CURRENT OPINION

The potential role of *Wolbachia* in controlling the transmission of emerging human arboviral infections

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Purpose of review
Wolbachia is a genus of Gram-negative intracellular bacteria that is naturally found in more than half of all arthropod species. These bacteria cannot only reduce the fitness and the reproductive capacities of arthropod vectors, but also increase their resistance to arthropod-borne viruses (arboviruses). This article reviews the evidence supporting a *Wolbachia*-based strategy for controlling the transmission of dengue and other arboviral infections.

Recent findings
Studies conducted 1 year after the field release of *Wolbachia*-infected mosquitoes in Australia have demonstrated the suppression of dengue virus (DENV) replication in and dissemination by mosquitoes. Recent mathematical models show that this strategy could reduce the transmission of DENV by 70%. Consequently, the WHO is encouraging countries to boost the development and implementation of *Wolbachia*-based prevention strategies against other arboviral infections. However, the evidence regarding the efficacy of *Wolbachia* to prevent the transmission of other arboviral infections is still limited to an experimental framework with conflicting results in some cases. There is a need to demonstrate the efficacy of such strategies in the field under various climatic conditions, to select the *Wolbachia* strain that has the best pathogen interference/spread trade-off, and to continue to build community acceptance.

Summary
Wolbachia represents a promising tool for controlling the transmission of arboviral infections that needs to be developed further. Long-term environmental monitoring will be necessary for timely detection of potential changes in *Wolbachia*/vector/virus interactions.

Keywords
arboviruses, chikungunya virus, dengue virus, Japanese encephalitis virus, prevention, transinfection, transmission, West Nile virus, *Wolbachia*, yellow fever virus, Zika virus

INTRODUCTION

Arthropod-borne viruses (arboviruses) are transmitted between vertebrate hosts and blood-feeding arthropod vectors including mosquitoes, sand flies, biting midges, mites, lice and ticks [1,2▪,3]. With the exception of African swine fever virus, which is a double-stranded DNA virus belonging to the *Asfarviridae* family [4], all other arboviruses have an RNA genome and belong to one of the following five families of viruses: *Flaviviridae*, *Togaviridae*, *Bunyaviridae*, *Rhabdoviridae* and *Reoviridae* [3]. The distribution of arboviruses across the globe is largely dependent on the distribution of susceptible vector species, which varies in response to climatic changes. Their spread is favoured by urbanization, human travel and livestock movements [1,5,6]. Arboviral infections cause a wide range of life-threatening manifestations, notably nervous system disease

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Curr Opin Infect Dis 2017, 30:108–116

DOI:10.1097/QCO.0000000000000342

KEY POINTS

- *Wolbachia* is a Gram-negative intracellular bacteria that is naturally found in more than 50% of all arthropod species, but is absent in the major arbovirus vector *A. aegypti*.
- *Wolbachia* alters the reproductive fitness of arthropod vectors through selective male killing, parthenogenesis, feminization of male embryos and cytoplasmic incompatibility. It also alters the competence of transinfected arthropod vectors for the transmission of arboviruses through competition for resources, immune-priming, induction of the phenoloxidase cascade and induction of microRNA-dependent immune pathways.
- Field releases of *Wolbachia*-transinfected *A. aegypti* mosquitoes have been successfully used in the Eliminate Dengue Programme to suppress the dissemination of DENV in Australia, but the true epidemiological impact on dengue-related morbidity and mortality is yet to be assessed. The evidence regarding the efficacy of *Wolbachia* to prevent the transmission of other arboviruses including chikungunya, JEV, WNV and Zika is still limited to the experimental framework.
- It is possible that the *Wolbachia* strains that confer the strongest interference with pathogen transmission do not spread easily into local vector populations because of deleterious fitness effects. Adequate selection of the *Wolbachia* strain is therefore crucial in the implementation of *Wolbachia*-based biocontrol strategies.
- There has been some concern about potential exposure of humans to *Wolbachia* via mosquito bites. However, there is currently no evidence that such exposure occurs (or at least, is medically relevant). In addition, there is no evidence that the field release of *Wolbachia*-infected mosquitoes has any adverse effects on the environment.

