The re-emerging arboviral threat: Hidden enemies

The emergence of obscure arboviral diseases, and the potential use of *Wolbachia* in their control

*Heverton Leandro Carneiro Dutra, Eric Pearce Caragata and Luciano Andrade Moreira**

Mayaro, Oropouche, and O'Nyong-Nyong share many traits with more prominent arboviruses, like dengue and yellow fever, chikungunya, and Zika. These include severe clinical symptoms, multiple animal hosts, and widespread vector species living in close proximity to human habitats, all of which constitute significant risk factors for more frequent outbreaks in the future, greatly increasing the potential of these hidden enemies to follow Zika and become the next wave of global arboviral threats. Critically, the current dearth of knowledge on these arboviruses might impede the success of future control efforts, including the potential application of *Wolbachia pipientis*. This bacterium inherently possesses broad anti-pathogen properties and a means of genetic drive that allows it to eliminate or replace target vector populations. We conclude that control of obscure arboviruses with *Wolbachia* might be possible, but successful implementation will be critically dependent on the ability to transinfect key vector species.

Keywords:
chikungunya; dengue; Mayaro; O'Nyong-Nyong; Oropouche; *Wolbachia*; Zika

DOI 10.1002/bies.201600175

Mosquitos Vetores: Endossimbiontes e Interação Patógeno-Vetor, Centro de Pesquisas René Rachou – Fiocruz, Belo Horizonte, MG, Brazil

***Corresponding author**
Luciano A. Moreira
E-mail: luciano@cpqrr.fiocruz.br

Abbreviations:
CHIKV, chikungunya virus; **DENV,** dengue virus; **IIT,** the incompatible insect technique; **JEV,** Japanese encephalitis virus; **MAYV,** Mayaro virus; **ONNV,** O'Nyong-Nyong virus; **OROV,** Oropouche virus; **WNV,** West Nile virus; **YFV,** yellow fever virus; **ZIKV,** Zika virus.

Introduction

The recent emergence of Zika virus (ZIKV) [1], and its associated developmental and neurological effects caught the world by surprise [2]. Yet, there are many notable examples of little known arthropod-transmitted viral pathogens rapidly transitioning into major health threats. This process has been facilitated by changes in environmental conditions, human behavior, viral genetics, and arthropod vector populations, leading to perturbations of the virus transmission cycle, and greater incidence of human outbreaks [3]. A case can be made that ZIKV is only the latest example of a poorly understood and neglected tropical disease that has rapidly spread across the globe with serious consequences.

Historically, the most serious vector-borne disease has been malaria, but more than a decade of concentrated effort, driven by expanded vector control programs, has finally seen case numbers decline (see World Health Organization (WHO) Malaria Report 2015: http://tinyurl.com/joc38e3 [accessed October 19, 2016]). Simultaneously, there has been resurgence in arboviral infections [4], with viruses such as dengue (DENV), chikungunya (CHIKV), West Nile (WNV), Japanese encephalitis (JEV), and yellow fever (YFV) still representing serious threats to global health. For this reason, the vast majority of arboviral research has focused on these current threats, and not on those that might arise in the future.

In this review, we will examine the historical factors underlying the emergence of major arboviruses including the recent emergence of ZIKV, and consider the similarities with three currently obscure arboviruses; Mayaro (MAYV), Oropouche (OROV), and O'Nyong-Nyong (ONNV) (Table 1), which could potentially emerge as future threats to human health. Finally, we will end the review by exploring the potential use of the endosymbiotic bacterium *Wolbachia pipientis* to combat current and emerging arboviral threats.

Table 1. Medically important arboviruses discussed in this review and their characteristics

Family/ virus	Year of first formal viral isolation	Place of isolation	Main sylvatic/endemic vector(s)	Main epidemic vector(s)	Main vertebrate hosts (reservoirs)	Main transmission cycle	Geographic distribution	Genome size (Kb)
Flaviviridae								
Dengue 1–4	1943 (DENV-1)	Asia	*Aedes spp.*	*Aedes aegypti* *Aedes albopictus*	Humans, other primates	Urban	Tropics worldwide	~10
Japanese encephalitis	1924	Asia	*Culex spp.*	*Culex tritaeniorhynchus*	Birds	Suburban	Asia, The Pacific	~11
West Nile	1937	Africa	*Culex spp.*	*Culex spp.*	Humans, birds, horses	Urban	Worldwide	~11
Yellow fever	1927	Africa	*Aedes spp.* *Haemagogus spp.* *Sabethes spp.*	*Aedes aegypti*	Non-human primates	Sylvatic	Africa, South America	~11
Zika	1947	Africa	*Aedes spp.*	*Aedes aegypti*	Humans, other primates	Urban	Tropics worldwide	~11
Togaviridae								
Chikungunya	1952–1953	Africa	*Aedes spp.*	*Aedes aegypti* *Aedes albopictus*	Humans, other primates	Urban	Tropics worldwide	~12
Mayaro	1954	South America	*Haemagogus spp.*	*Haemagogus spp.*	Humans, birds	Sylvatic	South America	~11.5
O'Nyong-Nyong	1959	Africa	*Anopheles spp.*	*Anopheles spp.*	Unknown	Sylvatic	Africa	~12
Bunyaviridae								
Oropouche	1955	South America	*Culicoides paraensis*	*Culicoides paraensis*	Unknown	Sylvatic	Central and South America	~12

Arboviral disease emergence follows a clear pattern

Arthropod-borne viruses, or arboviruses, are predominantly RNA viruses (with the exception of the DNA-based African swine fever virus [5]), mainly belonging to the families *Flaviviridae*, *Togaviridae*, and *Bunyaviridae* [6]. Arboviruses are maintained in nature through a complex biological transmission cycle between vertebrate hosts and hematophagous (blood feeding) arthropod vectors including mosquitoes, sandflies, ticks, and kissing bugs. Transmission occurs predominantly through vector saliva during bites, but there is some evidence of transmission from mother to progeny [7], or venereal transmission during mating [8], although the epidemiological impact is controversial [7].

Most arboviral transmission occurs among wild animals (sylvatic transmission cycle) while human infections occur accidentally during "spillover" events, either directly, or following infection in domestic animals [9]. These events are typically rare, but can become more frequent due to changes in transmission efficiencies in some vector species due to rapid evolution in RNA viruses [10], changing vector population dynamics [11], climate change [12], deforestation [13], and the increased frequency of international travel [14], among others. This increases the risk that transmission of that virus shifts from sylvatic to anthroponotic (human transmission cycle), which heightens the potential for major outbreaks, and this pattern has occurred for many of the current arboviral threats.

The old arboviruses are not going away

DENV and YFV have a long association with humans

Cases of DENV were reported as early as the 18th century, while infections of YFV date back to the 16th century [6, 15]. Both viruses originated in Africa, but were introduced to other regions starting in the colonial period [6, 16]. Both viruses have multiple genetic lineages. In the case of DENV, four genetically distinct serotypes, and for YFV, five distinct lineages, each with different geographical distributions (see [17] for a detailed review).

There is evidence that the transmission of both viruses was once primarily sylvatic, although DENV transmission has now shifted to be primarily anthroponotic through the primary vector *Ae. aegypti* [12], which has a close association with humans, and through *Ae. albopictus*, an aggressive, invasive mosquito species. For YFV, the situation is slightly more complicated; the primary transmission cycle still occurs in non-human primates, with human transmission the result of frequent spillover events. Urban transmission is dominant in Africa through *Ae. aegypti*, but rare in Latin America where the dominant vectors are *Haemogogus* mosquitoes, which are not well urbanized [6, 17, 18]. Both viruses have seen a recent resurgence in cases, incited by changes in environmental conditions affecting the prevalence of vectors [19], increased urbanization in disease endemic areas providing better access to hosts, and recent changes to viral genetics [20].

