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Rolf Hilgenfeld · Subhash G. Vasudevan
*Editors*

# Dengue and Zika: Control and Antiviral Treatment Strategies

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# Preface

Many emerging viruses have their origin in Africa. For example, Ebola virus was first recorded after the outbreak in the Democratic Republic of Congo (then known as Zaire) in 1976 and Chikungunya virus emerged and was isolated in Tanzania (formerly known as Tanganyika) in 1952. During that outbreak, a number of cases also occurred in Northern Mozambique. Furthermore, a particularly virulent strain of Dengue virus (serotype 3) first appeared in Mozambique, and antibodies to Zika virus have been detected in the Mozambican population decades ago (see Chap. 2 of this book).

Mozambique, with its tropical climate, seems to be an ideal breeding ground for newly emerging viruses. Yet, even though Africa, perhaps along with South East Asia and Latin America, carries the largest burden of outbreaks of emerging viruses, almost all scientific conferences dealing with these viruses take place in the USA or Europe. To address this gap, one of us (RH) started the Tofo Advanced Study Week (TASW) series on Emerging Viral Diseases in 2015, during the Ebola outbreak in West Africa, and managed to convince about 50 prominent Ebola researchers from around the world to gather in Tofo, Mozambique, for an in-depth discussion of the scientific and public health issues connected with the epidemic. Very importantly, this workshop provided a forum for many African scientists to report their results, when most of them did not have the opportunity to speak at the large Ebola conferences in Paris or Washington, D.C.

Building on this success, the 2nd Tofo Advanced Study Week on Emerging Viruses was held from Aug 28 to Sep 01, 2016, when the Zika virus epidemic was gripping the world. The meeting brought together experts from around the world to discuss Dengue and Zika epidemiology, diagnosis, immunopathology, mosquito control, and antiviral targets ranging from entry mechanism to viral replication enzymes or their protein interactions. The discussions also briefly considered the merits and issues of the Dengvaxia® vaccine that has been approved in more than 10 countries.

Given the very unique presence of experts from around the globe, this time we also endeavored to produce a scientific book that would collate the contributions written by the lecturers. Today, we are proud to present a collection of papers in this book entitled ***Dengue and Zika: Control and Antiviral Treatment Strategies*** arising from the Proceedings of the 2nd Tofo Advanced Study Week on Emerging Viral Diseases, published by Springer as a contributed volume in the Advances in Experimental Medicine and Biology series.

The industry perspective on antiviral development against flaviviral diseases was discussed briefly at the meeting and a comprehensive chapter on this topic from industry-based authors is included in the book. As in the first TASW meeting, the attendance and participation of young scientists from the African continent, as well as international scientists working on epidemiological aspects of arboviruses in Africa, was a key component. The abstracts of the presentations by these scientists are collectively provided in Chap. 25, and we envisage that these should allow international scientists interested in emerging viruses to connect, collaborate, and help extend and build virology-related research capacity in Africa. In total, the book contains 25 chapters written by leading international experts and reflects the current state of knowledge on Dengue and Zika and occasionally also other arboviruses. Moreover, we include transcripts of the discussions following each presentation that were recorded during the conference. In fact, Chap. 8 comprises the transcript of an in-depth discussion on flavivirus entry and on the NS1 protein as potential drug targets, where you can read the opinion of leading experts, which to a large part you will not find published anywhere else. We consider these lively discussions the "salt in the soup" of this book and very much hope that you will equally enjoy them.

We believe that the wide spectrum of topics covered in this book will bring the field up to date to consider the merits of a three-pronged approach to Dengue and Zika control involving vaccines, antivirals, and mosquito control. We would like to thank all the authors who contributed to this volume, as well as people who helped with producing it: Nina Eichler, Charlotte Flory, Antje Lindae, Jan Magonov, and the dedicated staff of Springer. Others have to be mentioned because they provided essential support with the organization of the meeting: Charlotte Flory, Nobina Morimoto, Linda Ngoromani, Eduardo Samo Gudo, and the staff of the conference venue, Hotel Tofo Mar. We also thank the organizations and companies that sponsored the meeting: Casa do Mar Guesthouse, Euroimmun AG, Gilead, Hotel Tofo Mar, Instituto Nacional de Saúde de Mocambique, Terra Agua Ceu Travel Agency, and Top-of-the-Dune Guesthouse. The German Centre for Infection Research (DZIF) supported the conference as a co-organizer.

We sincerely hope that you will be delighted by this lively and comprehensive compendium of flavivirus science and decide to join us at future Tofo Advanced Study Week meetings to discuss emerging viral diseases.

Lübeck, Germany — Rolf Hilgenfeld
Singapore, Singapore — Subhash G. Vasudevan


Photo taken by Subhash G. Vasudevan during a post-conference tour of the Kruger National Park. Dedicated to Dr. Wilfred LF Armarego (The Australian National University).

Participants of the 2nd Tofo Advanced Study Week on Emerging Viral Diseases, Praia do Tofo, Inhamabane, Mozambique, August 28 - September 01, 2016:
First row:Vanessa Monteiro, Imelda Rogério Chelene, Flora Inalda Mula, Onélia Cecília Guiliche, Aruna Sampath, Suzanne Kaptein, Maurice Demanou
Second row: Sádia Ali, Laura Rivino, Argentina Felisbela Muianga, Nicole Zitzmann, Edwige Rances, Francis Maluki Mutuku
Third row:Katja Fink, Katell Bidet, Beate Kümmerer, Anuja Mathew, Claudia Ohst, Ana Rita Chico, Aravinda de Silva, David Jans
Fourth row: Inocêncio Chongo, Paul Young, Subhash G. Vasudevan, Edward Jaszi, Sailas Witimosi, Scott O'Neill, Félix Rey
Fifth row: Vijaykrishna Dhanasekaran, Robert Lowen, John Oludayo Oludele, Jonas Schmidt-Chanasit, Charlotte Flory, Christoph Nitsche, Linda Ngoromani, Yi Shi, Norbert Heinrich
Sixth row: Joanna Miller, Kerstin Falk, Xavier Gundana, Jan Magonov, Rolf Hilgenfeld

# Contents

# Arboviruses: A Family on the Move

1

Paul R. Young

**Abstract**

Arboviruses are a diverse group of vector-borne viruses, many of whose members are the cause of significant human morbidity and mortality. Over the last 30 years, the emergence and/or resurgence of arboviruses have posed a considerable global health threat. The ongoing geographical expansion of the dengue viruses (DENV), along with the explosive outbreaks of West Nile virus (WNV), Chikungunya virus (CHIKV) and more recently, Zika virus (ZIKV) have all served as reminders that new epidemics may emerge at any time from this diversity. A clearer understanding of what mechanisms drive these dramatic changes in vector-host transmission cycles that result in the human population becoming significantly more exposed, will help to prepare us for the next emerging epidemic/pandemic. This Chapter seeks to provide a brief overview of the arboviruses, their mode of transmission and some of the known factors that drive their expansion.

