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# Challenges and options for disease vector control

*The outbreak of Zika virus in South America and increasing insecticide resistance among mosquitoes have rekindled efforts for controlling disease vectors*

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The ongoing Zika virus outbreak, which briefly threatened to put a stop to the 2016 Olympic Games in Brazil, has again focused attention on the threat of vector-borne diseases. One efficient strategy to battle such diseases, now underway in Brazil to fight the Zika virus, is to kill the insects that carry the pathogen. Vector control has been practised with varying effectiveness for at least half a century, especially for malaria; the Brazil outbreak again highlights the need for integrating existing methods including the use of insecticides, vaccines, drugs and low technology such as sanitation to keep insect vectors at bay. In addition, new approaches are being developed based on sophisticated methods such as genome editing of the insect vector and deliberate infection of mosquitoes with the *Wolbachia* bacteria. However, attempts to scale up these new methods from field trials to deployment have been slowed down or halted by environmental concerns or public resistance against genetically modified organisms.

## Epidemiology

The Zika outbreak in South America has stimulated cross-border collaboration of public health authorities and increased pressure to use new integrative approaches that are essential for combatting vector-borne diseases. The irony is that the Zika virus itself has a minimal global impact compared with other insect-borne pathogens, but it has attracted enormous publicity because of the risk of causing microcephaly in unborn children, combined with the coincidence of the outbreak during the Olympic year. The exact risk for the foetus is as yet unknown [1], but it is clear that Zika is otherwise a relatively mild disease with no symptoms in the majority of cases (http://www.cdc.gov/zika/symptoms/index.html). By contrast, other mosquito-borne diseases, including malaria, yellow fever, dengue and chikungunya (CHIKV), are major causes of both mortality and morbidity, responsible for hundreds of millions of cases each year and millions of deaths (http://www.who.int/whr/1996/media_centre/executive_summary1/en/index9.html). Of these malaria, caused by the parasitic protozoa *Plasmodium*, spread mostly by female *Anopheles* mosquitoes, is the most devastating, with nearly half the world's population at risk (http://www.who.int/features/factfiles/malaria/en/). In 2015, there were 214 million cases and an estimated 438,000 deaths, 90% of which occurred in Africa.

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One other major vector-borne disease, leishmaniosis, is also caused by a protozoan parasite, but spread by sand flies rather than mosquitos. Most of the other mosquito-borne diseases, including dengue, yellow fever and CHIKV, are viral. Dengue is the most serious one with 20 million cases a year spanning more than 100 countries and 2.5 billion people at risk of infection (same WHO ref). Yellow fever causes 200,000 infections and 30,000 deaths each year, mostly in Africa, but a safe and effective vaccine is now available. There are concerns though that the virus could spread into Southeast Asia along with its vector *Aedes aegypti*. CHIKV, transmitted by both *A. aegypti* and *A. albopictus*, is prevalent throughout most of the developing world and mostly dangerous for newborns, the elderly or people with chronic infections.

Mosquitos can also carry other infectious pathogens, including microscopic worms as in lymphatic filariasis, which can result in permanent physical disability. According to the WHO, 1.1 billion people in 55 countries are at risk and would require preventive chemotherapy to stop the spread of infection. There are also other vectors such as tsetse fly, which spreads sleeping sickness, again caused by a protozoan parasite, with 55 million people at risk.

## Environmental factors

The incidence of all these diseases could be reduced significantly by vector control, as has been proven for malaria. But the Zika outbreak in South America has highlighted the importance of the specific habitats of the

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different vectors and how in this case urbanization has created the conditions for the epidemic. "Humans have produced a new habitat called cities and towns, which have been colonized by the mosquito *Aedes aegypti*", said Steve Lindsay, a public health entomologist specializing in vector-borne diseases at Durham University in the UK. "This happens to be the best transmitter of arboviruses in the world, like Zika, yellow fever, dengue, chikungunya and perhaps others".

