193.

WHO (2018). Malaria. Retrieved June 16, 2018, from World Health Organization: http://www.who.int/news-room/fact-sheets/detail/malaria

WHO. (2021). *World Malaria Report 2021*. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2021.

YourGenome. (2016). How is malaria treated and prevented? Retrieved April 2018, from yg: https://www.yourgenome.org/facts/how-is-malaria-treated-and-prevented