DENV remains the most serious arboviral threat, with high levels of endemic transmission occurring in the Americas, South and Southeast Asia, and the Western Pacific [4], with nearly 4 billion people living in areas of endemic transmission [21]. Each year there are an estimated 100 million symptomatic cases, and $8–9 billion (USD) is spent on treatment and control, which makes the disease burden quite significant [22]. A dengue vaccine was first licensed only in 2015 [23], which meant that disease control has historically depended on mosquito control, and while effective, these programs must be continually maintained to suppress transmission [16]. Brazil has been severely affected by DENV in recent years, with around 1.4 million suspected cases in the first 32 weeks of 2016 (Epidemiological Alert issued by the Pan American Health Organization (PAHO)/WHO, October, 2016: http://tinyurl.com/hfpjnqu [accessed October 19, 2016]). See [24] for an extensive review of dengue in the Americas.

YFV remains a serious problem in Africa [25] and Latin America (see [17] for an in deep review of YFV epidemiology), and the fact that transmission still occurs at such high rates is particularly galling given that effective vaccines have been available for more than 70 years [26] (see [27] for a detailed review on YFV vaccine development). High YFV case rates likely occur due to low vaccination rates in areas of endemic transmission [17], which highlights the difficulties associated with combating arboviruses even when effective tools are available.

JEV and WNV still maintain strong enzootic transmission cycles

The case of YFV indicates that arboviruses that maintain strong enzootic transmission cycles can still cause serious disease outbreaks in humans. Two further examples of this pattern are WNV and JEV, which were both first isolated in the early 20th Century [28]. WNV transmission relies on both enzootic and anthroponotic transmission, with the former occurring in a wide range of vertebrates, including birds and horses. Birds are important WNV hosts, some are highly susceptible to infection, and others serve as asymptomatic carriers, while bird-to-bird transmission has also been observed [29–31]. The primary WNV vectors are mosquitoes from the genus *Culex* [30, 32], although many others mosquitoes may be capable vectors (Centers for Disease Control and Prevention (CDC) report on WNV detection in mosquitoes: http://tinyurl.com/zgqowf8 [accessed October 19, 2016]). The widespread prevalence of multiple host and vector species in urban environments is likely an important factor in WNV outbreaks.

For the first 50 years after its discovery, WNV was only associated with infrequent outbreaks involving mild disease in rural areas of Africa, the Middle East, Asia, and Europe [6]. The geographic distribution of cases expanded in the 1990s, and a severe, neuroinvasive form of the disease was characterized (see [33] for a detailed review on clinical manifestations of WNV infection). During this time, the virus moved to the USA [30], and by 2004 it was circulating in 48 states, and had spread to many other countries in the region [30]. Between 1999 and 2015 there were a reported 43,937 cases of WNV in the United States, with almost 2,000 deaths (CDC – WNV cases in the USA: http://tinyurl.com/gpduheo [accessed October 19, 2016]), with these outbreaks linked to the emergence of a new viral genotype [30]. Currently, WNV vaccines are only available for horses, although human vaccines are under development [34].

Like WNV, JEV is primarily transmitted by *Culex* mosquitoes, and has a stable, diverse enzootic transmission cycle, in farm animals, and aquatic birds [35, 36]. The virus was first isolated from a human brain in the 1920s, and is now known to be the most common cause of mosquito-borne encephalitis. There are 3 billion people living at risk in Asia and the Pacific, and an estimated 50,000 symptomatic cases and 10,000 deaths occur annually. As an estimated 99% of JEV cases are asymptomatic, anthroponotic transmission is likely extensive [36–38].

JEV transmission is either epidemic or endemic depending on the location, with epidemics in China, Nepal, and India driven by seasonal effects, and sporadic, but persistent cases occurring in places like Thailand and Indonesia [36]. The risk that JEV becomes a greater threat in the near future is quite high, given the genetic diversity of the virus [12], and the fact that several *Aedes* species are marginally competent vectors [39, 40]. Despite the availability of several effective vaccines [34], JEV remains a persistent threat to human health, with the majority of infections occurring in children, and in areas with established vaccination programs [38].

CHIKV and ZIKV emerged rapidly

CHIKV, which was first isolated in Tanzania in 1952–53, can cause serious illness and chronic health problems. The disease was named after a local word that translates as “that which bends up” due to bent postures induced by extreme pain during infection [41]. CHIKV initially circulated enzootically among non-human primates, with multiple arboreal mosquitoes of the genus *Aedes* implicated as vectors [42]. Transmission to humans was first observed in Asia, with *Ae. aegypti* identified as a major human vector [43].

The first major outbreak occurred in Kenya during 2004, and this involved about 14,000 cases [44]. The virus rapidly spread to surrounding areas in subsequent months, causing an outbreak in Mozambique that was initially misdiagnosed as dengue [45]. A large outbreak of 250,000 cases took place on La Reunion Island in 2005–06, which was surprising given the small size of the local *Ae. aegypti* population [46]. This outbreak was associated with a new CHIKV lineage, with mutations in the viral envelope glycoproteins enabling more effective transmission by *Ae. albopictus* [47]. This new lineage spread quickly to previously inaccessible locations [19], and caused large outbreaks in areas that had previously seen only sporadic cases [48].

CHIKV has recently become a matter of great concern in the Americas. The virus was introduced to the area in 2013,

and quickly became endemic given the high prevalence of vector populations [49]. In 2016, there have been nearly 200,000 suspected cases across Latin America (PAHO/WHO epidemiological alert, October 7, 2016: http://tinyurl.com/ztyln9m [accessed October 19, 2016]). The transition of CHIKV outbreaks from sporadic and low-level, to severe and widespread in just over a decade highlights the speed at which arboviruses can rise from obscurity to threaten human health.

The recent emergence of ZIKV shows many similarities with that of CHIKV. The virus was initially isolated in rhesus monkeys, in the Zika forest in Uganda in 1947, as part of an effort to identify naturally occurring YFV [50]. The following year, the same virus was found infecting *Ae. africanus* mosquitoes [51]. ZIKV was thought to circulate sylvatically among primates, as rhesus monkeys became infected after inoculation with macerated, infected mosquitoes [50].

ZIKV was first detected in humans in Nigeria in 1954 [52]. Serosurveillance studies in the 1970s revealed that infections were fairly prevalent with about 40% of the Nigerian population possessing neutralizing antibodies [53]. The virus was first detected outside of Africa in *Ae. aegypti* in 1969 in Malaysia [54], and in humans in Indonesia in the late 1970s [55]. Nevertheless, it was a further 30 years before the first major outbreak occurred, in the Micronesian Yap Islands [56], initially believed to be an outbreak of dengue [57]. Serological surveillance indicated that 70–80% of tested individuals were positive for ZIKV infection, but only 18–40% were symptomatic [58]. While there were no instances of severe complications, this did represent an increase in viral infectivity. In 2013, there was a second major ZIKV outbreak, this time in French Polynesia, where there were 30,000 suspected cases, affecting 50–66% of the population [59]. This represented the first association of ZIKV with increased prevalence of the autoimmune disorder Guillain-Barré syndrome [60].

The largest ZIKV outbreak to date began in Brazil at the end of 2014 [61], and by the end of 2015, there had been an estimated 1.3 million cases (PAHO/WHO epidemiological alert, December 1, 2015: http://tinyurl.com/hb3hscb [accessed: October 19, 2016]). More concerning was the association of ZIKV infection during pregnancy with increased occurrence of microcephaly [62], with more than 2,000 cases associated with the outbreak as of October 2016 (Epidemiological Alert issued by the PAHO/WHO, October, 2016: http://tinyurl.com/hovggl6 [accessed October 19, 2016]), and 2,000 confirmed cases of Guillain-Barré [63]. Current estimates suggest that there is a 1–13% chance of microcephaly associated with ZIKV infection during the first trimester of pregnancy [64].

As of May 2016, 47 countries and territories in the Americas, included the United States, have reported autochthonous cases of ZIKV (map of ZIKV infections: http://tinyurl.com/z7q6l43 [accessed October 19, 2016]) [65]. While there are predictions that the outbreak will burn itself out over the next few years [66], ZIKV will likely remain a serious and life-threatening problem in the short term. For a detailed review on ZIKV spread worldwide, refer to [58].