**Keywords**

Arthropod-borne viruses · Zoonotic infections · Virosphere · Arbovirus transmission · Climate change impacts

## 1.1 Introduction

Arboviruses (a term derived from the descriptor, **ar**thropod-**bo**rne **viruses**) are an amazingly diverse group of viruses that are transmitted from infected to susceptible hosts by a range of arthropod vectors that include mosquitoes, ticks, sand flies or biting midges [20, 21]. Following ingestion of a blood meal from an infected host, viruses multiply in the insect mid-gut and then invade underlying tissues to cause a spreading infection (collectively referred to as the extrinsic incubation period) that ultimately results in a high-titred viral load, particularly in the salivary glands. They are then passed on to humans or other vertebrates during insect biting. Most diseases caused by arboviruses are zoonoses, primarily infections of vertebrates that can occasionally cause incidental infection and disease in humans. Notable exceptions to this are the dengue viruses (DENV), as humans are the primary vertebrate host. Indeed, passage through humans is essential in maintaining the virus transmission cycle. The nature of this two-way dependency prompted Duane Gubler to once remark that "humans could

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be considered the vector for dengue virus infection in mosquitoes". While monkeys have been implicated as an alternative vertebrate host to humans for dengue in rural settings, it is unlikely that this sylvatic cycle contributes much to the current global impact of this apex arbovirus.

By definition, arboviruses are arthropod-borne, however some are grouped within the arboviruses despite no apparent association with an arthropod vector, primarily because of their close genetic relationship. The naming of individual arboviruses has had a somewhat eclectic history with no formal taxonomic approach having been established. Some refer to dialect names after the illness they induce (chikungunya, o'nyong-nyong, dengue), others recognise the name of the location where they were first discovered (West Nile, Bwamba, Ross River, Zika) and some reflect a characteristic clinical presentation (Western equine encephalitis, yellow fever) [21].

Over the course of the last two decades, a dramatic expansion in the territorial range of a number of arboviruses has seen a significant increase in global epidemic activity. These include West Nile virus and its emergence in New York in 1999 and subsequent march across the North American continent over the next 4 years and subsequent spread, both north and south over the following decade. Chikungunya virus with its sudden expansion on La Reunion in 2005 and spread across the Indian subcontinent, South East Asia and globally. The ongoing expansion of the dengue viruses across the tropical zone and beyond, and of course, the recent explosive epidemic of Zika virus in South America, on the other side of the world from its first isolation in an African forest some 70 years previously. One thing is certain; we will see more of these outbreaks in the years to come [1, 9]. As a brief introduction to the research efforts detailed in the following Chapters, this review provides an overview of the group of viruses we collectively refer to as arboviruses, and addresses some of the issues that are helping to drive their expansion.

## 1.2 Who Are They?

More than 500 arboviruses have been recognised worldwide [21], a number that is undergoing rapid and exponential revision as researchers interrogate the virosphere using deep sequencing [19]. Estimates have suggested that the arboviruses we have recognised to date may represent less than 1% of the total. Only some of the currently known arboviruses, some 150, are known to cause human disease [21]. Some infect humans only occasionally or cause only mild illness, whereas others are of significant medical importance, causing large epidemics.

Most arboviruses causing human disease belong to three families; Togaviridae (genus *Alphavirus*), Flaviviridae (genus *Flavivirus*) and Bunyaviridae (*Bunyavirus*, *Orthobunyavirus*, *Nairovirus* and *Phlebovirus* genera), with members of three further families, Rhabdoviridae, Orthomyxoviridae and Reoviridae also contributing (Fig. 1.1). The alphaviruses and flaviviruses are enveloped, linear single-stranded, positive-sense RNA viruses. They are spherical in shape, with an underlying capsid and measure from 40 to 70 nm. The bunyaviruses are enveloped, segmented, circular negative-strand RNA viruses. They are generally spherical and measure 80–120 nm in diameter.

The most important group, at least from a human disease perspective, are the flaviviruses with a number of viruses in this group being of global health concern; dengue virus (DENV), West Nile virus (WNV), Zika virus (ZIKV) and yellow fever virus (YFV) [11]. Others, including Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBEV), Venezuelan equine encephalitis virus (VEEV) and St. Louis encephalitis virus (SLEV) are usually restricted to specific regions. However, the spread of arboviruses across several regions have lead to major international health concerns. WNV with its jump from the Middle-East into the Americas, chikungunya virus (CHIKV) moving into islands in the southwest Indian Ocean, and from there to Southeast