(encephalitis, meningitis, seizures, stroke, myelitis, polyradiculoneuritis and myositis), liver disease (heptatitis and fulminant hepatic failure) and haemorrhagic disease (with thrombocytopenia, coagulopathies, bruising and bleeding) [2▪,3,7–10]. There is currently no antiviral treatment for any arboviral infection; nor have nonspecific treatments like corticosteroids made a difference [3,11]. Supportive treatment remains the mainstay and includes the management of fever, seizures, headaches or raised intracranial pressure (if any) and maintenance of vital functions. Because of the limited treatment options, and the wide extent of these diseases, better preventive measures are urgently needed. As shown in Table 1 [6,12–17,18▪▪,19–24], these measures could be implemented at the level of the human host, at the level of the vector or at the interface between the two.

Preventive measures at the level of the human host are often not available or prove difficult to develop. Effective vaccines are only available for yellow fever virus (YFV) [20], Japanese encephalitis virus (JEV) [21], dengue [22] and tick-borne encephalitis [23]; there is currently none approved for other widespread arboviruses, notably chikungunya, West Nile virus (WNV) and Zika [25–27]. Research on chemoprophylaxis is still in its early stages [24]. Preventive measures at the level of the vector include radiological, chemical and genetic interventions to eradicate arthropod vectors or limit their reproductive capacities (the ability to produce viable and abundant offspring) [13–16]. However, chemical interventions are limited by the increasing development of resistance to insecticides [12], whereas genetic modifications raise ecological concerns about the potential long-term health and environmental risks [28▪▪,29]. As one approach alone is unlikely to be

Table 1. Summary of strategies that could be used to prevent the transmission of arboviruses to humans

Level of action	Ultimate goal	Strategies that could be used
Vector	Reduce the prevalence of vectors and their capacity to transmit viruses	Direct killing of vectors by spreading of insecticides [12]
		Limitation of vectors' reproduction by [13–16]:
		Destroying breeding sites and promoting good sanitary conditions
		Releasing sterile or genetically modified vectors
		Introducing biological control agents
		Wolbachia-based methods: population replacement with transinfected vectors displaying reduced vector competence [17,18▪▪], or population suppression (Incompatible Insect Technique) [19]
Host–vector interface	Avoid bites [6]	Use of bed nets
		Use of repellents
		Sensitization of travellers and communities at risk
Human host	Reduce host susceptibility to arboviruses	Vaccine [20–23]
		Chemoprophylaxis [24]

sufficient and/or always affordable, there is an urgent need for the development of novel strategies for vector control, prompting a high level of interest in using the bacterium *Wolbachia* to control the transmission of arboviruses.

Wolbachia is a genus of Gram-negative intracellular bacteria belonging to the order *Rickettsiales* and the family *Anaplasmataceae*. These bacteria only infect invertebrate organisms and are naturally found in more than 50% of all arthropod species and in several nematodes [1,30,31]. However, *Wolbachia* is naturally absent from *Aedes aegypti* (also called *Stegomyia aegypti*), but can be introduced [1,32]. *A. aegypti* is a widespread human blood-feeding mosquito responsible for the transmission of several arboviruses including dengue, yellow fever, Zika, Murray valley, La Crosse, chikungunya and Rift valley fever viruses. Generally, the different strains of *Wolbachia* are named according to the host in which they were first discovered. For instance, *Wolbachia pipientis* (*w*Pip) was the first strain discovered in the mosquito *Culex pipiens* [33]. Similarly, *w*Mel was first isolated from the common fruit fly *Drosophila melanogaster*, whereas *w*Alb was first isolated from *Aedes albopictus* [34]. Several studies have demonstrated that *Wolbachia* increases arthropods' resistance to viruses [35–37] and/or alters their reproductive capacities [17,38]. More recently, researchers of the Eliminate Dengue Programme in Australia have demonstrated that the transfer of this bacterium into wild populations of the mosquito *A. aegypti* represents an effective measure to control the transmission of dengue [18▪▪]. This has led various public health authorities, including the WHO, to advocate the use of *Wolbachia*-based strategies to control the spread of dengue and other arthropod-borne viruses [39].

Here, we review the scientific evidence supporting the use of *Wolbachia*-based strategies to control the transmission of these arboviral infections and discuss the related risks, challenges and limitations.

BENEFICIAL EFFECTS OF *WOLBACHIA* FOR CONTROLLING THE TRANSMISSION OF ARBOVIRAL INFECTIONS

Wolbachia can be used for the control of arboviral diseases in one of two strategies: the reduction of vectors' reproductive capacity and the induction of resistance to RNA viruses.