Obscure arboviruses with global epidemiological potential

Mayaro virus is already endemic to Latin America

MAYV was firstly isolated in 1954, from the serum of febrile patients in Trinidad [67]. There are two main genetic lineages: the widespread D, and the less common L [68]. Infection produces indistinguishable symptoms to the closely related CHIKV [69], and these can last for over a year [70]. Autochthonous MAYV transmission has since been detected across Latin America [71, 72, 73], (see CDC arbovirus catalog: http://tinyurl.com/jjo8fs2 [accessed October 19, 2016]), but the disease remains poorly understood [73]. Cases are not reported frequently, and this might be due to the high degree of co-circulation with dengue and other similar viruses [12]. An estimated 1% of all febrile dengue-like illness in northern South America is caused by MAYV [74], as evidenced by the high rates of detection during serosurveillance in the region [75]. This would suggest that there might be tens of thousands of cases per year, and given the issues surrounding monitoring and detection of obscure arboviruses, many of this will be misdiagnosed, or go undiagnosed [74].

The transmission cycle of MAYV resembles the sylvatic transmission of YFV, with non-human primates acting as the main reservoirs. However, there is a wider range of potential hosts, as MAYV has been isolated in birds [76], and neutralizing antibodies have been detected in rodents, sloths, lizards, marsupials, and horses. Likewise, there appears to be a broad and complex range of potential vectors. The primary vectors are likely mosquitoes from the genus *Haemagogus* [12], however, MAYV has also been detected in *Gigantolaelaps* mites, and *Sabethes*, *Psorophora*, and *Mansonia* mosquitoes (see CDC, MAYV natural host range: http://tinyurl.com/zssdfmf [accessed October 19, 2016]) [77].

A major point of concern is that MAYV has also been detected in the field in two of the most abundant mosquito genera: *Culex* and *Aedes* [78] (http://tinyurl.com/zssdfmf). Experimental evidence suggests that the virus is highly infectious to *Ae. aegypti* upon artificial oral infection, and that they are capable of transmitting the virus to rodents at a high rate [79]. Similarly, MAYV can replicate in *Ae. albopictus* cell lines [80], and live mosquitoes have been shown to transmit the virus at low rates after experimental infection, making that species a potential secondary vector [81].

Historically, MAYV outbreaks have been sporadic, however, spillover events have occurred following deforestation, and increased tourism to endemic areas, both of which bring the virus into closer proximity to larger human populations, and to their associated urban vectors [12, 72]. Given the close genetic relationship with CHIKV [82], it is plausible that MAYV could also evolve to become more infectious to humans or anthropophilic mosquitoes, and experience similarly high levels of outbreaks. Fortunately, there is a promising vaccine candidate under development [83], and while it may take some time to become commercially available, this may eventuate before MAYV ever becomes a serious threat. Nonetheless, the potential impact of MAYV is not something that should be dismissed out of hand, and further research

and surveillance are essential to ensure that it does not become the next Zika.

Oropouche virus is still poorly understood

OROV is a pathogen of great epidemiological importance, which causes an acute febrile illness that can progress into meningitis [84]. OROV was first isolated in 1955 in Trinidad, from foresters, and from *Coquillettidia venezuelensis* mosquitoes [85]. In addition to humans, OROV has been isolated from sloths [86], while neutralizing antibodies have been detected in birds, monkeys, and rodents (see CDC arbovirus catalog: http://tinyurl.com/z7zxmhy [accessed October 19, 2016]). The virus is likely endemic to many areas in Central and South America [87], with the majority of reported cases occurring in the Brazilian Amazon, and central plateau [88]. There are three OROV genotypes, although it is unclear if any of these are of greater epidemiological importance [89].

OROV is maintained through both urban and sylvatic transmission cycles, and potentially has a wide range of associated vectors. The primary vector is likely the biting midge *Culicoides paraensis*, which has a wide distribution from Argentina to the northern United States [90]. The virus has also been isolated from potential secondary vectors including the mosquitoes *Aedes serratus*, *Ochlerotatus serratus* [86], *Co. venezuelensis* [85], and *Cx. quinquefasciatus* [91], which is a competent vector if challenged with a high viral titer [92]. *Ae. albopictus* is unlikely to be a vector, as it cannot transmit or sustain infection with the virus at high rates [81]. To our knowledge, there have been no published studies on the potential of *Ae. aegypti* as a vector, which is an important deficit to address.

An estimated 500,000 cases have been reported in the last 50 years across Latin America [89]. In a recent serosurveillance assay from 2007 to 2008, OROV neutralizing antibodies were detected in nearly 21% of 631 residents from the city of Manaus, in the Brazilian Amazon [93]. Similar antibody levels were detected in studies in Iquitos, Peru from 1992 in both urban and rural populations [94], suggesting that the virus is quite pervasive across the region [91].

While large-scale OROV outbreaks have not yet been seen, the widespread distribution of potential vectors such as *Cx. quinquefasciatus* mosquitoes and *Culicoides* midges is a significant risk factor [95]. Yet critically, for a disease that is both widely prevalent and that can have severe symptoms, there is much about OROV that remains a mystery, as evidenced by the dearth of published studies on the virus, and the lack of vaccine candidates in development.

O'Nyong-Nyong virus is a CHIKV-like virus endemic to Africa

O'Nyong-Nyong and CHIKV share similar symptoms, are difficult to distinguish clinically [69], and are highly similar genetically, to the point where it was previously thought that ONNV was a genetic subtype of CHIKV [96]. More recent evidence suggests that the two viruses actually diverged several thousand years ago [97]. Nevertheless, there is typically cross-reactivity observed between the two viruses in antibody-based serological assays, which hinders detection [98].

ONNV is unique among the *Alphavirus* genus as it is primarily transmitted by anopheline mosquitoes, with prominent vectors including *An. gambiae* and *An. funestus* [99]. There are suggestions that *Ae. aegypti* may play some role in transmission, with moderate disseminated infection rates observed after experimental infection [96]. The virus has no described sylvatic transmission cycle, with humans being the only known natural host [97].

ONNV was first isolated from human serum, and in anopheline mosquitoes in Uganda in 1959 [100]. Subsequent epidemics have been large-scale, but sporadic and restricted to Africa (see CDC arbovirus catalog: http://tinyurl.com/jtex8gt [accessed October 19, 2016]). The first of these occurred between 1959 and 1962, with 2 million identified cases occurring across Uganda, Mozambique, and Senegal, but no reported fatalities [101]. The virus was regularly detected during serological surveillance until 1969, but then went undetected for 35 years, when there was a large outbreak in southern Uganda [102]. Antibodies to ONNV are still detected at high rates across Africa during serosurveillance [103]. To date, there has been only one case of imported ONNV reported outside of Africa [104].

As is the case for many pathogens of moderate epidemiological importance, there have been few scientific studies on ONNV [105]. Furthermore, given the issues with misdiagnosis, and likely under-reporting of cases, the extent to which ONNV actually impacts human health is unclear. ONNV's close relationship with CHIKV could be beneficial for potential treatments, as preliminary data indicate that a CHIKV vaccine candidate also offers protection against ONNV [106]. It is also relevant to say that there have been no studies that have examined the potential for pre-exposure with an alphavirus to enhance infection with ONNV, or vice versa. Given their genetic similarity, there is also a risk that ONNV could follow the same path as CHIKV and quickly emerge as a severe threat [97].

As with OROV, the scarcity of vector competence data means there is uncertainty about the distribution of potential vectors, and how well ONNV could spread. Certainly, the distribution of *An. gambiae*, and *An. funestus* implies that outbreaks could occur across Africa, and if other anophelines prove to be suitable vectors, there is potential to affect billions of people [107]. Likewise, potential viral genetic changes that promote more effective infection in *Ae. aegypti* could also lead to large-scale outbreaks, given the pervasiveness of that species [19]. Given the fact that a large part of the world's population will have had contact with that mosquito, there is a clear risk that ONNV infection could be far more common in the future.