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But while urban environments encourage the *Aedes* mosquito carrying arboviruses, they are hostile for most species of the *Anopheles* mosquito that transmits malaria, according to Jo Lines, Reader of Malaria Control and Vector Biology at the London School of Hygiene and Tropical Medicine. Indeed, regional or habitat differences are highly relevant for vector control: for instance, the subgenera of *Anopheles* that transmits malaria in Africa are adapted to agricultural environments, such as rice fields, but other subspecies favour different habitats. "Those *Anopheles* mosquitos transmitting malaria in SE Asia were thoroughly adapted to the forest", Lines said. "When you cut the forest down, they retreat with the forest". Thus, the widespread deforestation that has occurred during the past 20–30 years in the lowlands of Vietnam and Thailand has led to the virtual eradication of malaria in those countries, Lines explained. "[T]he mosquitos that are abundant in rice fields in Africa are important vectors because they bite people and are long lived. So rice does grow malaria in Africa. It doesn't do so in SE Asia because although there are plenty of *Anopheles* in the rice fields, they bite animals and are short lived". This has the corollary that there is no such prospect of malaria eradication at the moment in the affected African countries, despite hopes raised by the success of insecticide-treated nets. "You would have to cover the entire continent of Africa in concrete and make sure there was no standing water in there for the *Anopheles* mosquito, then we would eradicate malaria at a stroke", Lines commented.

## Growing resistance

Between 2000 and 2015, a targeted campaign halved the prevalence of malaria in sub-Saharan Africa and reduced the incidence of critical disease by 40% [2]. This success was based largely on distributing insecticide-treated bed nets, which are effective against malaria because the *Anopheles* mosquito bites mostly at night when people are sleeping, unlike the *Aedes* mosquito, which bites more during the day. Unfortunately, this success has been undermined by growing resistance of mosquitoes to the most successful insecticide group, the pyrethroids that target the nervous systems of insects. Multiple resistance mechanisms are involved, and it is still unclear which ones play the main role or exactly how they interact [3].

Generally, growing resistance by *Anopheles* mosquitos in particular to pyrethroids threatens to reverse all the gains made since 2000, according to a recent study, which reported that mosquito survival is increasing, while no effective new insecticides are expected to reach the market for at least another five years [4]. This is a concern not just for malaria but all mosquito-borne diseases, given that insecticides play a major role in vector control, according to Christian Lengeler, Unit Head at the Swiss Tropical and Public Health Institute in Basel. This, he said, has led to a three pronged effort to develop new vector control tools as quickly as possible. "Firstly in the short term, we will re-purpose and/or re-formulate existing insecticides not used so far for public health. One example is pirimiphos-methyl, an old organophosphate insecticide that was re-marketed in a new formulation about two years ago, and which is currently the best insecticide for indoor residual spraying (IRS)", Lengeler explained. "Then in the medium to long-term, we aim to develop new active ingredients for truly new insecticides including a novel mechanism of action. This is a long-term task, taking at least 15 years to complete, with the first products expected around 2020–2022". Lengeler added that other methods are also needed to reinforce insecticides. These include spatial or airborne insect repellents and attractive toxic sugar baits (ATBS), oral insecticides comprising a toxin such as boric acid coated in a sugar component to encourage feeding, along with a scent to attract the insects to the bait [5].

These three approaches have almost universal support except among the most diehard environmentalists, since insecticides can be contained locally with minimal contamination. In contrast, the two principle high-tech approaches, gene drive and *Wolbachia* infection, are much more controversial, with backers and detractors even within the vector control community.

## New technologies

Gene drive takes advantage of the fact that only female mosquitoes spread malaria by inducing female sterility. The technique involves genome manipulation of mosquitos to install a transgene that confers female sterility. But gene drive goes beyond this: additional genetic elements copy the alien genes to the maternally inherited allele with the result that all offspring carry the transgene [6]. This can very quickly transform a whole insect population and substantially reduce its ability to transmit diseases.

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In contrast, the *Wolbachia* approach works by suppressing other microorganisms in the mosquito, including the agents of vector born disease. *Wolbachia* is a genus of bacteria which infects arthropods and often confers protection against subsequent RNA virus infection in particular, which has been shown to reduce transmission of both dengue and chikungunya virus [7,8].