ALTERATION OF VECTORS' FITNESS AND REPRODUCTIVE CAPACITIES

Wolbachia sp. can induce significant alterations of the reproductive biology of their host including selective male killing, parthenogenesis (a form of asexual reproduction in which viable embryos develop from unfertilized eggs), feminization of genetically male embryos and cytoplasmic incompatibility [38]. Cytoplasmic incompatibility refers to the failure of *Wolbachia*-infected males to produce viable offspring when mating with either uninfected females or females infected with a different strain of *Wolbachia* [40,41]. In the first scenario, the cytoplasmic incompatibility is said to be unidirectional because it will promote the expansion of only one subpopulation composed of *Wolbachia*-infected mosquitoes. In the second scenario, the cytoplasmic incompatibility may be bidirectional because it can result in the development of divergent subpopulations, each infected with one of two or more opposing *Wolbachia* strains [1,42,43]. However, infected females can mate successfully with infected males and this provides them with an evolutionary advantage over uninfected females [40,41]. The selective expansion of *Wolbachia*-infected subpopulations of vectors is responsible for their ability to invade and progressively replace wild populations following large-scale field releases [44,45]. Alternatively, if only male infected mosquitoes are released into an uninfected or incompatible population (the 'Incompatible Insect Technique'), the vector population may crash, which then leaves an ecological niche for repopulation by noninfected vectors [19].

INDUCTION OF VIRAL RESISTANCE IN ARTHROPOD VECTORS

Wolbachia is thought to induce resistance to arboviruses through four complementary mechanisms (Fig. 1): competition for resources, preactivation of the immune system (also referred to as immune-priming), induction of the phenoloxidase cascade and induction of microRNA-dependent immune pathways that are essential for host defence against viruses [46,47▪▪].

Competition for resources

Autophagy is a cellular degradation and recycling process by which unnecessary or dysfunctional cellular components are incorporated in lysosomes for digestion. The resulting nutrients are made available for further metabolic processes [48]. *Wolbachia* is not only able to induce autophagy in arthropod vector's cells but also to hijack the autophagy system in order to ensure its own survival both *in vitro* and *in vivo* [49]. As both flaviviruses and alphaviruses are dependent on the autophagy pathway to replicate [50,51], it has been hypothesized that *Wolbachia* interferes with the replication of some arboviruses