MAYV, OROV, and ONNV are perhaps the most prominent of the future arboviral threats (Fig. 1), but they are by no means the only viruses with that potential. Tropical forests with rich biodiversity, such as the Amazon, represent breeding grounds for future arboviral emergence, and could potentially shelter hundreds of undescribed arboviruses [108]. These may regularly cause infection in humans, but remain uncharacterized due to their clinical similarity to better-known pathogens.

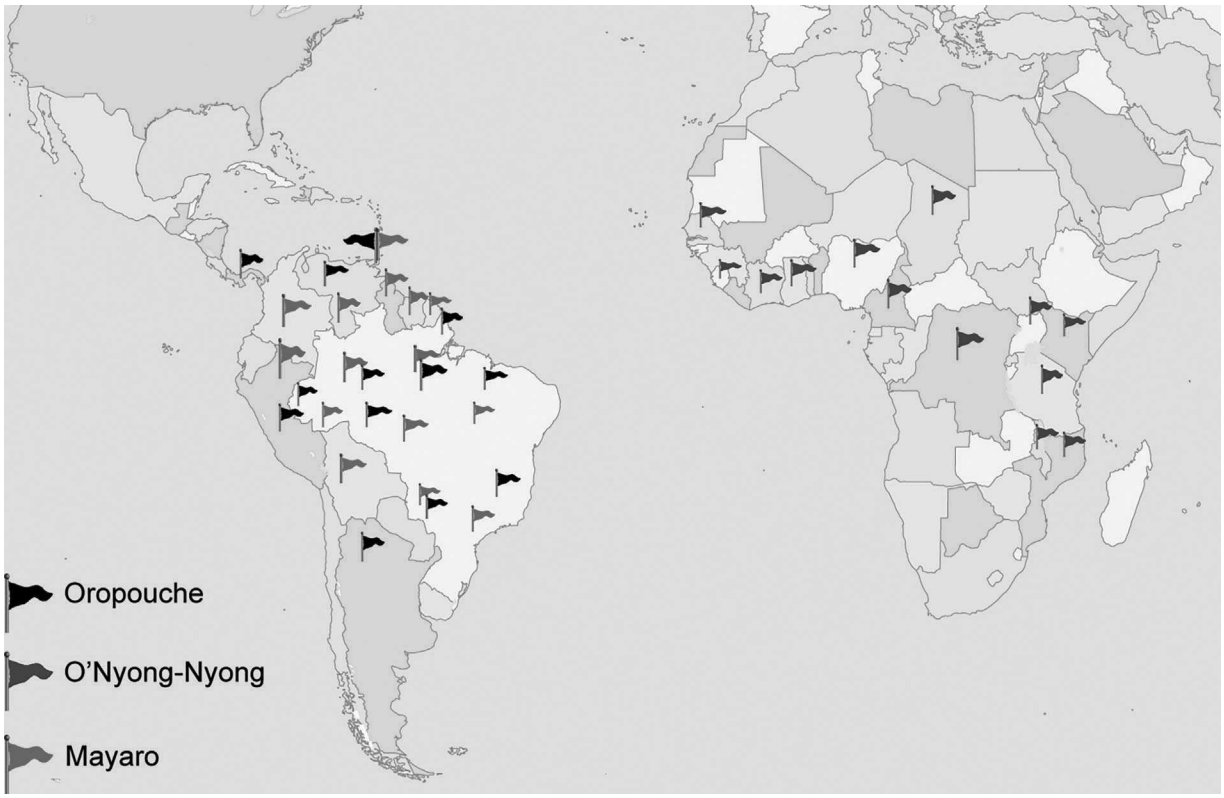

Figure 1. Historical distribution of Mayaro, Oropouche, and O'Nyong-Nyong viruses. Information based on historical accounts of urban, suburban, and sylvatic detection of each virus, taken from available literature. Infections with Mayaro (red flag) and Oropouche (black flag) have occurred in Latin America and the Caribbean, while O'Nyong-Nyong (blue flag) has been restricted to Africa. Each flag represents either autochthonous detection of the virus, or detection of specific antibodies against the virus through serosurveillance, in vertebrate (human and non-human) or invertebrate (arthropods) hosts. Map template kindly provided by Free Vector Maps (https://freevectormaps.com).

Wolbachia could be used to combat obscure arboviral diseases

The repertoire of vector control techniques is expanding

Over the past 50 years, a great deal of research has focused on developing vaccines or drugs to combat arboviral disease. While this has unfortunately proven to be more difficult than predicted, the near future will likely bring greater success as there are many promising vaccine candidates under development [83, 109, 110]. But, what the continued outbreaks of YFV and JEV tell us is that even with a widely available and effective vaccine, it is difficult to control a vector-borne disease using a single technique in isolation.

For that reason, there has long been a parallel aim of reducing disease burden through vector population control, and while initially successful [111], gains in that area have been eroded by the development of resistance to commonly used insecticides, and economic, environmental, and logistical issues associated with deployment of these chemicals [112]. As our civilization has grown more urbanized and increasingly interconnected, the vector populations that live in our proximity have thrived, and in that sense the recent resurgence of arboviral diseases was not unexpected [12]. Efforts to counteract increased transmission levels have led to the development of several new and innovative solutions for vector population control, including natural biocontrol agents such as entomopathogenic microbes like Bti, novel chemically derived insecticides like the insect growth regulator hormone analog pyriproxyfen, and GM approaches that involve mosquitoes or microbes that produce anti-pathogen effectors, or mosquitoes modified to crash local vector populations after release into the field [112]. Many of these approaches are complementary in nature, which would allow them to be deployed simultaneously, allowing for more effective disease prevention [112]. Many have also been trialed in the field, where they have shown great potential (see [112] for a review on these approaches). One promising example involves the endosymbiotic bacterium *Wolbachia pipientis*, which is an encouraging example of translational research that could potentially be applied to combat multiple arboviral diseases [113]. We will limit our subsequent discussion to *Wolbachia*, as this approach has not been reviewed in the context of potential application to obscure and emerging arboviruses.

Wolbachia is a common bacterial symbiont that naturally infects around 40% of all known terrestrial insects, as well as other arthropod taxa, and filarial nematodes [114]. The spread of this bacterium into wild arthropod populations is driven by high rates of maternal transmission, and by parasitic manipulations of host reproductive biology. Of these manipulations, cytoplasmic incompatibility (CI), wherein

infection modifies sperm and prevents effective reproduction between uninfected females and infected males, and between individuals carrying different strains of *Wolbachia*, is the most common and potentially has the greatest usefulness for vector control [113].

Wolbachia is a promising control option for many vectors and diseases

Wolbachia-based biological control can be divided into two main approaches: population suppression/incompatible insect technique (IIT) and population replacement/transmission blocking [113]. The bacterium has most commonly been used against mosquito vectors, but could foreseeably be applied to other taxa in the future. To work effectively, there must be a *Wolbachia* infection in the target vector. This can occur naturally, or be generated artificially through the process of transinfection [115, 116], which involves injecting developing embryos with purified *Wolbachia* from another host species (reviewed here [117]).

The population suppression approach relies on the release of incompatible males infected with CI-inducing *Wolbachia*. These males mate with wild, *Wolbachia*-free females, causing reduced egg hatch rates, and the wild population crashes over several generations [118]. In essence, the released males act as if they were sterile, making the IIT similar in method and effect to the sterile insect technique, without the radiation or chemical-induced competitiveness issues. The IIT involves an inundative release strategy, where large numbers of males are repeatedly released into geographically isolated areas [113]. It also necessitates an effective method for pupal sexing in order to avoid accidental releases of females [118]. The IIT has been successfully tested in the field for *Cx. pipiens fatigans* in the late 1960s, and more recently for *Cx. quinquefasciatus*, *Ae. albopictus*, and the filariasis vector *Aedes polynesiensis* (IIT approach reviewed here [119]).

