Institution: University of Glasgow (UofG)		
Unit of Assessment: UoA 1 Clinical Medicine		
Title of case study: A biological control approach based on mosquito release to block dengue transmission in Malaysia		
Period when the underpinning research was undertaken: 2016–present		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Prof Steven Sinkins	Professor in Microbiology and Tropical Medicine	2016–present
Period when the claimed impact occurred: 2016–present		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact

Dengue virus is endemic in Malaysia (~100,000 cases annually), and the primary mosquito vector *Aedes aegypti* is difficult to control. However, when *Wolbachia* symbiotic bacteria are introduced into this mosquito, transmission of dengue can be blocked. UofG produced a line of *Ae. aegypti* carrying a heat-stable *Wolbachia* strain, and then partnered with the Malaysian Institute for Medical Research on the '*Wolbachia* Malaysia' biological control programme (2016–present). Releases of UofG mosquitoes reduced dengue cases by at least 60% across residential sites in Greater Kuala Lumpur, with very high community acceptance. The Malaysian Government has subsequently expanded the intervention, resourced through disinvesting from insecticidal control. Plans have been developed to enable other countries affected by dengue to use the UofG approach.

2. Underpinning research

Dengue is a mosquito-borne virus that is ranked among the top ten global health threats by the World Health Organization (WHO). Control relies on suppressing the primary vector *Ae. aegypti* with insecticides and/or by breeding site clearance, but is not very effective and alternative approaches to dengue control are urgently required.

Prof Steven Sinkins' research has focused on *Wolbachia* as a strategy for targeted biological control of dengue (work started at the University of Lancaster but over 90% carried out after he moved to UofG in 2016). *Wolbachia* are maternally inherited bacterial symbionts found in many insects (but not naturally in *Ae. aegypti*) that can induce the reproductive manipulation 'cytoplasmic incompatibility'. When *Wolbachia*-free female mosquitoes mate with *Wolbachia*-carrying males, the resulting eggs die; by contrast, *Wolbachia*-carrying females produce offspring regardless of the *Wolbachia* status of the male they mate with, and thus benefit from a reproductive advantage. All their offspring will also carry *Wolbachia*. Therefore, cytoplasmic incompatibility allows rapid spread and then maintenance of *Wolbachia* at high frequency in insect populations. Following laboratory transfer of *Wolbachia* strains into *Ae. aegypti* by embryo microinjection, some can inhibit transmission of mosquito-borne viruses such as dengue through a variety of cellular perturbations. For example, in 2017, Sinkins demonstrated a role for disruption of intracellular lipid metabolism and transport in the virus-blocking activity of *Wolbachia* [3.1].

Work published by Sinkins' group in 2018 indicated that different strains of *Wolbachia* vary widely in their ability to block virus transmission; their effects on host reproduction; and whether there are any negative effects on the host such as shortened lifespan [3.2]. To assess these effects, Sinkins transferred various *Wolbachia* strains—including *w*Mel and *w*Au (derived from *Drosophila*) and *w*AlbA and *w*AlbB (derived from the mosquito *Aedes albopictus*)—into *Ae. aegypti* [3.2]. Australian researchers had previously used *w*Mel-carrying *Ae. aegypti* for vector control in Queensland, where it led to a reduction in non-endemic dengue outbreaks. However, suboptimal densities and reduced dengue inhibition were seen with *w*Mel at high rearing temperatures [3.2], which would reduce efficacy and potentially increase the risk of selection for dengue virus resistance (analogous to the selection of antimicrobial resistance at suboptimal drug doses). By contrast, the *w*AlbB strain was not susceptible to the effects of rearing larvae at high temperatures and still blocked dengue transmission effectively. Thus, the decision was taken to use *w*AlbB for biological control of dengue in Malaysia.

From 2017–2019, field trials of the UofG *w*AlbB line of *Ae. aegypti* were conducted by the Malaysian Institute for Medical Research (IMR; led by Dr WA Nazni), working in partnership with UofG (led by Sinkins, who was also the overall Principal Investigator for this research) and the University of Melbourne (led by Prof Ary Hoffmann). Following extensive community engagement regarding the intervention (known as '*Wolbachia* Malaysia'), *w*AlbB-carrying *Ae. aegypti* were released at six residential sites in Greater Kuala Lumpur characterised by hyperendemic (high transmission) dengue [3.3]. Control sites (i.e. with no releases) were matched to the release sites for size, building type and dengue transmission rate. The UofG *Wolbachia* strain was maintained at high frequency within the mosquito population even after releases had stopped. Malaysian case reporting data indicated that the incidence of dengue was reduced at all release sites versus the control sites (40% to 80% reduction in cases) [3.3]. The Malaysian trial was the first to use *w*AlbB in the field for blocking dengue transmission, confirming the utility of this strain. It was also the first reported demonstration of the efficacy of a *Wolbachia*-based intervention for dengue control in a hyperendemic setting. The six release sites in Great Kuala Lumpur had all previously been classified as dengue hotspots, but they were no longer classified as such after the *Wolbachia* Malaysia intervention.