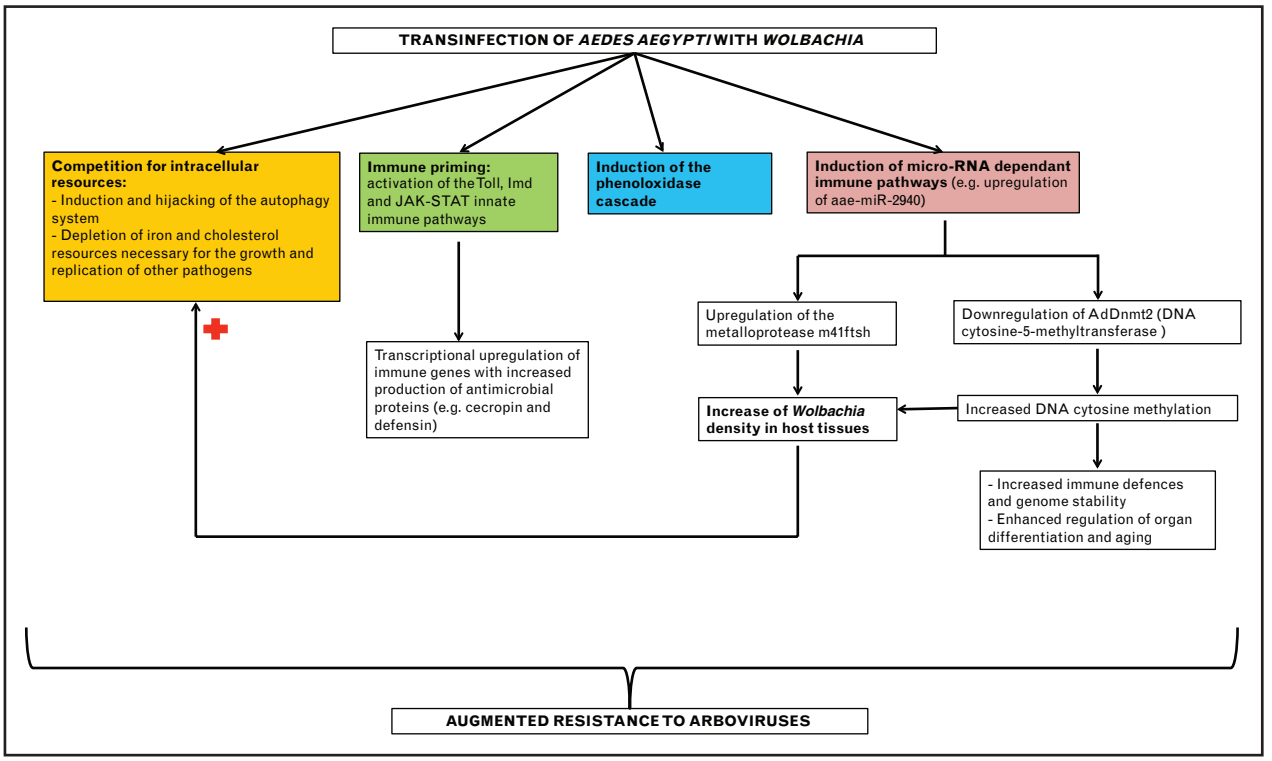

FIGURE 1. Mechanistic pathways of *Wolbachia*-induced resistance to arboviruses in *Aedes aegypti* following transinfection (the plus sign indicates that the increase of *Wolbachia* density in host tissues aggravates the competition for intracellular resources).

through its ability to manipulate the autophagy system, thus reducing the amount of nutrients available for viruses.

Wolbachia-mediated antiviral resistance might also be favoured by competition with viruses for iron and cholesterol. The bacterium is known to manipulate host cell iron reserves, as does the dengue virus (DENV) and the chikungunya virus (CHIKV) [52▪,53]. Like other members of the order *Rickettsiales*, *Wolbachia* is unable to synthesize cholesterol *de novo* and therefore relies on host cell cholesterol reserves for its replication and growth [54]. Similarly, mosquito-borne flaviviruses and alphaviruses have been shown to rely on host cell cholesterol for cell invasion, replication, virion assembly, infectivity and release from the infected cells [55–62].

Immune-priming

Transinfection of *Wolbachia* into heterologous arthropod vectors (i.e. vectors that are not naturally infected by any, or that specific *Wolbachia* strain; such as the mosquito *A. aegypti*) preactivates their immune system, enabling it to combat microbes (including viruses) more effectively. This is done by inducing three major signalling pathways of the innate immune system: Toll, Imd (immune deficiency) and Janus kinase-signal transducer and activator of transcription (JAK-STAT) [1,46]. Toll (from the German adjective 'toll' meaning 'wonderful') are transmembrane proteins encoded by the eponymous gene in *Drosophila* [63]. The JAK-STAT pathway is made up of one cell surface receptor called JAK and two proteins acting as STAT [64]. Activation of these signalling pathways leads to the transcriptional upregulation of antimicrobial peptide genes – such as those that encode drosomycin, cecropin and defensin – and several other immune genes [65–68], resulting in increased resistance of arthropod vectors to various arboviruses [1,69–75].

Induction of the phenoloxidase cascade

The phenoloxidase cascade is important in mosquitoes' immune response to viruses [76], and *Wolbachia* has been recently shown to trigger this pathway both in homologous and heterologous host vectors [77].

Induction of miRNA-dependent immune pathways

Wolbachia upregulates the microRNA aae-miR-2940 in mosquitoes [78] and this has two consequences: the upregulation of the metalloprotease m41ftsh and the downregulation of the DNA cytosine-5-methyltransferase gene, AaDnmt2, thus favouring DNA

cytosine methylation. The latter is indispensable for host immune defence, gene regulation, genome stability, organ differentiation and ageing [79].

It is also noteworthy that both the metalloprotease m41ftsh and DNA cytosine methylation are essential for maintaining a high density of *Wolbachia* infection in host cells [78]. Therefore, the upregulation of microRNAs could potentiate the competition for resources (Fig. 1), as a high density of *Wolbachia* creates unfavourable conditions for viruses by decreasing the amount of available resources (iron, cholesterol and other lipids) [35,80].

APPLICATION OF THE *WOLBACHIA*-BASED STRATEGY FOR CONTROLLING THE TRANSMISSION OF ARBOVIRAL INFECTIONS: CURRENT RESULTS, POTENTIAL RISKS AND FUTURE CHALLENGES

The phenotypic effects of *Wolbachia* on arthropod vectors' reproduction and resistance to viruses make it a promising tool for controlling the transmission of arboviral infections. Indeed, *Wolbachia* has already been successfully used to control the transmission of dengue, whereas its role in combating other infections is still being assessed.