The population replacement strategy is primarily dependent on the release of *Wolbachia*-infected female mosquitoes, but can also be facilitated by the co-release of infected males. The aim of this strategy is to drive *Wolbachia* into naturally uninfected field mosquito populations, relying on high levels of maternal transmission and CI, and low associated fitness costs to promote self-sustaining infection [120]. This strategy is currently being utilized against *Ae. aegypti* [121] (www.eliminatedengue.com [accessed: October 19, 2016]), and relies on the conferral of resistance to viral infection seen with some *Wolbachia* strains, which makes infected mosquitoes poor vectors of key pathogens of medical importance including DENV, CHIKV, and *Plasmodium falciparum* [122, 123], with similar effects recently characterized against ZIKV [124, 125] (see [113] for a review on this effect). The high prevalence of CI seen in different *Wolbachia*-infected hosts, and the fact that pathogen blocking occurs against many major arboviruses of high epidemiological importance gives *Wolbachia* great potential as a control agent [126], and this could also extend to future arboviral threats to human health if several key obstacles can be overcome (Table 2).

Controlling obscure diseases with *Wolbachia* will require preparation

The greatest impediment to controlling these disease with *Wolbachia* is the lack of knowledge on the distribution and identity of current or potential vectors of these diseases. Without this information it will be nearly impossible to effectively implement any type of vector control strategy. The transmission of MAYV, OROV, and ONNV appears convoluted, potentially relying on multiple vector species with different niches. This makes identification of the key vector species a critical step, as transinfections in multiple species may be required to adequately control disease transmission, and this must be planned well in advance of deployment to the field.

The need to generate transinfections is an issue that will likely prove a significant barrier to the extension of the *Wolbachia* approaches to new pathogens. Historically, there has been a great deal of difficulty associated with generating transinfections in new host taxa, as seen in anopheline mosquitoes, where the first stable infection took nearly a decade [122]. Similar issues might arise with the potential vectors of OROV and MAYV, as there are currently no *Wolbachia* transinfections of biting midges and *Haemagogus* mosquitoes, and it may prove difficult or even impossible to generate *Wolbachia* infections in these organisms.

Table 2. Impediments to control of obscure arboviruses with *Wolbachia*

Issue 1: Lack of knowledge about disease	
MAYV	Many potential vectors, but it is unclear which of these play a major role in the anthroponotic transmission cycle
ONNV	Unclear if there is a sylvatic transmission cycle. Unclear if *Anopheles gambiae* and *Anopheles funestus* are the only vectors, or if *Aedes aegypti* is an important vector
OROV	More clarity about the involvement of potential secondary vectors in disease transmission required. Vector competence of key vector species like *Aedes aegypti* must be established
Issue 2: Potential difficulties in generating *Wolbachia* infections in vectors	
MAYV	No previous *Wolbachia* transinfections in key vectors: *Haemagogus*, *Sabethes*, *Psorophora*, and *Mansonia* mosquitoes, *Gigantolaelaps* ticks. Unclear if *Wolbachia* can inhibit MAYV
ONNV	No *Wolbachia* transinfections in either anopheline species implicated in transmission. Difficulties associated with transinfection of anophelines. Unclear if *Wolbachia* can inhibit ONNV
OROV	No previous *Wolbachia* transinfections in key vectors: *Culicoides paraensis* midges, or the mosquitoes *Aedes serratus*, *Ochlerotatus serratus*, *Coquillettidia venezuelensis*. Potential vector *Culex quinquefasciatus* is naturally infected with a *Wolbachia* strain that is unlikely to inhibit ONNV infection. This must be cleared by antibiotic treatment prior to attempting transinfection. Unclear if *Wolbachia* can inhibit OROV

Additionally, the generation of transinfections necessitates that the species be colonized in the laboratory, and this may prove difficult for some vectors.

Post-transinfection, there is no guarantee that desired phenotypes such as CI and viral inhibition will occur. Furthermore, it is currently unclear if *Wolbachia* can even inhibit MAYV, ONNV, or OROV. To that end, examination of these key phenotypes in the natural host, or in target vector cell culture must be performed in order to assist with selecting the right *Wolbachia* strain for transinfection. In the event these effects do not occur, there is scope to adapt the intended strategy. For instance, if the *Wolbachia* infection does not inhibit the target pathogen, the IIT approach could be used instead. Likewise, if there are multiple vectors, and a transmission blocking approach is only feasible for one, the others could be targeted using the IIT, or one of the other vector control approaches. However, if this proves to be impractical, it may be necessary to begin transinfections again with a different strain.

While developing *Wolbachia* infections in the vectors of obscure arboviruses will likely prove complicated, the recent advances in *Wolbachia*-based control of malaria [127, 128], and the expansion of field-deployment of *Wolbachia*-infected *Ae. aegypti* for the control of DENV, CHIKV, and now ZIKV, suggest that the bacterium does possess the type of broad utility that will make it a useful tool to combat both current and future arboviral threats.

Conclusion and outlook

The emergence of arboviral diseases as serious epidemiological threats is driven by modifications of the virus transmission cycle, specifically changes in the dynamics of interactions between virus, vector, and human host. When emergence does occur, history suggests that these diseases tend to persist as serious threats to human health, in spite of the numerous strategies that have been used to reduce disease transmission.

This pattern will likely continue in the future, as there is great diversity of obscure or undiscovered arboviruses, which currently persist in sylvatic transmission cycles, or have gone uncharacterized due to their clinical similarity to better-known diseases. These viruses, including MAYV, ONNV, and OROV, have high levels of associated risk, as they can cause severe disease, their likely vectors have widespread distributions, and they are genetically similar to viruses that have previously emerged to become serious health threats.

The key to controlling these obscure pathogens lies in better characterization of their transmission cycles. Define these, and specific control strategies can be implemented, potentially using some of the novel approaches that have been developed in the past decade, including *Wolbachia*. Given what we have learned about the control of viruses like DENV, a multifaceted approach appears the best option to prevent the rapid emergence of another serious health threat like ZIKV, and the time to start preparing is now.

The authors have declared no conflict of interest.