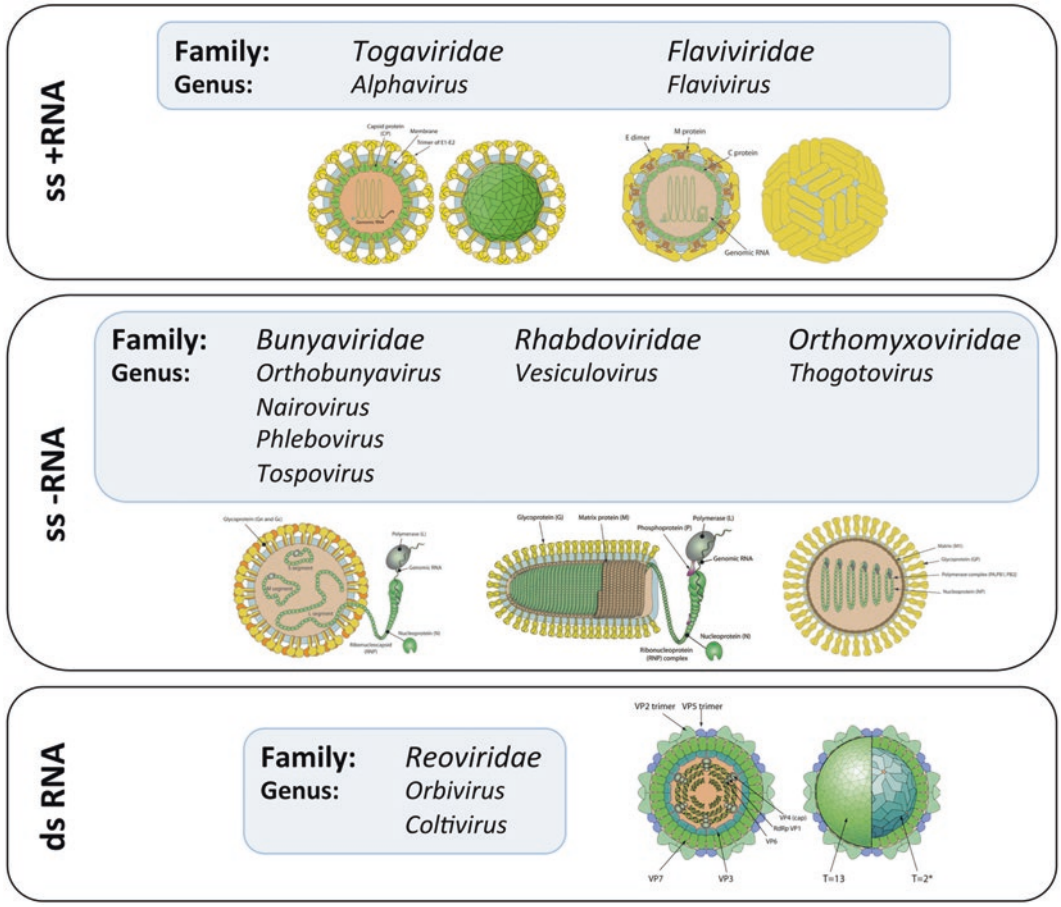


**Fig. 1.1 Arboviruses and virion schematics.** Viruses are grouped according to genome composition: single-stranded positive-sense RNA, ss + RNA; single-stranded negative-sense RNA, ss-RNA; double-stranded RNA, dsRNA. Arboviruses that are associated with human disease are mostly found within the Togaviridae, Flaviviridae and Bunyaviridae families. Virus schematics provided by ViralZone, Swiss Institute of Bioinformatics

Asia and the Americas, and Zika virus which spilled out of Africa to Southeast Asia, the islands of Polynesia and then to Brazil in an explosive epidemic in 2015–2016 (Fig. 1.2).

## 1.3 How Are They Maintained and Spread?

Three key elements are required for effective maintenance of arbovirus transmission: the vector (mosquito, tick, sandfly, biting midge), the vertebrate host(s) and appropriate environmental conditions. Some transmission cycles are relatively simple (involving one vector and one host, e.g., DENV and ZIKV) while some are highly complex (involving multiple vectors and hosts, e.g., JEV, WNV and Rift Valley Fever virus, RVFV). The epidemiology of human arboviral disease usually involves one of two transmission cycle scenarios (Fig. 1.3). In the first, the virus is stably and naturally maintained via transmission between vectors and wild animals in a sylvatic (jungle) cycle with spillover occurring when an infected arthropod bites either a domestic animal or human that has strayed into that ecological niche. This mode of infection results in small clusters of cases initiated at the same site. The second is the urban cycle where a person or domestic animal, infected via the sylvatic mode

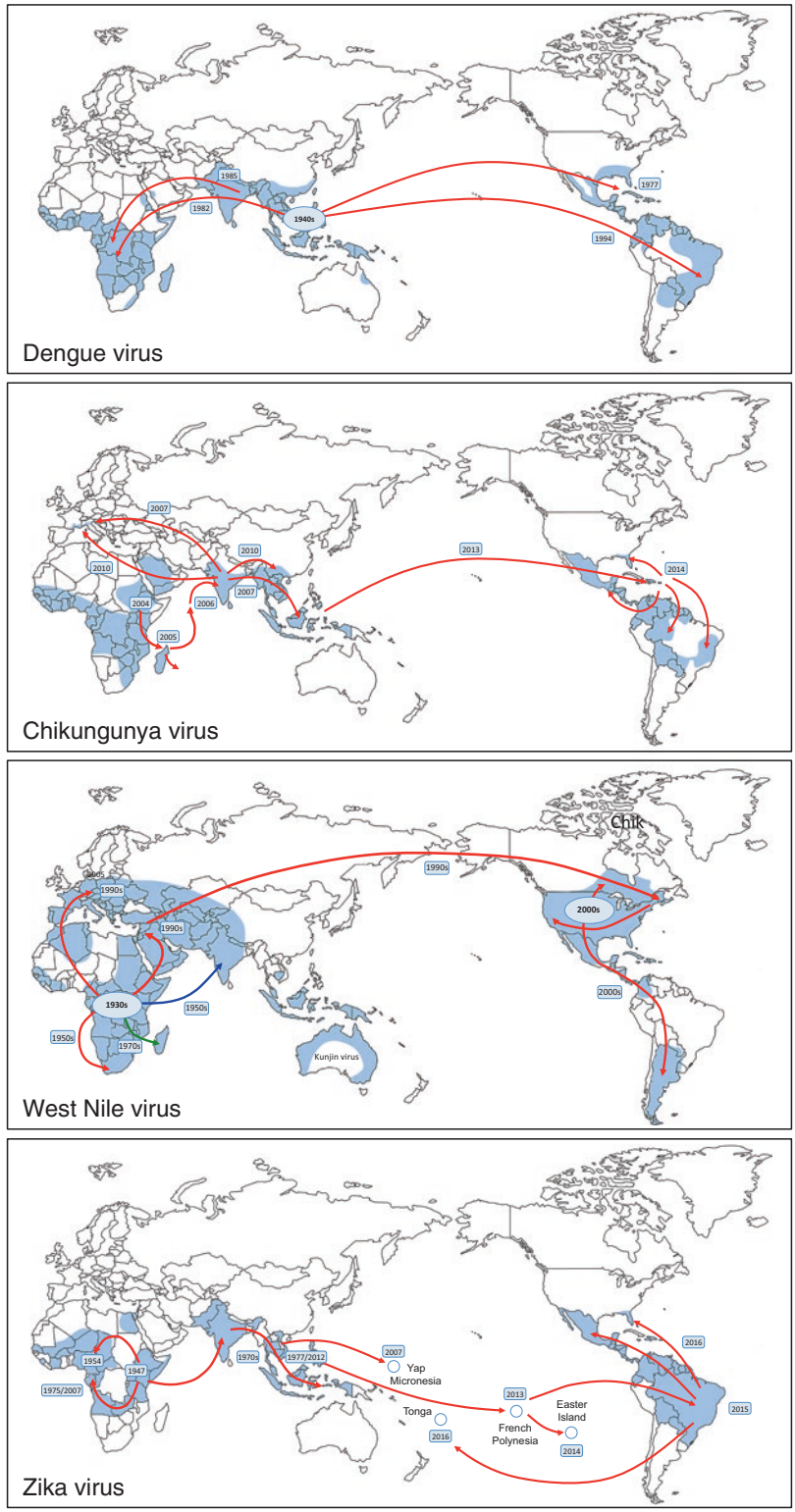


**Fig. 1.2 Arbovirus epidemiology.** Four examples of arboviruses that have emerged as globally distributed threats to human health. Geographical regions shaded in blue indicate historical, ongoing and recent viral activity with dates highlighting key epidemic translocation events. Dengue virus (DENV); the four serotypes of dengue virus

or moving from another area with urban activity, acts as an amplifier host in the transfer of the virus to other persons or domestic animals in the community. These cases occur as epidemics or epizootics in nature (Fig. 1.3) The vector involved in the urban cycle may be the same or different to that in the sylvatic cycle and indeed, there may be multiple vector species playing a role in transmission in either cycle.

