Infecting mosquitoes with bacteria so they can't infect us with viruses like Zika and dengue

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Tiny bug, major disease spreader. Credit: Dr. Paul Howell, USCDCP

Mosquitoes and their itchy bites are more than just an annoyance. They transmit dangerous viruses with deadly consequences – making them the most lethal animal on Earth. It's the *Aedes aegypti* and *Aedes albopictus* mosquito species that are behind outbreaks of dengue virus, Zika virus, yellow fever virus and Chikungunya virus, responsible for over 100 million human cases around the world annually. And they're expanding

their habitat around the world as the global climate warms, bringing them into contact with more potential victims who have less immunity and increased susceptibility to these mosquito-transmitted viruses.

A vaccine can provide the recipient with immunity to one or two of these viruses at a time. But there's another way to tackle these diseases: by going after the insects. Targeting the mosquito population as a whole or their ability to transmit disease takes aim at all these viruses at the same time.

As the U.S. enters another mosquito season, mosquito control districts in Florida and California are preparing new strategies to combat mosquitoes and the viruses they transmit. They're trying out one of two new mosquito management methods made possible by a bacterium called *Wolbachia pipientis*.

A bacterium that's our enemy's enemy

Wolbachia are bacteria naturally found in insects throughout the world. They live inside a host organism's cells. From there, *Wolbachia* are able to manipulate their host in many ways – things like increasing the number of eggs a host lays or even changing the host's sex from male to female by manipulating its hormones.

Researchers discovered in 2008 that *Wolbachia* in fruit flies protect their hosts from fruit fly viruses. That realization got them wondering: Could *Wolbachia* also protect *Aedes aegypti* mosquitoes from viruses that cause human diseases?

Aedes aegypti mosquitoes don't naturally carry *Wolbachia*. But consistent with the fruit fly studies, when researchers infected *Aedes aegypti* in the lab, the viruses they carry replicated less. Fewer of the infectious bits of the disease-carrying virus inside the mosquito meant disease

transmission was limited – they were less likely to be passed on when mosquitoes fed on their prey.

Researchers in Australia, the United States and elsewhere are currently investigating the reasons why *Wolbachia* limit viruses. Some hypothesize *Wolbachia* improves the mosquitoes' immunity to the virus, while other research, including my own, suggests *Wolbachia* steals key nutrients the virus needs. Both may be true.

The real need to employ this strategy now is motivating field trials to release *Wolbachia*-infected mosquitoes in several regions of the world.

Vector competency: The female approach

Only female mosquitoes bite and transmit viruses. Thus, the most powerful approach to reducing virus spread is limiting viruses in the female mosquito.

Wolbachia bacteria are transmitted from mother to offspring. If you introduce *Wolbachia*-infected female mosquitoes to a population, all offspring will have *Wolbachia* – and therefore be less likely to transmit disease-causing viruses.

This strategy is used by the Eliminate Dengue program, a nonprofit collaboration employing seven research institutes around the world. In test areas, Eliminate Dengue has successfully incorporated *Wolbachia* into mosquito populations.

In this context, an interesting aspect of *Aedes aegypti* behavior is their tendency not to travel far. In fact, a highway is a sufficient barrier to prevent mosquito spread. When researchers set up a release site in one city or town, they don't see their mosquitoes travel to other areas.

Researchers in Vietnam working with Wolbachia-infected mosquito pupae. Credit: AP Photo/Na Son Nguyen

This allows for controlled studies, as well as the release of these mosquitoes only where it's been approved. The limited spread and isolated sites used were important factors in the decision to allow mosquito releases in the United States.

Eliminate Dengue is not yet active in the U.S. Instead, the U.S. is taking a different approach, looking to male rather than female mosquitoes.

Population control: The male approach

MosquitoMate is a company developed out of the University of Kentucky in Lexington by medical entomologist Stephen Dobson. Partnering with the Florida Keys Mosquito Control District, they started the release of 40,000 *Wolbachia*-infected male mosquitoes per week this spring.

The strategy relies on a phenomenon called cytoplasmic incompatibility (CI) to reduce mosquito populations. CI occurs when a male mosquito infected with *Wolbachia* mates with an uninfected female. Because *Wolbachia* is transmitted through the female egg, the offspring will be *Wolbachia*-free. But *Wolbachia* has already altered the father's sperm DNA in a way that allows offspring to survive only if the fertilized egg has *Wolbachia*. Since the infected males will come in contact only with the naturally occurring *Wolbachia*-free population, their offspring will die during embryonic development – the eggs won't hatch.

And unfortunately for the mosquitoes, females store sperm inside them to continuously fertilize their eggs. This means that the female mosquito's first mate will be the father of all her offspring. So even if a female just mates again, once she's partnered with a *Wolbachia*-infected male, all her offspring will not be viable.

The Florida Keys Mosquito District is not limiting its attack to just one approach. Beyond *Wolbachia* and more traditional strategies, they're also partnering with Oxitec, a genetic engineering company. Like MosquitoMate, Oxitec also releases male mosquitoes. But, in place of *Wolbachia*, Oxitec genetically modifies its mosquito to contain a self-limiting gene that causes offspring to die.

The goal remains the same: Release males into the environment that will mate with females and cause all offspring to die, eventually leading to a mosquito population crash.

Male and female strategies share one goal

Each *Wolbachia* mosquito strategy has its strengths: The female approach is broad-reaching and should directly decrease disease transmission. The male strategy effectively lowers the local mosquito population, without releasing female nuisance mosquitoes.

The male release strategies are an important "right-now" fix, but they'll require an annual, costly release because male mosquitoes – with either MosquitoMate's *Wolbachia* or Oxitec's self-limiting gene – cannot pass on to the next generation their crucial trait. When these males are not being released, fertile wild males will mate with females and the population will rebound.

Eliminate Dengue's female release strategy is sustainable long-term, but it takes extensive monitoring to ensure the initial establishment of mosquitoes. While MosquitoMate and Oxitec do not disclose their costs, Eliminate Dengue hopes to make their system affordable at a cost of approximately US$1 per person.

Some members of the public have advocated against these kinds of mosquito release programs, particularly when the mosquitoes have been genetically modified, as with Oxitec's transgenic insects. While the United States Department of Agriculture received 2,600 responses to the Oxitec plan, only one response was filed regarding MosquitoMate's non-GMO strategy.

In the U.S., mosquito control districts are taking a cautious approach. They're first trying the two nonpermanent male strategies in small areas. The Florida Keys will be evaluating mosquitoes on their Stock Island release site for 12 weeks. We should know how effective male *Wolbachia*-infected mosquitoes are at reducing populations by late summer.