References

1. **Chang C, Ortiz K, Ansari A, Gershwin ME**. 2016. The Zika outbreak of the 21st century. *J Autoimmun* **68**: 1–3.
2. **Haug CJ, Kieny MP, Murgue B**. 2016. The Zika challenge. *N Engl J Med* **374**: 1–3.
3. **Liang G, Gao X, Gould EA**. 2015. Factors responsible for the emergence of arboviruses; strategies, challenges and limitations for their control. *Emerg Microbes Infect* **4**: e18.
4. **Stanaway JD, Shepard DS, Undurraga EA, Halasa YA**, et al. 2016. The global burden of dengue: an analysis from the Global Burden of Disease Study 2013. *Lancet Infect Dis* **3099**: 1–2.
5. **Śmietanka K, Woźniakowski G, Kozak E, Niemczuk K**, et al. 2016. African swine fever epidemic, Poland, 2014–2015. *Emerg Infect Dis* **22**: 1201–7.
6. **Gubler DJ**. 2002. The global emergence/resurgence of arboviral diseases as public health problems. *Arch Med Res* **33**: 330–42.
7. **Grunnill M, Boots M**. 2016. How important is vertical transmission of dengue viruses by mosquitoes (Diptera: culicidae)? *J Med Entomol* **53**: 1–9.
8. **Mavale M, Parashar D, Sudeep A, Gokhale M**, et al. 2010. Venereal transmission of chikungunya virus by *Aedes aegypti* mosquitoes (Diptera: culicidae). *Am J Trop Med Hyg* **83**: 1242–4.
9. **Kreuder Johnson C, Hitchens PL, Smiley Evans T, Goldstein T**, et al. 2015. Spillover and pandemic properties of zoonotic viruses with high host plasticity. *Sci Rep* **5**: 14830.
10. **Weaver SC**. 2005. Host range, amplification and arboviral disease emergence. *Arch Virol Suppl* **19**: 33–44.
11. **Maciel-de-Freitas R, Avendanho FC, Santos R, Sylvestre G**, et al. 2014. Undesirable consequences of insecticide resistance following *Aedes aegypti* control activities due to a dengue outbreak. *PLoS ONE* **9**: e92424.
12. **Weaver SC, Reisen WK**. 2010. Present and future arboviral threats. *Antiviral Res* **85**: 328–45.
13. **Gould EA, Higgs S**. 2009. Impact of climate change and other factors on emerging arbovirus diseases. *Trans R Soc Trop Med Hyg* **103**: 109–21.
14. **Kilpatrick AM, Randolph SE**. 2012. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. *Lancet* **380**: 1946–55.
15. **Howe GM**. 1979. A world geography of human diseases. *Med Hist* **23**: 242.
16. **Gubler DJ**. 2011. Dengue, urbanization and globalization: the unholy trinity of the 21(st) Century. *Trop Med Health* **39**: 3–11.
17. **Monath TP, Vasconcelos PFC**. 2015. Yellow fever. *J Clin Virol* **64**: 160–73.
18. **Goenaga S, Fabbri C, Dueñas JCR, Gardenal CN**, et al. 2012. Isolation of yellow fever virus from mosquitoes in Misiones province, Argentina. *Vector Borne Zoonotic Dis* **12**: 986–93.
19. **Kraemer MUG, Sinka ME, Duda KA, Mylne A**, et al. 2015. The global compendium of *Aedes aegypt*i and *Ae. albopictus* occurrence. *Sci Data* **2**: 150035.
20. **Vasconcelos PF**. 2010. Yellow fever in Brazil: thoughts and hypotheses on the emergence in previously free areas. *Rev Saude Publica* **44**: 1144–9.
21. **Brady OJ, Gething PW, Bhatt S, Messina JP**, et al. 2012. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. *PLoS Negl Trop Dis* **6**: e1760.
22. **Shepard DS, Undurraga EA, Halasa YA, Stanaway JD**. 2016. The global economic burden of dengue: a systematic analysis. *Lancet Infect Dis* **3099**: 1–7.
23. **Scott LJ**. 2016. Tetravalent dengue vaccine: a review in the prevention of dengue disease. *Drugs* **76**: 1301–12.
24. **Dick OB, San Martín JL, Montoya RH, Del Diego J**, et al. 2012. Review: the history of dengue outbreaks in the Americas. *Am J Trop Med Hyg* **87**: 584–93.
25. **Grobbelaar AA, Weyer J, Moolla N, Jansen van Vuren P**, et al. 2016. Resurgence of yellow fever in Angola, 2015–2016. *Emerg Infect Dis* **22**: 1854–5.
26. **Vasconcelos PFC, Monath TP**. 2016. Yellow fever remains a potential threat to public health. *Vector Borne Zoonotic Dis* **16**: 566–7.
27. **Beck AS, Barrett ADT**. 2015. Current status and future prospects of yellow fever vaccines. *Expert Rev Vaccines* **14**: 1479–92.
28. **Smithburn KC, Hughes TP, Burke AW, Paul JH**. 1940. A neurotropic virus isolated from the blood of a native of Uganda. *Am J Trop Med Hyg* **s1–20**: 471–92.
29. **Bowen RA, Nemeth NM**. 2007. Experimental infections with West Nile virus. *Curr Opin Infect Dis* **20**: 293–7.