The primary arboviral hosts are mammals and birds with the potential for virus dispersal depending on the type of vertebrate host involved [21]. Migratory birds can facilitate virus movement over large distances, such as occurred with the spread of WNV through the Americas, whereas transmission through most terrestrial hosts result in virus activity that is restricted to a particular region.

Animal hosts that are essential for arbovirus transmission and for the maintenance of virus populations are referred to as reservoir hosts, with the immune status of these hosts impacting on transmission rates. Their long co-evolution with their viral passengers is characterised by high titre viraemia that enables vector mediated virus transmission to occur, often in the absence of overt disease. A wide variety of reservoir host species have been implicated in arbovirus diseases. These include birds, mammals (including primates), rodents, marsupials and bats. Individual arboviruses may have more than one host species involved in transmission cycles. For example, birds (herons in particular) are considered to be the major maintenance hosts for the flavivirus JEV. In Asia however, pigs have also been shown to amplify the virus to high titres. Feeding mosquitoes can therefore be readily infected, with transmission of the virus to humans who live in close proximity. The life cycle of Ross River virus (RRV) in Australia involves complex relationships between multiple vectors and zoonotic (marsupials, horses, possums, bats) reservoirs across multiple environments including urban, inland (freshwater wetlands) and coastal (estuarine wetlands) regions [3].

Host species may move virus from an area of active transmission to another location. Movement by viraemic waterbirds has been suggested as a mechanism of spread for a number of arboviruses including Murray Valley encephalitis virus (MVEV), JEV, WNV and Eastern equine encephalitis virus (EEEV). Arboviruses can also be introduced into new areas by the movement of humans, particularly as air travel now enables movement between two destinations anywhere in the world, all within the time window of a typical viraemic period. Infected arthropod vectors may also disseminate disease if they are carried on air, marine, rail or road transport. This has been pro-

**Fig. 1.2** (continued) continue to spread across the globe, with serotype subsets cycling in sequence with developing local herd immunity and virus evolution. The sudden and dramatic expansion of dengue in the early 1940s with the influx of naive adult hosts during the Pacific campaign of WWII seeded much of the subsequent global epidemic activity. After successful vector eradication programs in the first half of the twentieth century, dengue was re-introduced into the Americas, first into Cuba in 1977 with subsequent spread throughout tropical South America as its vector, *A. aegypti* reclaimed its earlier territory. Chikungunya virus (CHIKV); CHIKV exploded out of Africa following a large epidemic on the island of La Reunion in 2005. A single mutation in the virion surface protein facilitated a spillover into a new mosquito host, *A. albopictus* and further, global spread, reaching the Americas in 2014. West Nile virus (WNV); WNV was known to circulate within Africa from the 1930s when it was first isolated, spreading to the Middle East and Europe in the 1990s. What is thought to be a single transportation event resulted in WNV landing in New York from Israel in 1999. The subsequent march of WNV west across the North American continent was driven primarily by migration of its bird hosts, resulting in its wide distribution across the Americas over the subsequent decade. Zika virus (ZIKV); ZIKV was first isolated in 1947 but it wasn't until 1954 that the first human cases were reported in Nigeria. While its spread across Africa and into India and South East Asia were noted, it wasn't until a large epidemic on the island of Yap in 2007 highlighted the potential importance of ZIKV to human health. The subsequent epidemic in French Polynesia in 2013/2014 was thought to be the seed for its emergence in Brazil in 2015. The cause of the explosive nature and severity of the resulting epidemic over 2015–2016 is still the subject of considerable conjecture – the presence of a naïve population primed with a high level of potentially enhancing dengue-specific antibody, viral genome mutation or a combination of both along with additional factors remain possibilities