To date, evidence of environmental risks posed by either gene drive or *Wolbachia* approaches is elusive. The main issue for gene drive is how well it will work on a large scale rather than whether it poses any threat to the ecosystem, according to Nikolai Windbichler who specializes in genome engineering for insect control at Imperial College London, UK. "I think that ultimately the technology will be found to be a very effective, environmentally friendly way to control harmful insect vector or pest populations," he said. "It will not be found to be a

magic bullet, but neither will it ultimately come to be regarded as particular risky, despite current perceptions".

Some GM approaches work by producing "self-limiting" insects that die before they reach maturity. One example is a strain of *Aedes aegypti* called OX513A engineered by Oxitec, a biotech spin-out from Oxford University in the UK. These are genetically modified males that produce offspring that die before reaching reproductive stage. They carry a modified gene for the non-toxic protein tTAV (tetracycline repressible activator variant), the expression of which inhibits other key genes leading to death of the cell and ultimately the whole insect [9]. Vector control would involve releasing a large number of these self-limiting males in the target area where they outcompete normal males in producing offspring.

According to Gabriel da Luz Wallau, Public Health Researcher in the Entomology Department of the Aggeu Magalhaẽs Research Center in Brazil, where OX513A has been trialled, the strength of the approach lies in use of the antibiotic tetracycline as an antidote: it binds to the tTAV protein blocking its function and therefore enables the insects in the laboratory to reach reproductive age. "So every time a GM mosquito mates with a natural female it will generate offspring that can only grow in the presence of this antibiotic", da Luz Wallau explained. "As the antibiotic is very rare in nature, all offspring from such crosses will become a dead end, diminishing the mosquito population". The US Food and Drug Administration has already confirmed that OX513A poses negligible risk either to humans or the environment (http://www.fda.gov/downloads/AnimalVeterinary/DevelopmentApprovalProcess/GeneticEngineering/GeneticallyEngineeredAnimals/UCM487379.pdf).

### Limits of gene drive

Such GM techniques have been shown to work against dengue, Zika and CHIKV by reducing populations of the *Aedes* mosquito, but the *Anopheles* malaria mosquito is a much more challenging target, owing to its genetic diversity, according to Lengeler. Yet, the prospects for GM approaches to control *Anopheles* have greatly improved in recent years through the arrival of CRISPR-Cas9, according to Elizabeth McGraw, whose laboratory studies the evolutionary basis of interactions between insects and microbes at Monash University in Australia. "As an evolutionary biologist, I have always been sceptical about whether genetic modification of mosquitoes could be an effective tool for biocontrol in the field", she said. "Modifications of genomes often lead to non-target effects and reductions in mosquito fitness that would hinder performance and spread of the GM mosquitoes. With the emergence of CRISPR-Cas9 approaches, however, it is possible to make very targeted changes in mosquito genomes that do not affect other genes or prove costly for mosquitoes to carry".

> *"... there is growing interest in Integrated Vector Management (IVM) as it embraces all existing methods including future high-tech ones, such as gene drive"*

McGraw is still sceptical though of approaches that limit reproduction across a whole population, including the release of sterile males. "I do not think strategies that reduce mosquito fecundity are necessarily sustainable in the field, given that selection will drive toward individuals in the population with higher fecundity. I think the best opportunities will come from targeting mosquito genes that control the insect's susceptibility to pathogens or its ability to transmit pathogens to humans. The identification of genes that underpin pathogen transmission in mosquitoes is the first step to further progressing this approach".

### *Wolbachia*

There is just as much debate over the merits, efficacy and safety of the *Wolbachia* approach. One study has suggested that mosquitoes infected with the bacteria *Wolbachia* were more likely in turn to become infected with the West Nile virus, even though it blocked dengue virus, casting doubts over the safety of deliberate *Wolbachia* infection to constrain insect-borne diseases [10].