30. **Chancey C, Grinev A, Volkova E, Rios M**. 2015. The global ecology and epidemiology of West Nile Virus. *Biomed Res Int* **2015**: 1–20.
31. **Komar N, Langevin S, Hinten S, Nemeth N**, et al. 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerg Infect Dis* **9**: 311–22.
32. **Rizzoli A, Jiménez-Clavero M, Barzon L, Cordioli P**, et al. 2015. The challenge of West Nile virus in Europe: knowledge gaps and research priorities. *Eurosurveillance* **20**: 21135.
33. **Beckham JD, Tyler KL**. 2015. Arbovirus infections. *Contin Lifelong Learn Neurol* **21**: 1599–611.
34. **Ishikawa T, Yamanaka A, Konishi E**. 2014. A review of successful flavivirus vaccines and the problems with those flaviviruses for which vaccines are not yet available. *Vaccine* **32**: 1326–37.
35. **Burke DS, Leake CJ**. 1998. Japanese encephalitis virus: ecology and epidemiology. In: Monath T. ed; *The Arboviruses: Epidemiology and Ecology*. Berlin Heidelberg: Springer. p 63–92.
36. **Erlanger TE, Weiss S, Keiser J, Utzinger J**, et al. 2009. Past, present, and future of Japanese encephalitis. *Emerg Infect Dis* **15**: 1–7.
37. **Mackenzie JS, Williams DT, Smith DW**. 2006. Japanese encephalitis virus: the geographic distribution, incidence, and spread of a virus with a propensity to emerge in new areas. *Perspect Med Virol* **16**: 201–68.
38. **Campbell GL, Hills SL, Fischer M, Jacobson JA**, et al. 2011. Estimated global incidence of Japanese encephalitis: a systematic review. *Bull World Health Organ* **89**: 766–74.
39. **Rosen L, Tesh RB, Lien JC, Cross JH**. 1978. Transovarial transmission of Japanese encephalitis virus by mosquitoes. *Science* **199**: 909–11.
40. **Takashima I, Rosen L**. 1989. Horizontal and vertical transmission of Japanese encephalitis virus by *Aedes japonicus* (Diptera: culicidae). *J Med Entomol* **26**: 454–8.
41. **Robinson MC, Lumsden WH**. 1955. An epidemic of virus disease in Southern Province, Tanganyika territory, in 1952–1953. *Trans R Soc Trop Med Hyg* **49**: 28–32.
42. **Higgs S, Vanlandingham D**. 2015. Chikungunya virus and its mosquito vectors. *Vector-Borne Zoonotic Dis* **15**: 231–40.
43. **Dash AP, Bhatia R, Sunyoto T, Mourya DT**. 2013. Emerging and re-emerging arboviral diseases in Southeast Asia. *J Vector Borne Dis* **50**: 77–84.
44. **Geser A, Henderson BE, Christensen S**. 1970. A multipurpose serological survey in Kenya. 2. Results of arbovirus serological tests. *Bull. World Health Organ*. **43**: 539–52.
45. **Sergon K, Yahaya AA, Brown J, Bedja SA**, et al. 2007. Seroprevalence of chikungunya virus infection on Grande Comore Island, union of the Comoros, 2005. *Am J Trop Med Hyg* **76**: 1189–93.
46. **Renault P, Solet J-L, Sissoko D, Balleydier E**, et al. 2007. A major epidemic of chikungunya virus infection on Reunion Island, France, 2005–2006. *Am J Trop Med Hyg* **77**: 727–31.
47. **Tsetsarkin KA, Weaver SC**. 2011. Sequential adaptive mutations enhance efficient vector switching by chikungunya virus and its epidemic emergence. *PLoS Pathog* **7**: e1002412.
48. **Dash PK, Parida MM, Santhosh SR, Verma SK**, et al. 2007. East Central South African genotype as the causative agent in reemergence of chikungunya outbreak in India. *Vector Borne Zoonotic Dis* **7**: 519–27.
49. **Leparc-Goffart I, Nougairede A, Cassadou S, Prat C**, et al. 2014. Chikungunya in the Americas. *Lancet* **383**: 514.
50. **Dick GW, Kitchen S, Haddow A**. 1952. Zika virus (I). Isolations and serological specificity. *Trans R Soc Trop Med Hyg* **46**: 509–20.
51. **Haddow AJ, Williams MC, Woodall JP, Simpson DI**, et al. 1964. Twelve isolations of Zika virus from *Aedes* (Stegomyia) *africanus* (Theobald) taken in and above a Uganda forest. *Bull World Health Organ* **31**: 57–69.
52. **MacNamara F**. 1954. Zika virus: a report on three cases of human infection during an epidemic of jaundice in Nigeria. *Trans R Soc Trop Med Hyg* **48**: 139–45.
53. **Fagbami AH**. 1979. Zika virus infections in Nigeria: virological and seroepidemiological investigations in Oyo state. *J Hyg (Lond)* **83**: 213–9.
54. **Marchette NJ, Garcia R, Rudnick A**. 1969. Isolation of Zika virus from *Aedes aegypti* mosquitoes in Malaysia. *Am J Trop Med Hyg* **18**: 411–5.
55. **Olson JG, Ksiazek TG, Suhandiman T**. 1981. Zika virus, a cause of fever in Central Java, Indonesia. *Trans R Soc Trop Med Hyg* **75**: 389–93.
56. **Duffy MR, Chen T-H, Hancock WT, Powers AM**, et al. 2009. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med* **360**: 2536–43.
57. **Durand MA, Bel M, Ruwey I, Marfel M**, et al. 2005. An outbreak of dengue fever in Yap State. *Pac Health Dialog* **12**: 99–102.
58. **Musso D, Gubler DJ**. 2016. Zika virus. *Clin Microbiol Rev* **29**: 487–524.
59. **Aubry M, Finke J, Teissier A, Roche C**, et al. 2015. Seroprevalence of arboviruses among blood donors in French Polynesia, 2011–2013. *Int J Infect Dis* **41**: 11–2.
60. **Cao-Lormeau V-M, Roche C, Teissier A, Robin E**, et al. 2014. Zika virus, French Polynesia, South Pacific, 2013. *Emerg Infect Dis* **20**: 1085–6. http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(16)00562-6/abstract
61. **Cardoso CW, Paploski IAD, Kikuti M, Rodrigues MS**, et al. 2015. Outbreak of exanthematous illness associated with Zika, chikungunya, and dengue viruses, Salvador, Brazil. *Emerg Infect Dis* **21**: 2274–6.
62. **Calvet G, Aguiar RS, Melo ASO, Sampaio SA**, et al. 2016. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. *Lancet Infect Dis* **16**: 653–60.
63. **Brasil P, Sequeira PC, Freitas AD, Zogbi HE**, et al. 2016. Guillain-Barré syndrome associated with Zika virus infection. *Lancet* **387**: 1482.
64. **Johansson MA, Mier-y-Teran-Romero L, Reefhuis J, Gilboa SM**, et al. 2016. Zika and the risk of microcephaly. *N Engl J Med* **375**: 1–4.
65. **Likos A, Griffin I, Bingham AM, Stanek D**, et al. 2016. Local mosquito-borne transmission of Zika virus – Miami-Dade and Broward Counties, Florida, June-August 2016. *MMWR Morb Mortal Wkly Rep* **65**: 1032–8.
66. **Ferguson NM, Cucunubá ZM, Dorigatti I, Nedjati-Gilani GL**, et al. 2016. Countering the Zika epidemic in Latin America. *Science* **353**: 353–4.
67. **Casals J, Whitman L**. 1957. Mayaro virus: a new human disease agent. I. Relationship to other arboviruses. *Am J Trop Med Hyg* **6**: 1004–11.
68. **De Figueiredo MLG, Figueiredo LTM**. 2014. Emerging alphaviruses in the americas: chikungunya and Mayaro. *Rev Soc Bras Med Trop* **47**: 677–83.
69. **Suhrbier A, Jaffar-Bandjee M-C, Gasque P**. 2012. Arthritogenic alphaviruses – an overview. *Nat Rev Rheumatol* **8**: 420–9.
70. **Halsey ES, Siles C, Guevara C, Vilcarromero S**, et al. 2013. Mayaro virus infection, Amazon Basin region, Peru, 2010–2013. *Emerg Infect Dis* **19**: 1839–42.
71. **Llagonne-Barets M, Icard V, Leparc-Goffart I, Prat C**, et al. 2016. A case of Mayaro virus infection imported from French Guiana. *J Clin Virol* **77**: 66–8.
72. **Auguste AJ, Liria J, Forrester NL, Giambalvo D**, et al. 2015. Evolutionary and ecological characterization of Mayaro virus strains isolated during an outbreak, Venezuela, 2010. *Emerg Infect Dis* **21**: 1742–50.
73. **Patiño-Barbosa AM, Bedoya-Arias JE, Cardona-Ospina JA, Rodriguez-Morales AJ**. 2016. Bibliometric assessment of the scientific production of literature regarding Mayaro. *J Infect Public Health* **9**: 532–4.
74. **Forshey BM, Guevara C, Laguna-Torres VA, Cespedes M**, et al. 2010. Arboviral etiologies of acute febrile illnesses in Western South America, 2000–2007. *PLoS Negl Trop Dis* **4**: e787.
75. **Abad-Franch F, Grimmer GH, de Paula VS, Figueiredo LTM**, et al. 2012. Mayaro virus infection in Amazonia: a multimodel inference approach to risk factor assessment. *PLoS Negl Trop Dis* **6**: e1846.
76. **Calisher CH, Gutierrez E, Maness KS, Lord RD**. 1974. Isolation of Mayaro virus from a migrating bird captured in Louisiana in 1967. *Paho Bull* **8**: 243–8.
77. **Pauvolid-Corrêa A, Soares Juliano R, Campos Z, Velez J**, et al. 2015. Neutralising antibodies for Mayaro virus in Pantanal, Brazil. *Mem Inst Oswaldo Cruz* **110**: 125–33.
78. **Pereira Serra O, Fernandes Cardoso B, Maria Ribeiro AL, dos Santos FAL**, et al. 2016. Mayaro virus and dengue virus 1 and 4 natural infection in culicids from Cuiabá, state of Mato Grosso, Brazil. *Mem Inst Oswaldo Cruz* **111**: 20–9.
79. **Long KC, Ziegler SA, Thangamani S, Hausser NL**, et al. 2011. Experimental transmission of Mayaro virus by *Aedes aegypti*. *Am J Trop Med Hyg* **85**: 750–7.
80. **Mezencio JMS, de Souza W, Fonseca MEF, Rebello MA**. 1989. Replication of Mayaro virus in *Aedes albopictus* cells: an electron microscopic study. *Arch Virol* **104**: 299–308.
81. **Smith GC, Francy DB**. 1991. Laboratory studies of a Brazilian strain of *Aedes albopictus* as a potential vector of Mayaro and Oropouche viruses. *J Am Mosq Control Assoc* **7**: 89–93.
82. **Friedrich-Janicke B, Emmerich P, Tappe D, Gunthe S**, et al. 2014. Genome analysis of mayaro virus imported to Germany from French Guiana. *Emerg Infect Dis* **20**: 1255–7.
83. **Weise WJ, Hermance ME, Forrester N, Adams AP**, et al. 2014. A novel live-attenuated vaccine candidate for Mayaro fever. *PLoS Negl Trop Dis* **8**: e2969.
84. **Fauquet CM, Mayo MA, Maniloff J, Desselberger U**, et al. 2005. *Virus Taxonomy*. San Diego: Academic Press.