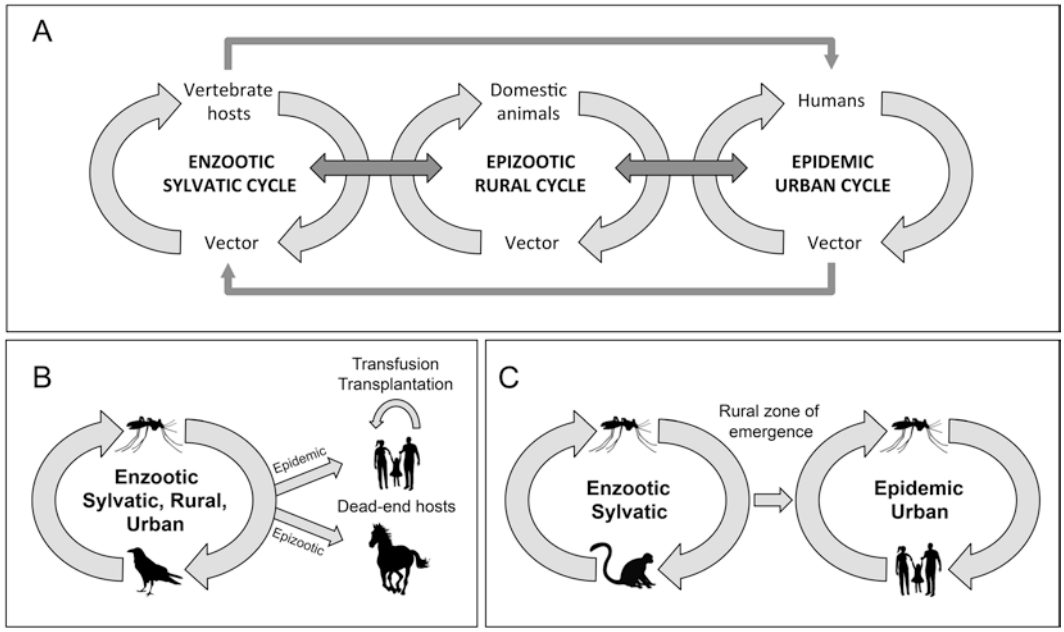


**Fig. 1.3 Arbovirus transmission cycles.** A. Enzootic (low level endemic virus transmission within native animals), epizootic (higher level epidemic transmission, usually within domestic animals) and epidemic cycles within humans are inextricably linked for many arboviruses, with spillover events driving the dynamics of each cycle. B. For some arboviruses (e.g., WNV) the epidemic and epizootic spillover from the enzootic cycle are unimportant for arbovirus survival, as these are dead-end hosts that do not act as reservoirs for further rounds of transmission. Exceptions are driven by specific human activity; e.g., transfusion and transplantation. C. For some arboviruses (e.g., DENV and ZIKV), the epidemic cycle in humans can be self-sustaining given the high levels of viraemia resulting in efficient transmission between vector and humans without the need for an enzootic amplification host. Nevertheless, occasional spillover events from the enzootic sylvatic cycle have been recorded

posed as the most likely mechanism for introduction of WNV into the USA in 1999.

Some hosts that become infected may not be sufficiently viraemic or may not be infected with sufficient regularity to contribute to the stable maintenance of virus populations and are referred to as incidental hosts. Incidental hosts may or may not show symptoms. For many arbovirus infections, humans are usually an incidental host, often being a dead end in the transmission chain.

Arthropod-borne viruses are distinguished from other animal viruses because of their ability to infect both vertebrate and invertebrate hosts. The virus replicates within the cells of the arthropod vector before being transferred to a susceptible host [16]. Occasionally, arthropods may also transmit viruses by mechanical transmission with the vector simply transferring the virus from an infected to a susceptible host without replication in the vector itself. Direct transfer from an infected to an uninfected vector during co-feeding on a naïve host has also been reported.

Invertebrate hosts include mosquitoes, sandflies, ticks and culicoides (biting midges) although most arboviruses have been recovered from mosquitoes. While transmission of arboviruses most often follows the bite of the infected arthropod, transmission has also been reported in other ways. European TBEV can be acquired by drinking the milk of infected goats, VEEV (in cotton rats) apparently via urine or faeces infecting the nasopharynx, WEEV possibly through aerosol from a patient and WNV and DENV has been transmitted by blood transfusion. DENV, JEV, WNV and CHIKV have all been transmitted from mother to foetus following infection during pregnancy, but this is considered rare. In contrast, an unusually high rate of maternal to foetal transmission has been observed in the recent ZIKV outbreak in Brazil. The finding of Zika virus in a

range of bodily fluids including semen, tears and sweat, as well as the apparently high rate of CNS invasion following foetal infection remains to be fully explained [18].

## 1.4 What Diseases Do They Cause?

The vast majority of arboviral infections lead to either an asymptomatic or non-specific mild illness. Only a handful of those who are infected develop clinical symptoms for which the individual arbovirus is known. For the flaviviruses, the case to infection ratio varies considerably, from very low (e.g. around 1:300 for encephalitis due to JEV) to quite high (1:4 for fever as a result of DENV infection). It may be higher during epidemic (rather than endemic) disease activity, and will be modified by a range of other factors, including host susceptibility and virus strain. The major burden of disease is at the extremes of life, the very young and the elderly. For alphavirus infections, particularly those causing arthritis, the ratio of symptomatic to asymptomatic infection is typically higher than that of the flaviviruses, from 1:40 to 1:3. If clinical manifestations arise after infection they do so after an intrinsic incubation period lasting from a few days to a week or more. During that time the virus replicates at the site of inoculation, then further amplifies within the reticuloendothelial system before it becomes viraemic and spreads to target organs.

Symptomatic arbovirus infection often presents as a systemic febrile illness. In the early stages, this illness may be non-specific or even suggestive of other viral illnesses, including gastrointestinal and respiratory infections. In a developing world setting featuring an increased burden of disease, this can be particularly problematic, often delaying appropriate clinical management. On-going development of low cost, point-of-care diagnostics to provide early and effective diagnosis, remains an important goal of current research efforts. Headache is common and may be severe and accompanied by meningitis. Muscle and joint aches and pains are common, especially with alphavirus infections where many also develop joint swelling and stiffness. Rash may be present and is usually generalised and maculopapular, although occasionally vesicular. Petechial rashes are less common and may be an early indicator of haemorrhagic fever. In the vast majority of cases, febrile illness is followed by recovery. In the remainder, illness may progress to one of the more severe forms of disease, sometimes following a few days of remission. These can be broadly grouped into those arboviruses causing haemorrhagic fever, encephalitis or polyarthralgic illness (for further discussion see [21]).

## 1.5 What Is Driving Arbovirus Expansion?

As noted above, humans are often no more than incidental hosts for arbovirus infection. However, their behaviour, along with environmental factors can play a significant role in the activity and spread of these viruses [20] with many human activities known to encourage transmission [4, 7, 17, 21]. The construction of dams and extensive areas of irrigation promotes the breeding of large numbers of mosquitoes that is otherwise unusual for these geographical locations. For instance, the development of rice fields encourages breeding of *Cx. tritaeniorhynchus* in Sarawak that in turn fosters the spread of JEV, and *Mansonia uniformis* and *Anopheles gambiae* in Kenya spreading CHIKV, o'nyong-nyong virus (ONNV) and Sindbis virus (SINV). The seasonal removal of old vegetation in Sarawak leads to heavily polluted pools that support large populations of culicines. Driving cattle into marginal forest areas in India promotes the growth and transport of ticks, and the incursion of people into forest areas exposes them to infection with YFV and the tick-borne diseases. In many countries, the practice of using large containers for water storage has helped to increase *Aedes aegypti* populations and the consequent transmission of DENV, CHIKV and other viruses vectored by this species.

Environmental conditions, particularly rainfall, temperature and humidity, also have an important role to play in arbovirus transmission

cycles with the result that arbovirus activity is often seasonal. For example, the alphaviruses transmitted by mosquitoes in temperate regions cause disease in summer during periods of increased vector activity [2]. In tropical areas, human infections caused by arboviruses usually occur during the wet season, with increased virus activity again coinciding with periods of high vector numbers. As mosquito larvae and pupae are aquatic, the abundance of arthropod vectors is directly affected by the amount of rainfall and flooding in a particular region. Rainfall is also required to maintain permanent water bodies, or in some cases create temporary water bodies that provide a sanctuary and breeding grounds for water birds that act both as mechanisms for introducing the virus into that area and for amplifying the virus. Humidity can also play a role, with increased humidity facilitating increased survival of mosquitoes. Temperature can also affect the length of the extrinsic incubation period with most studies showing that the extrinsic incubation period for mosquitoes is shorter at 30 °C than at lower temperatures thereby ensuring that mosquitoes become 'infectious' in a shorter time after ingestion of an infected blood meal. High external temperatures on the other hand may have adverse effects on vector survival.