3. References to the research

1. Geoghegan V, Stainton K, […], **Sinkins SP** (2017) Perturbed cholesterol and vesicular trafficking associated with dengue blocking in Wolbachia-infected Aedes aegypti cells. Nat Commun;8:526 (doi:10.1038/s41467-017-00610-8).
2. Ant TH, Herd CS, Geoghegan V, Hoffmann AA, **Sinkins SP** (2018) The Wolbachia strain wAu provides highly efficient virus transmission blocking in Aedes aegypti. PLoS Pathog;14(1):e1006815 (doi:10.1371/journal.ppat.1006815).
3. Nazni WA, Hoffmann AA, […], **Sinkins SP** (2019) Establishment of *Wolbachia* strain *w*AlbB in Malaysian populations of *Aedes aegypti* for dengue control. Curr Biol;29(24):4241–4248.e5 (doi:10.1016/j.cub.2019.11.007). Publication of this paper was accompanied by an editorial: doi:10.1016/j.cub.2019.11.046.

Grants

- Sinkins SP (PI): Wellcome Trust Translational Award '*Wolbachia*-based control of virus transmission by the *Aedes* mosquitoes' (2016–2021; GBP1,884,251).
- Sinkins SP (PI): Wellcome Trust Senior Research Fellowship in Basic Biomedical Science (second renewal) '*Wolbachia*-mediated arbovirus inhibition in mosquitoes' (2016–2021; GBP1,180,019).

4. Details of the impact

Dengue mainly occurs in urban and semi-urban areas of tropical and sub-tropical countries. Infection is characterised by fever and a variety of other symptoms (e.g. headache, joint pain, muscle pain). Although symptoms usually resolve within a few days, some patients develop severe dengue associated with haemorrhage or shock syndrome, which requires urgent medical attention and can be fatal. A sharp rise in the global incidence of dengue has been noted by WHO over the past few decades, with approximately 80–100 million clinically apparent cases annually.

Dengue is endemic in Malaysia and so exerts considerable burden on public health and the economy. For example, 101,357 cases of dengue and 237 dengue-associated deaths were reported during 2016. To address this problem, UofG researcher **Prof Steven Sinkins** partnered with Malaysian and University of Melbourne colleagues to develop and pilot the use of *Ae. aegypti* carrying the heat-resistant *Wolbachia* strain *w*AlbB for biological control of dengue in hot climates [3.2, 3.3]. The impacts of this research were two-fold. First, the success of the Greater Kuala Lumpur field trial [3.3] in reducing dengue transmission in six sites (over 40,000 residents) convinced the Malaysian Ministry of Health to expand this approach to additional dengue hotspots. Second, other countries affected by dengue, including Cambodia, the Maldives and Paraguay, have consulted on trialling the *Wolbachia* line.

The Wolbachia Malaysia intervention drives a paradigm shift in the national strategy to reduce transmission of dengue

Malaysia has a population of approximately 32 million, with 76.6% resident in urbanised areas such as Greater Kuala Lumpur (population: 7.6 million). Kuala Lumpur is the capital of Malaysia and currently ranks as one of the most rapidly growing cites in Southeast Asia, both in terms of population and economic development. Many residents live in high-rise complexes that are characterised by high densities of people (e.g. approximately 15,000 in the Mentari Court complex) and *Ae. aegypti* (which is detected even on the upper levels of these buildings). Consequently, this mode of city living has created hotspots for dengue transmission. *Ae. aegypti* bites during the daytime, which precludes use of certain vector control measures (e.g. bed nets). The Malaysian Ministry of Health had adopted a strategy of insecticidal spraying ('fogging') and encouraging removal of rubbish to limit breeding sites. Yet, despite the high financial cost involved, this approach had limited effect on the rate of dengue transmission. An urgent need therefore existed for innovation in vector control.

In 2016, Sinkins approached the IMR to develop a collaboration and obtained Wellcome Trust funding to launch the '*Wolbachia* Malaysia' project [5.A, 5.B]. The IMR Director highlighted heat stability of the UofG *w*AlbB strain as "*an important consideration in the decision to deploy it in our release trials—mosquito lines carrying other Wolbachia strains that were generated and used elsewhere are much less effective at the temperatures experienced in Malaysia*" [5.A].