This has led some in the vector control field to question the wisdom of using *Wolbachia*, including Wallau, who has found that the bacterium can be transmitted to other insect species. "We detected many horizontal transfer events of *Wolbachia* strains very closely related to the wMel strain which is being used to infect *Aedes aegypti*", he said. "So our main concern was about the possibility of such massive release of mosquitoes infected with *Wolbachia* increasing the chance of this *Wolbachia* ending up in another insect species such as bees or butterflies and hence generating some unexpected consequences such as shortening the life of such species. Life shortening is one of the features that *Wolbachia* induces in *Aedes* mosquitoes, diminishing the chance of mating and hence reducing mosquito population. Moreover, it is known that *Wolbachia* effects on arthropod hosts are species specific, so if a horizontal transfer occurs we could end up with other host reproductive manipulation effects such as male feminization, cytoplasmic incompatibility and so on, leading to negative effects on other insect species".

But these risks remain to be demonstrated as significant while many others advocate the use of *Wolbachia* because of its broad applicability. "The great thing about a *Wolbachia*-based approach is that you don't really have to revise the strategy to target say CHIKV if you already are using it against [dengue virus]", explained Matthew Aliota from the Veterinary Medicine University of Wisconsin. "The same mosquito transmits both, and in many locations around the globe you have co-circulation of both viruses. Our group and a Brazilian group also recently demonstrated that this would likely be effective against Zika virus". McGraw commented that field trials now need to show that laboratory experiments can be scaled up and that the risks are negligible. This, however, means that *Wolbachia*-infected mosquitoes, as GM methods, are unlikely to be approved soon, which highlights the continuing need for a combined approach, including low-tech methods.

### Integrated approaches

"Low-tech methods such as house improvement and larval source management are slowly but surely gaining recognition", commented Henk van den Berg, from the Laboratory of Entomology at Wageningen University in the Netherlands. "When used in combination with insecticides they also have potential to delay insecticide resistance. I am much in favour of the low-tech methods, which are available and do not require long evaluations".

 

The various methods are not mutually exclusive, and it is likely that combining multiple methods will be more effective than any one individually. Indeed, there is growing interest in Integrated Vector Management (IVM) as it embraces all existing methods including future high-tech ones, such as gene drive. IVM, defined by the WHO as "a rational decision-making process for the optimal use of resources for vector control", takes into account the relevant insects and pathogens, local infrastructure and environmental concerns combining with the availability of chemical, biological and basic control measures within a coherent strategy. "The basic point is that we need to use multiple tools and attack the vectors from different areas", Lindsay said.

## References


1. Anderson KB, Thomas SJ, Endy TP (2016) The Emergence of Zika Virus: A Narrative Review. *Ann Intern Med* 165: 175 – 183
2. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, Battle KE, Moyes CL, Henry A, Eckhoff PA et al (2015) The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature* 526: 207 – 211
3. Liu N (2015) Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. *Annu Rev Entomol* 60: 537 – 559
4. Baleta A (2016) Insecticide resistance threatens malaria control in Africa. *Lancet* 374: 1581 – 1582
5. Müller GC, Beier JC, Traore SF, Toure MB, Traore MM, Bah S, Doumbia S, Schlein Y (2010) Successful field trial of attractive toxic sugar bait (ATSB) plant-spraying methods against malaria vectors in the *Anopheles gambiae* complex in Mali, West Africa. *Malar J* 9: 210
6. Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, Katsanos D, Gribble M, Baker D, Marois E, Russell S *et al* (2016) A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. *Nat Biotechnol* 34: 78 – 83
7. Hedges L, Brownlie J, O'Neill S, Johnson K (2008) *Wolbachia* and virus protection in insects. *Science* 322: 702
8. Aliota MT, Walker EC, Uribe Yepes A, Dario Velez I, Christensen BM, Osorio JE (2016) The wMel Strain of *Wolbachia* Reduces Transmission of Chikungunya Virus in *Aedes aegypti*. *PLoS Negl Trop Dis* 10: e0004677
9. Harris AF, McKemey AR, Nimmo D, Curtis Z, Black I, Morgan SA, Oviedo MN, Lacroix R, Naish N, Morrison NI *et al* (2012) Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nat Biotechnol* 30: 828 – 830
10. Dodson BL, Hughes GL, Paul O, Matacchiero AC, Kramer LD, Rasgon JL (2014) *Wolbachia* enhances West Nile virus (WNV) infection in the mosquito *Culex tarsalis*. *PLoS Negl Trop Dis* 8: e2965