Global climate change will significantly impact on arbovirus transmission cycles over time [7]. The amount and extent of rainfall, frequency and heights of high tides, temperature, humidity and consequent movement of vertebrate hosts and human populations will all contribute. The extent and timing of these environmental changes is unknown, but because of the complex interactions between these viruses, their hosts and vectors as well as the environment, it is likely that even minor changes will affect arbovirus activity in different regions. This may result in an increased number of cases and/or a greater geographical spread of these viruses [5, 12–14]. Climate change impacts on arbovirus transmission are already being played out, such as in the dramatic resurgence of West Nile virus in the US in 2012. This emergence was linked to a record-breaking drought across the US in combination with sporadic, end of season rains and local complacency with regards vector control. Mosquito numbers in metropolitan areas surged, with consequent increased transmission of WNV.

As noted above, the last two decades have seen a dramatic increase in the emergence and/or re-emergence of a number of serologically distinct arboviruses [6, 15, 21]. Ecological factors have played a pivotal role in this expansion with a rich array of demographic, cultural and societal changes impacting arbovirus transmission between vectors and hosts. Understanding some of these mechanisms will provide insight into future predictions of arboviral activity, disease risk assessment and control.

Southeast Asia has experienced an exponential increase in the number of arbovirus related epidemics; YFV and RVFV cases are on the rise in Africa; South America has seen the re-emergence of DENV and YFV and the emergence of ZIKV; and the incursion into North America and Europe of some arboviruses previously restricted to the tropical zone (e.g., CHIKV and DENV) all serve to emphasize that no region of the globe is resistant to these threats. Their spread has been linked to a range of complex factors.

It is recognized that biodiversity plays an important role for arbovirus maintenance with African, Southeast Asian and South American tropical regions, particularly their rainforests, considered reservoirs for many of these arboviruses. However, it is the demographic and societal changes in the human population during the past two to four decades that has had the biggest impact on the revival of arbovirus infections. Unprecedented population growth has been the underlying driver of many of the changes that have affected transmission dynamics. These include rapid urbanization, deforestation, new dams, an expansion in irrigation, and a lack of closed water storage containers. The resulting increase in mosquito populations and their closer contact with human communities has contributed to increased virus, and hence disease transmission. The changing demographics that have resulted from modern transportation have also played a significant role in the distribution and transmission dynamics of arboviruses. While the

geographic distribution of some arboviruses and their mosquito vectors has expanded, resulting in recurrent and larger outbreaks (e.g., DENV), others have invaded new geographic regions having taken advantage of susceptible mosquito vectors and hosts to become established (e.g., WNV, CHIKV and ZIKV). Clearly, factors such as the absence of herd immunity and a lack of vector control have been instrumental in the re-emergence of several arboviral infections (e.g., CHIKV, JEV, and more recently, ZIKV).

The changing epidemiological patterns of arboviruses are complex and unique to each virus, however virus evolution can also be an important driver of the emergence of these new disease threats. One clear example of how virus evolution has re-defined the epidemiology of an arbovirus infection is the re-emergence and spread of CHIKV. Sequence analyses have shown that CHIKV originated from Africa and was later introduced in to Asia with the delineation of three phylogenetic distinct clusters: East-, Central- and South-African (ECSA), Asian, and West-African clusters [10]. Analysis of CHIKV strains isolated from the Indian Ocean outbreaks indicated that it was more closely related to the ECSA cluster than the Asian or West African clusters. However, 90% of the CHIKV strains isolated revealed a nucleotide mutation leading to an alanine to valine change at position 226 in the virus E1 glycoprotein. This single amino acid change was of particular interest as it was exclusively found in CHIKV isolated from *Ae. albopictus*. This mutation was subsequently shown to be associated with adaptation to *Ae. albopictus* with an increased fitness in this vector attributable to the loss of cholesterol dependence for virus growth. This adaptation has allowed CHIKV to replicate and disseminate more efficiently in *Ae. albopictus*.

More recently, another arbovirus that has generated significant interest is ZIKV. First isolated from sentinel primates in the Zika forest of Uganda in 1947, it was also isolated in sub-Saharan Africa and South East Asia [8]. Few human cases were previously noted but in 2007, major human outbreaks were reported on Yap Island, Micronesia. Preliminary phylogenetic data showed two distinct ZIKV lineages circulating in Africa and a third lineage formed by the Micronesia and Malaysia strains [8]. The subsequent spread of ZIKV to the Americas in 2015 and the extensive epidemic it caused is now being attributed, in part, to specific mutations found in these circulating South American viruses.

## 1.6 Conclusion

In a world of rapid travel and transportation, many other arboviruses have the potential to spread geographically and cause serious outbreaks. What is of concern is that most of these new introductions are not detected until an epidemic or some unusual situation signals the alarm, often too late to effect control. The world is finally coming to grips with the notion of epidemic preparedness and the realization that significant and coordinated effort will be required to effectively deal with the inevitable future threats to global health posed by arboviruses on the move.

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# 2 Historical Perspective of Arboviruses in Mozambique and Its Implication for Current and Future Epidemics

Eduardo Samo Gudo, Kerstin Falk, and Julie Cliff

**Abstract**

Mozambique is a tropical country situated in the Southern part of Africa, a region where data on the burden and epidemiology of arbovirus is presently quite scarce although the frequency of outbreaks caused by arboviruses is rapidly increasing. Outbreaks of dengue fever have been reported in Mozambique, Angola and Tanzania and a recent unprecedented outbreak of Yellow fever has been recorded in Angola. These new outbreaks collectively suggest that arboviruses, and specifically flavivirus infections, are endemic in Mozambique.

Although recent data on arbovirus activity is scarce, the work of *Kokernot* et al. [R.H. Kokernot, K.C. Smithburn, A.F. Gandara, B.M. Mc'Intosh and C.S. Heymann Anais Inst Med Trop (1960), 17:201–230] describes seroepidemiological and entomological studies carried out in several parts of Mozambique during the 1950s. Complementary seroepidemiological investigations on arboviruses that were conducted in the early 1980s also found serological evidence of several arboviruses which included Dengue, Chikungunya, Zika, Rift Valey Fever, Sinbdis virus, Wesselsbron, Bunyamwera, Pongola and Bawamba Fever and Yellow Fever.