85. **Anderson CR, Spence L, Downs WG, Aitken TH**. 1961. Oropouche virus: a new human disease agent from Trinidad, West Indies. *Am J Trop Med Hyg* **10**: 574–8.
86. **Pinheiro FP, Pinheiro M, Bensabath G, Causey OR**, et al. 1962. Epidemia de vírus Oropouche em Belém. *Rev do Serv Espec Saúde Pública* **12**: 15–23.
87. **Saeed MF, Wang H, Nunes M, Vasconcelos PF**, et al. 2000. Nucleotide sequences and phylogeny of the nucleocapsid gene of Oropouche virus. *J Gen Virol* **81**: 743–8.
88. **Nunes RMT, Vasconcelos HB, Medeiros DBDA, Rodrigues SG**, et al. 2007. A febre do Oropouche: uma revisão dos aspectos epidemiológicos e moleculares na Amazônia Brasileira. *Cad Saúde Coletiva* **15**: 303–18.
89. **Vasconcelos HB, Azevedo RSS, Casseb SM, Nunes-Neto JP**, et al. 2009. Oropouche fever epidemic in Northern Brazil: epidemiology and molecular characterization of isolates. *J Clin Virol* **44**: 129–33.
90. **Mellor PS, Boorman J, Baylis M**. 2000. *Culicoides* biting midges: their role as arbovirus vectors. *Annu Rev Entomol* **45**: 307–40.
91. **Cardoso BF, Serra OP, Da Silva Heinen LB, Zuchi N**, et al. 2015. Detection of Oropouche virus segment s in patients and in *Culex quinquefasciatus* in the state of Mato Grosso, Brazil. *Mem Inst Oswaldo Cruz* **110**: 745–54.
92. **Hoch AL, Pinheiro FP, Roberts DR, Gomes ML**. 1987. Laboratory transmission of Oropouche virus by *Culex quinquefasciatus* Say. *Bull Pan Am Health Organ* **21**: 55–61.
93. **Mourão MPG, Bastos MS, Gimaque JBL, Mota BR**, et al. 2009. Oropouche fever outbreak, Manaus, Brazil, 2007–2008. *Emerg Infect Dis* **15**: 2063–4.
94. **Watts DM, Phillips I, Callahan JD, Griebenow W**, et al. 1997. Oropouche virus transmission in the Amazon River basin of Peru. *Am. J Trop Med Hyg* **56**: 148–52.
95. **Carpenter S, Groschup MH, Garros C, Felippe-Bauer ML**, et al. 2013. Culicoides biting midges, arboviruses and public health in Europe. *Antiviral Res* **100**: 102–13.
96. **Vanlandingham DL, Hong C, Klingler K, Tsetsarkin K**, et al. 2005. Differential infectivities of O'Nyong-Nyong and chikungunya virus isolates in *Anopheles gambiae* and *Aedes aegypti* mosquitoes. *Am J Trop Med Hyg* **72**: 616–21.
97. **Brault AC, Tesh RB, Powers AM, Weaver SC**. 2000. Re-emergence of chikungunya and O'Nyong-Nyong viruses: evidence for distinct geographical lineages and distant evolutionary relationships. *J Gen Virol* **81**: 471–9.
98. **Blackburn N, Besselaar T, Gibson G**. 1995. Antigenic relationship between chikungunya virus strains and O'Nyong-Nyong virus using monoclonal antibodies. *Res Virol* **146**: 69–73.
99. **Williams MC, Woodall JP, Corbet PS, Gillett JD**. 1965. O'Nyong-Nyong fever: an epidemic virus disease in East Africa. 8. virus isolations from *Anopheles* mosquitoes. *Trans R Soc Trop Med Hyg* **59**: 300–6.
100. **Williams MC, Woodall JP**. 1961. O'Nyong-Nyong fever: an epidemic virus disease in East Africa. II. Isolation and some properties of the virus. *Trans R Soc Trop Med Hyg* **55**: 135–41.
101. **Shore H**. 1961. O'Nyong-Nyong fever: an epidemic virus disease in East Africa. *Trans R Soc Trop Med Hyg* **55**: 361–73.
102. **Marshall TF, Keenlyside RA, Johnson BK, Chanas AC**, et al. 1982. The epidemiology of O'Nyong-Nyong in the Kano Plain, Kenya. *Ann Trop Med Parasitol* **76**: 153–8.
103. **Posey DL, O'Rourke T, Roehrig JT, Lanciotti RS**, et al. 2005. Short report: O'Nyong-Nyong fever in West Africa. *Am J Trop Med Hyg* **73**: 32.
104. **Tappe D, Kapaun A, Emmerich P, de Mendonca Campos R**, et al. 2014. O'Nyong-Nyong virus infection imported to Europe from Kenya by a traveler. *Emerg Infect Dis* **20**: 1766–7.
105. **Rios-González CM**. 2016. Bibliometric study of international scientific production in O'Nyong-Nyong virus during the years 1962–2016. *J Infect Public Health* in press, DOI: 10.1016/j.jiph.2016.05.006
106. **Partidos CD, Paykel J, Weger J, Borland EM**, et al. 2012. Cross-protective immunity against O'Nyong-Nyong virus afforded by a novel recombinant chikungunya vaccine. *Vaccine* **30**: 4638–43.
107. **Sinka ME, Bangs MJ, Manguin S, Chareonviriyaphap T**, et al. 2011. The dominant *Anopheles* vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic précis. *Parasit Vectors* **4**: 89.
108. **Mourão MPG, De Bastos MS, de Figueiredo RMP, De Gimaque JBL**, et al. 2015. Arboviral diseases in the western Brazilian Amazon: A perspective and analysis from a tertiary health & research center in manaus, state of Amazonas. *Rev Soc Bras Med Trop* **48**: 20–6.
109. **Larocca RA, Abbink P, Peron JPS, de A. Zanotto PM**, et al. 2016. Vaccine protection against Zika virus from Brazil. *Nature* **536**: 474–8.
110. **Guy B, Lang J, Saville M, Jackson N**. 2016. Vaccination against dengue: challenges and current developments. *Annu Rev Med* **67**: 387–404.
111. **Gubler DJ**. 1998. Resurgent vector-borne diseases as a global health problem. *Emerg Infect Dis* **4**: 442–50.
112. **McGraw EA, O'Neill SL**. 2013. Beyond insecticides: new thinking on an ancient problem. *Nat Rev Microbiol* **11**: 181–93.
113. **Caragata EP, Dutra HLC, Moreira LA**. 2016. Exploiting intimate relationships: controlling mosquito-transmitted disease with *Wolbachia*. *Trends Parasitol* **32**: 207–18.
114. **Zug R, Hammerstein P**. 2012. Still a host of hosts for *Wolbachia*: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. *PLoS ONE* **7**: e38544.
115. **Xi Z, Khoo CCH, Dobson SL**. 2005. *Wolbachia* establishment and invasion in an Aedes aegypti laboratory population. *Science* **310**: 326–8.
116. **McMeniman C, Lane R, Cass B**. 2009. Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science* **323**: 141–4.
117. **Hughes GL, Rasgon JL**. 2014. Transinfection: a method to investigate *Wolbachia*-host interactions and control arthropod-borne disease. *Insect Mol Biol* **23**: 141–51.
118. **Bourtzis K, Dobson SL, Xi Z, Rasgon JL**, et al. 2014. Harnessing mosquito-*Wolbachia* symbiosis for vector and disease control. *Acta Trop* **132**: S150–63.
119. **Lees RS, Gilles JR, Hendrichs J, Vreysen MJ**, et al. 2015. Back to the future: the sterile insect technique against mosquito disease vectors. *Curr Opin Insect Sci* **10**: 156–62.
120. **Hoffmann AA, Iturbe-Ormaetxe I, Callahan AG, Phillips BL**, et al. 2014. Stability of the *w*Mel *Wolbachia* Infection following invasion into *Aedes aegypti* populations. *PLoS Negl Trop Dis* **8**: e3115.
121. **Hoffmann AA, Montgomery BL, Popovici J, Iturbe-Ormaetxe I**, et al. 2011. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* **476**: 454–7.
122. **Bian G, Joshi D, Dong Y, Lu P**, et al. 2013. *Wolbachia* invades Anopheles stephensi populations and induces refractoriness to *Plasmodium* infection. *Science* **340**: 748–51.
123. **Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G**, et al. 2009. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, chikungunya, and *Plasmodium*. *Cell* **139**: 1268–78.
124. **Dutra HLC, Rocha MN, Dias FBS, Mansur SB**, et al. 2016. *Wolbachia* blocks currently circulating Zika virus isolates in Brazilian *Aedes aegypti* mosquitoes. *Cell Host Microbe* **19**: 771–4.
125. **Aliota MT, Peinado SA, Velez ID, Osorio JE**. 2016. The *w*Mel strain of *Wolbachia* reduces transmission of Zika virus by *Aedes aegypti*. *Sci Rep* **6**: 28792.
126. **Yakob L, Walker T**. 2016. Zika virus outbreak in the Americas: the need for novel mosquito control methods. *Lancet Glob Heal* **4**: e148–9.
127. **Baldini F, Segata N, Pompon J, Marcenac P**, et al. 2014. Evidence of natural *Wolbachia* infections in field populations of *Anopheles gambiae*. *Nat Commun* **5**: 3985.
128. **Shaw WR, Marcenac P, Childs LM, Buckee CO**, et al. 2016. *Wolbachia* infections in natural *Anopheles* populations affect egg laying and negatively correlate with *Plasmodium* development. *Nat Commun* **7**: 11772.