Initial successes in the Eliminate Dengue Programme

Dengue is the most important mosquito-borne viral disease of humans with an estimated 2.5 billion people at risk in more than 100 countries worldwide, and 50–100 million infections acquired each year [81]. It is transmitted principally by the mosquito *A. aegypti*, which is present in more than 150 countries and is not naturally infected by *Wolbachia* [82▪▪]. The Eliminate Dengue Programme emerged in 2008 from the work of Professor Scott O'Neill and colleagues [83] (www.eliminatedengue.com). Early efforts focused on using the life-shortening *w*MelPop strain to reduce the number of dengue vectors reaching maturity. This approach took account of the fact that mature mosquitoes are more likely to transmit dengue, as the DENV must incubate in the mosquito for several days before becoming infectious [83]. However, as transinfection of *A. aegypti* with the *w*MelPop strain induced significant fitness costs [reduction of the longevity of infected adult females and reduction in the viability of eggs, whether or not they were in diapause (i.e. physiological state of dormancy induced by unfavourable environmental conditions)] [45], there were some concerns about its ability to rapidly invade wild mosquito populations following test releases of *Wolbachia*-infected mosquitoes. Indeed, the greater the fitness costs, the higher the initial *Wolbachia* frequencies required for invasion. According to mathematical predictions, as the fitness cost of infection approaches 0.5, spatial spreading of *Wolbachia* slows to zero [84]. For this reason, researchers of the Eliminate Dengue Programme turned to the *w*Mel strain that has a lower fitness cost but still confers sufficient resistance to DENV [35,37]. In 2011, they reported stable transinfection of *A. aegypti* with *w*Mel [83,85]. They subsequently demonstrated that this strain reduced the capacity of *A. aegypti* to transmit dengue and successfully invaded wild mosquito populations [86,87]. This laid the foundations for the large-scale release of *Wolbachia*-infected mosquitoes in dengue-endemic areas in Australia, resulting in successful suppression of DENV replication in and dissemination by mosquitoes as confirmed by vector competence experiments carried out 1 year following field release [18▪▪]. The success of the Eliminate Dengue Programme in Australia has led to further trial releases of *Wolbachia*-carrying *A. aegypti* in other dengue-endemic countries throughout the world, notably Colombia, Indonesia, Vietnam and Brazil [82▪▪]. Recent mathematical models have demonstrated that this strategy could reduce the transmission of DENV by 70% [82▪▪,88▪▪]. However, the true epidemiological impact (reduction of the incidence of dengue and the relative risk of infection between *Wolbachia*-treated and untreated areas) of *Wolbachia*-based biocontrol strategies for dengue is yet to be properly assessed through prospective cohort studies and cluster randomized trials [89▪▪].

The potential use of *Wolbachia* to control other arboviral infections

Although *Wolbachia*-infected mosquitoes were initially generated for the biocontrol of dengue, there is increasing evidence from experimental studies that they could also be used to control the transmission of other arboviruses, notably CHIKV [90], JEV [91▪] and YFV [92]. Concerning WNV, results are more controversial. In 2009, it was reported for the first time that *Wolbachia* could increase resistance to WNV in *Culex quinquefasciatus* [80]. However, subsequent reports highlighted the fact that most *C. quinquefasciatus* populations are naturally infected with *Wolbachia* but are still capable of transmitting WNV. Moreover, it appears that transinfection with the *w*AlbB strain from *A. albopictus* enhances WNV infection in *Culex tarsalis*, a naturally uninfected mosquito which is an important vector of WNV in North America [93▪]. Finally, it has been demonstrated recently that *Wolbachia*-infected mosquitoes are highly resistant to infection with two currently circulating Zika virus isolates

from the Brazilian epidemic. *Wolbachia*-infected *A. aegypti* also did not carry infectious Zika virus in the saliva, suggesting that *Wolbachia* can be used to block the transmission of Zika fever [94▪▪].

Potential risks

Wolbachia-infected mosquitoes are not considered to be genetically modified as *Wolbachia* is a naturally occurring symbiont of invertebrates. Moreover, volunteers that are bitten by *Wolbachia*-infected mosquitoes do not show any specific antibody production against *Wolbachia,* which probably means that there is no transmission of the bacteria from mosquitoes to humans [95]. As *Wolbachia* is an obligate intracellular bacterium, it cannot survive in the environment (air, soil, water and leaves), but horizontal transmission between arthropods does occur in nature [96]. Therefore, arthropod predators of mosquitoes could become infected with *Wolbachia* strains transinfected into their prey. The potential impact of such stochastic events is difficult to predict, but considering the ubiquity of *Wolbachia* in arthropod populations, deleterious effects on natural predators seem highly unlikely.