Notably the first description of Chikungunya virus in 1952–1953 in Tanzania also included reported cases in northern Mozambique. Furthermore, DENV serotype 3 was for the first time described in northern Mozambique in 1984 and 1985. Since several arboviral infections result in acute self limiting fever they have remained unsuspected for several decades. However, it is well known that during the 1980's intensive malaria control initiatives which included massive distribution of bed nets, community education and indoor and outdoor spraying campaigns were implemented. It is possible that these measures may have influenced the epidemiology of arboviruses. However, the impact of these interventions in controlling the spread of arboviruses is not known.

In conclusion, the old literature on arboviruses in Mozambique is relevant for assessing the gaps and current risk of occurrence of these pathogens at the region, particularly in a time in which they are spreading worldwide.

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**Keywords**

*Arboviruses* · *Dengue* · Chikungunya · Zika virus · Malaria seroepidemiology

## 2.1 General Description of Mozambique in the Context of Spread of Arboviruses

Mozambique is situated on the southeast coast of Africa, with a total land area of around 800,000 sq. km, which is slightly less than the size of the state of California, and has a population of close to 30 million people. The climate is tropical with two distinct seasons: the rainy season from November through April and the dry season during the rest of the year. Due to its geographical location, Mozambique has long been considered a unique and important hub-"tropical corridor" – for the movement of arboviruses in the region. Its ecological characteristics combined with its geographical location, make Mozambique a player in the international epidemiology and spread of arboviruses. Mozambique is a multi-cultural country with strong commercial and cultural trade with different regions in the world, specially with Asia where strong relationships with countries such as India and China have been established. Because of this, Mozambique is at particular risk of importation from and exportation of infectious diseases to Asia and other places in the region. For this reason, tracking the epidemiology of arboviruses in Mozambique is of utmost importance to understanding the regional and global pattern of their spread. Historical data demonstrate an active arboviral acitivity in Mozambique, which may have played an important role in the current epidemiology of arboviruses in the region and other continents with intense past and current trade links with Mozambique. In this context, we review the detailed studies in order to identify the gaps that exist at present to control arbovirus activity in Mozambique.

The seroepidemiological study conducted by [9] was an exhaustive investigation based on detecting, in residents of Mozambique, neutralizing antibodies against arboviruses that had previously been isolated in East Africa and the Union of South Africa. The study was undertaken to obtain information on the etiology of "unexplained fevers", so that useful information can be obtained, and to draw the attention of the medical authorities to the potential role of these viruses as causal agents of human diseases in Mozambique [9].

**Arbovirus Activity in Mozambique** The study conducted by Kokernot et al. was carried out in the months of July and August of 1957 and covered 29 different localities stretching over 8000 km of the entire country of Mozambique (Fig. 2.1). The map in the figure shows that both coastal and interior terrains were sampled. In this study Kokernot et al. collected blood samples from indigenous Mozambicans and then tested for different arboviruses using serological assays for detection of neutralizing antibodies. A total of 29 localities across the country were surveyed, which gives a good representativeness of the country.

The study enrolled 30 local residents in each of the selected locations who had never traveled outside the residence area in their lifetime. These samples were subjected to neutralization tests with 15 different viruses and a summary of the results is presented in Table 2.1. The viruses with high prevalence rates of neutralizing antibodies included Chikungunya (21.0%), Wesselsbron (15.9%), Bunyamwera (24.1%), Pongola (23.2%) and Bwamba Fever (24.7%) (Table 2.1).

This study, together with a concurrent entomological survey conducted in several localities across the country by [17] between 1957 and 1959, found that *Aedes* mosquitoes with potential to transmit different species of arbovirus were present in several parts of the country. The fact that these two studies were conducted in the same period, reinforced significantly the belief that the country was an important tropical corridor with potentially high arboviral activity. However, since then, research into arbovirus prevalence has been abandoned in Mozambique and there are no

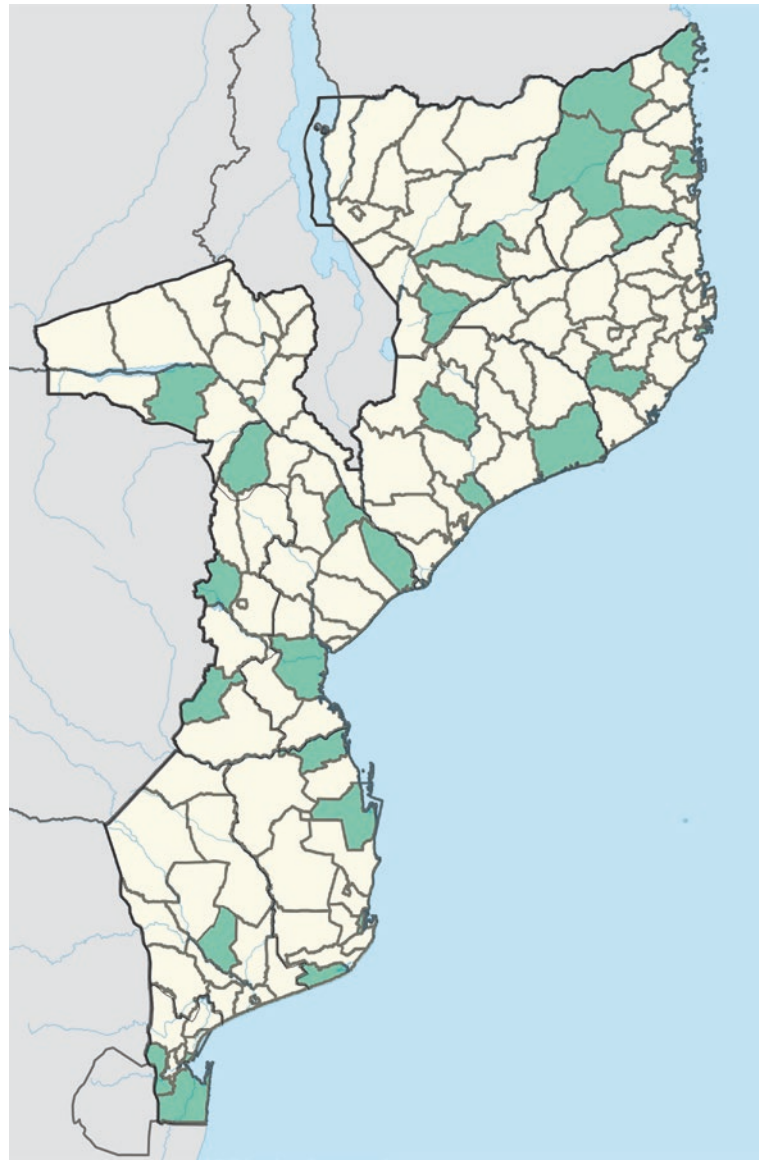

**Fig. 2.1** Administrative districts of Mozambique. In 1957, shaded areas have been surveyed for CHIKV antibodies (all with positive results) by [9]. (Taken from [5]. © Gudo, E.S.. Original image credit: Americo Feriano Jose)

records of similar detailed surveys covering the entire country. This long drought in information on arboviral activity was broken very recently when a Dengue outbreak caused by Dengue serotype 2 was recorded in Pemba city in northern Mozambique [11], where a high density of *Aedes* was also found during the outbreak [8]. There is strong reason to believe that several other outbreaks of dengue and other arboviruses occurred in the 1980s and 1990s, but due to lack of diagnostic capacity and lack of awareness as well as other health priorities, they were under-reported.