The results of the Greater Kuala Lumpur field trial [3.3] encouraged the Malaysian Ministry of Health to upscale the UofG vector control approach [5.C]. In May 2019, the ministry convened an International Expert Consultation Workshop on the release of *Wolbachia*-infected *Ae. aegypti* as a novel tool for dengue control and prevention across the country. As Principal Investigator of the field trial [3.3], Sinkins was invited to attend this workshop, along with other global experts in vector control (e.g. the WHO vector-borne diseases co-ordinator for the Western Pacific region) [5.C]. The workshop reviewed the results of dengue control trials conducted so far using the UofG *Wolbachia* line, and informed the decision taken by the Malaysian Government to roll-out the intervention nationally, while concurrently disinvesting in the use of fogging as a means of vector control [5.C]. Since July 2019, *Wolbachia* releases have been conducted at 19 additional 'operational' sites in Selangor, Greater Kuala Lumpur, Putrajaya and Penang Island, meaning that to date over 140,000 people in Malaysia are now living in *Wolbachia*-protected areas that were previously designated as dengue hotspots [5.C]. A representative of the Ministry of Health stated: "*There have been major health benefits associated with this roll-out of the Wolbachia intervention. While the further programme of roll-out has been somewhat delayed by the COVID-19 pandemic during this period, nevertheless, there have been some promising results to date, with strong dengue reductions seen in the intervention sites and thousands of dengue cases averted*" [5.C]. The success of the programme was also highlighted by the international media [5.D].

Primary benefits of the Wolbachia Malaysia intervention

Reducing the number of dengue cases

With no specific treatment or proven safe and effective vaccine available, the UofG dengue control approach reduces virus transmission and so is limiting outbreaks of dengue [5.A, 5.C]. Data collected by the Malaysian National Dengue Surveillance System indicated that there were 2,341 confirmed dengue cases in four of the *Wolbachia* Malaysia release sites in Greater Kuala Lumpur during the 3-year period prior to the start of the intervention; however, there were 793 dengue cases in the 3-year period after the start of the intervention, a 67% reduction in cases [database not publicly available]. By contrast, case numbers in the control sites for the same time periods before and after the start *Wolbachia* Malaysia were very similar. Comparable reductions in dengue case numbers are being seen in the expanded operational sites to date, and thus the UofG strategy of targeting dengue hotspots in areas of high population density is substantially reducing the national rate of infection [5.A, 5.C].

In addition, *Ae. aegypti* is the primary vector for transmission of Zika virus and chikungunya virus, both of which cause intermittent outbreaks in Malaysia. The UofG *Wolbachia*-carrying

Ae. aegypti line has been shown to block transmission of these viruses [3.2]; therefore, an additional benefit of the *Wolbachia* Malaysia intervention is that nationwide release will help to futureproof the country against epidemics of Zika and chikungunya [5.A–5.C].

Efficient use of financial and human resources
Dengue exerts a large economic burden in Malaysia, with the Ministry of Health spending approximately USD80 million annually on dengue vector mosquito control (primarily insecticidal fogging). The UofG strategy of weekly releases over a short period of time resulted in a very high frequency of *Wolbachia* in the local mosquito population that remained stable after releases stopped [3.3], thereby offering a sustainable and cost-effective method of long-term vector control. By contrast, another *Wolbachia*-based strategy uses continuous release of only male *Wolbachia* mosquitoes for suppression, which represents a large recurring expense. UofG took the decision not to commercialise the intervention so as to maximise uptake and impact in the low-income and middle-income countries (LMICs) where dengue primarily occurs. Consequently, a no-cost licence arrangement was agreed with the Malaysian Ministry of Health, with the same terms applying to other LMICs (see below). According to the Deputy Director of General Health (Research and Technical Support), the fact that UofG provided the *Wolbachia Ae. Aegypti* line without cost was "*an important consideration in our decision to roll out this programme*" [5.C]. The Malaysian Government has moved away from fogging in the *Wolbachia* intervention areas, and has invested in infrastructure (e.g. a facility for mass rearing female mosquitoes and eggs infected with *Wolbachia*). The *Wolbachia* Malaysia programme also safeguards jobs by redeploying existing staff (e.g. spraying teams and community health officers).

Changing public attitudes to dengue control
A key factor to the success of *Wolbachia* Malaysia was understanding and acceptance of the intervention by the local community. Public engagement was led by the Malaysian Institute for Health Behavioral Research and took the WHO 'communication for behavioural impact' approach to dengue control, which has been used by the Ministry of Health since 2001. Activities to promote changes in attitude and behaviour included educational talks; distribution of flyers; stakeholder meeting and briefing sessions; and development of the *Wolbachia* Malaysia website [5.B]. As a result of these efforts, the acceptance rate among residents was high (>95%) [3.3]. The fact that *Wolbachia* Malaysia does not involve release of genetically modified mosquitoes also helped acceptance of the approach. By contrast, some public and media opposition occurred in Malaysia when biotechnology company Oxitec previously trialled releases of its genetically modified strain of *Ae. aegypti*.