Future challenges

Taking into account the initial successes of the Eliminate Dengue Programme, the WHO currently encourages affected countries to boost the development and implementation of *Wolbachia*-based mosquito control interventions against other arboviral infections [39]. Nevertheless, before the *Wolbachia*-based strategy to control the transmission of arboviral infections can be implemented worldwide, various issues need to be addressed.

Choosing the optimum *Wolbachia* strain

Future studies will have to determine which *Wolbachia* strain shows the optimum trade-off between pathogen interference, the strength of cytoplasmic incompatibility and other potential fitness effects. Indeed, it is possible that *Wolbachia* strains that confer the strongest interference with pathogen transmission do not spread easily into local vector populations because of deleterious fitness effects [84]. These deleterious fitness effects could take the form of a reduced lifespan of larval and/or adult stages [97,98], decreased egg viability [45,99] or greater susceptibility of *Wolbachia*-infected mosquitoes to some insecticides, and thus should be carefully monitored. However, the experience to date with *w*Mel in *A. aegypti* suggests that this strain is likely to be well tolerated by other vector species.

Monitoring evolutionary changes

Evolutionary changes occurring in *Wolbachia*, the arboviruses or the arthropod hosts should be monitored over time as they could modulate the efficacy of the *Wolbachia*-based prevention strategy [28▪▪]. Furthermore, it is still too early to say to what extent the *Wolbachia*-mediated viral resistance in vectors could trigger the emergence of potentially more virulent strains of arboviruses.

Obtaining community acceptance

Adequate public engagement is indispensable for the success of *Wolbachia*-based mosquito control strategies. Indeed, all public health interventions need to be well explained in order to be approved by local regulatory authorities and to ensure the support of the vast majority of people within the target communities [100]. The lessons learned from the Eliminate Dengue Programme should be applied, and adapted to local conditions, for other arboviral diseases and the respective communities affected.

Accounting for geographical specificities

Wolbachia-based biocontrol strategies might not be equally efficient or applicable in all geographical areas. Indeed, in regions endemic for two or more arboviral diseases with different vectors, the need to allow spread of a newly released *Wolbachia*-infected vector could require that the application of insecticides be halted (at least temporarily), thus allowing other vectors to thrive, and potentially leading to increased risks of a disease outbreak. The same concern could arise in areas where a disease is transmitted by two or more vector species. For instance, dengue and Zika viruses are transmitted by *A. aegypti* and *A. albopictus*, and both species have increased viral resistance after transinfection with *w*Mel [17,86]. However, large-scale field releases are currently restricted to *Wolbachia*-transinfected *A. aegypti*. Moreover, in areas where rare vector species are more important for disease transmission than the most widespread ones, *Wolbachia*-based vector control strategies might be less cost-effective than insecticides that target all potential vectors at the same time. Finally, it is still unclear whether the results obtained with the Eliminate Dengue Programme can be replicated for dengue or other arboviral infections in the tropics, where arthropod vectors' density and efficiency are expected to be higher because of higher temperatures [101].

CONCLUSION

The naturally occurring endosymbiont *Wolbachia* has several effects on reproduction and vector

competence in arthropod vectors and therefore represents a promising tool for controlling the transmission of arboviral infections with apparently almost no health or environmental risk. Indeed, mass releases of *Wolbachia*-transinfected *A. aegypti* have already been used successfully in Australia to block the transmission of DENV with no known adverse effects. However, more research is required before the same strategy could be used for other infections. Indeed, it needs to be confirmed if the *w*Mel strain is the optimum one, in terms of both pathogen interference and rate of spread, for other vectors of arboviruses. Furthermore, implementation of *Wolbachia*-based prevention strategies should account for geographical specificities and be accompanied by adequate public engagement programmes to ensure community acceptance. These strategies should also be adequately monitored over a long period for timely detection of potential adverse effects or changes in *Wolbachia*/vector/virus interactions.

Acknowledgements

The authors acknowledge the institutions supporting their research: the Wellcome Trust (J.K.T., L.B.), the National Institute of Health Research (T.S.), the Health Protection Research Unit in Emerging and Zoonotic Infections (M.B., T.S.) and the European Union's Horizon 2020 research and innovation programme - grant agreement No. 734584 (T.S.).

Disclaimer: The views expressed in this article are those of the authors and not necessarily those of the National Health Service (NHS), The National Institute of Health Research (NIHR) and the Department of Health or Public Health England.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

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