Secondary benefits of the Wolbachia Malaysia intervention
Improving quality of life for residents
Fogging involves regular release of insecticide as fine droplets dispersed from a large and noisy machine that is transported around neighbourhoods on open trucks. During fogging, which tends to occur in the early morning or late afternoon, residents are requested to leave all doors and windows open so the fog can kill any mosquitoes within properties. Adverse health effects of this method of vector control are thought to be low; however, the physical process of fogging can affect quality of life. As *Wolbachia* Malaysia removes the need for regular fogging, quality of life has improved for people living in dengue hotspots [5.C].

Protecting the environment
Fogging is also implicated in negative effects on the environment. For example, it can give rise to insecticide-resistant mosquitoes and affect non-target insects such as bees, and their predators such as birds and bats. The *Wolbachia* Malaysia programme protects the environment by reducing the need for fogging [5.C]. In addition, this strategy uses mosquitoes that are of a species already prevalent in the target area, and avoids the release of genetically modified organisms that could impact non-target species (*Wolbachia* is not infectiously transmitted between insects, only inherited).

Roll-out of the UofG vector control strategy to other LMICs

Plans for deployment of the UofG *Wolbachia* strain of *Ae. aegypti* have been developed in association with Health Ministries, and other institutes/agencies, in Cambodia [5.E], the Maldives [5.F] and Paraguay [5.G], albeit with disruptions owing to the COVID-19 pandemic. In addition, material transfer agreements have been issued by UofG to institutes/agencies in Thailand, Saudi Arabia and Burkina Faso.

The Director of the National Center for Parasitology, Entomology and Malaria Control highlights the rationale for taking this approach to dengue control in Cambodia: "*We were aware of the emphasis placed by Prof Sinkins on local leadership and the importance of local expertise to the success of Wolbachia interventions, and this fitted with our model of working. Wolbachia has great potential to help us reduce the incidence of this disease in a cost-effective, sustainable and environmentally low-impact manner*" [5.E]. In January 2020, the Maldives Ministry of Health and WHO Maldives invited Sinkins to attend a situation assessment in Malé on the use of *Wolbachia Ae. aegypti* to prevent transmission of dengue [5.F]. This meeting resulted in consensus to run a pilot study, with WHO Maldives working in partnership with Maldives National University to develop new insectary and molecular screening infrastructure. These activities were delayed by COVID-19, but the Ministry of Health plans to move forward with this programme in 2021 [5.F]. The Acting Representative of WHO Maldives states: "*A successful long-term dengue control programme will have great benefits for the Maldives. It will also have positive impacts on the environment through reductions in insecticide fogging, and for tourism, which is important for the economy*" [5.F]. To address high numbers of dengue cases in Paraguay, the Ministry of Health formed a Technical Committee—involving Sinkins and other partner organisations—to implement a pilot study of the *Wolbachia* intervention [5.G]. Sinkins is providing the *Wolbachia* technology, as well as technical support and guidance, during this project.

5. Sources to corroborate the impact

PDFs uploaded for all listed items.

A. Testimonial from the Director of the IMR regarding (1) the relationship with UofG; (2) the decision to use the UofG model of dengue vector control; (3) the role of UofG researchers in the *Wolbachia* Malaysia field trial in Greater Kuala Lumpur; (4) roll-out to additional sites within Malaysia; (5) futureproofing Malaysia against epidemics caused by Zika and chikungunya viruses.
B. The *Wolbachia* Malaysia website provides information on all aspects of the programme, including the vector control strategy and public awareness campaigns. UofG is credited as a collaborating institution on the landing page.
C. Testimonial from the Deputy Director General of Health (Research and Technical Support), Malaysian Ministry of Health, regarding (1) Sinkins' role in the Expert Consultation Workshop; (2) the purpose of this meeting; (3) the change in national strategy for dengue control; (4) benefits of this approach.
D. Examples of media coverage of the Kuala Lumpur field study [3.3]: BBC News (November 2019) and Sky News (January 2020).
E. Testimonial from the Director of the National Center for Parasitology, Entomology and Malaria Control, Cambodia, to (1) confirm the relationship with UofG; (2) outline the decision to use the UofG model of dengue vector control.
F. Testimonial from Acting WHO Representative, WHO Maldives, to (1) confirm the relationship with UofG; (2) outline the decision to use the UofG model of dengue vector control.
G. Testimonial from the Minister of Public Health and Social Wellbeing, Paraguay, to (1) confirm the relationship with UofG; (2) outline the decision to use the UofG model of dengue vector control.