## 2.2 Initial Report of Zika in Mozambique

Public health interest in ZIKV has increased dramatically since 2015 when the virus experienced an explosive spread in south and central America and the Caribbean [3, 12], with reports of its association with an increase of cases of microcephaly and other neurologic disorders in Brazil prompting the World Health Organization (WHO) to declare the ZIKV epidemic in South

**Table 2.1** Summary of neutralization tests performed with 15 viruses in the presence of sera from indigenous donors living in Mozambique. [9]

| | Children | | | Adults | | | All ages | | |
|---|---|---|---|---|---|---|---|---|---|
| | Number of | Neut.poisitive | | Number of | Neut.poisitive | | Number of | Neut.poisitive | |
| Virus per group | participants | No. | % | participants | No. | % | participants | No | % |
| Group A | | | | | | | | | |
| Chikungunya | 404 | 16 | 4.0 | 467 | 175 | 37.5 | 871 | 191 | 21.9 |
| Semliki | 388 | 13 | 3.4 | 449 | 31 | 6.9 | 837 | 44 | 5.3 |
| Sindbis | 399 | 21 | 5.3 | 456 | 43 | 9.4 | 855 | 64 | 7.5 |
| Middelburg | 142 | 1 | 0.7 | 160 | 2 | 1.3 | 302 | 3 | 1.0 |
| Group B | | | | | | | | | |
| Wesselsbron | 403 | 40 | 9.9 | 467 | 98 | 21.0 | 870 | 138 | 15.9 |
| Spondweni | 399 | 5 | 1.3 | 459 | 30 | 6.5 | 858 | 35 | 4.1 |
| H 366 | 404 | 10 | 2.5 | 467 | 29 | 6.2 | 871 | 39 | 4.5 |
| Uganda S | 151 | 2 | 1.3 | 185 | 10 | 5.4 | 336 | 12 | 3.6 |
| Zika | 107 | 2 | 1.9 | 142 | 8 | 5.6 | 249 | 10 | 4.0 |
| Bunyamwera group | | | | | | | | | |
| Bunyamwera | 404 | 66 | 16.3 | 466 | 144 | 30.9 | 870 | 210 | 24.1 |
| Without group | | | | | | | | | |
| Pongola | 403 | 65 | 16.1 | 467 | 137 | 29.3 | 870 | 202 | 23.2 |
| Bwamba fever | 149 | 20 | 13.4 | 142 | 52 | 36.6 | 291 | 72 | 24.7 |
| Rift valley fever | 397 | 5 | 1.3 | 436 | 18 | 4.1 | 833 | 23 | 2.8 |
| Simbu | 57 | 0 | 0 | 61 | 0 | 0 | 118 | 0 | 0 |
| AR 344 | 53 | 0 | 0 | 57 | 0 | 0 | 110 | 0 | 0 |

Note: This table is translated and reproduced from Ref. [9]

America a Public Health Emergency of International Concern (PHEIC) [15].

Despite the fact that ZIKV was first described in Africa in 1947 [13] few studies had been conducted on the continent to understand its epidemiology. However, the study published by [9] was one of the few conducted on the continent at that time and demonstrated that neutralizing antibodies against ZIKV were found in Mozambicans in several parts of the country [6]. We recently noticed that the Kokernot et al. study had been ignored by most current descriptions or reviews on historical data of ZIKV on the continent and worldwide [7, 16]. We believe that the study conducted by Kokernot et al. has been ignored, most likely because the manuscript was published in Portuguese and also due to its poor indexing [6]. However this study highlights that Mozambique was considered a hotspot for the occurrence of arboviruses, including ZIKV since the 1950s, and may have played a role in the spread of the virus in the region and to other regions. Although no recent study has been conducted to assess circulation of ZIKV in Mozambique and few studies had been conducted in the region, old data on ZIKV in Mozambique may suggest that the risk of current circulation of the virus in Mozambique is considerable.

Mozambique does not have a surveillance system for birth defects, which makes it difficult to correlate any potential link between microcephaly cases and Zika. On the other hand, there is much discussion in the scientific arena suggesting that the most studied African strains of ZIKV may not be involved in causing microcephaly. To confirm this, studies should be conducted in Mozambique and other countries in sub- Saharan Africa using newly isolated Zika virus strains as

well as historic isolates to investigate the potential for these strains to cause Zika-associated microcephaly.

## 2.3 First Report of Chikungunya in 1953 Is Tightly Bounded to Mozambique

Chikungunya virus (CHIKV) has re-emerged in 2005 as an important cause of infectious disease, mostly after the occurrence of large epidemics of CHIKV on the Indian Ocean islands and in Asia [2, 14]. Since then, the virus has rapidly expanded to become endemic in South America [14]. We revisited old literature to understand the potential role of Mozambique in the current global epidemiology of CHIKV and found that indeed, during the initial discovery of CHIKV in 1952/3 in Tanzania, a few cases were also reported in villages in northern Mozambique. This is reflected in the serosurvey by [9] that, conducted a few years later, detected neutralizing antibodies for CHIKV in all sampled places with a prevalence rate of 21.9%. Given the geographical situation, together with its commercial trade relationships to other parts of the region and the world, there is the potential that Mozambique may have contributed to the spread of CHIKV. Molecular epidemiological studies would be needed to explore this further.

The recent report of a severe case of CHIKV case with intestinal bleeding in northern Mozambique [1] suggests that several cases of severe CHIKV infection had been under-reported or misdiagnosed as malaria for many years and decades. Severe disease caused by CHIKV had not been considered until 2005 when a massive CHIKV outbreak hit Reunion Island [10]. This recent report of a severe case of CHIKV has increased the concern that CHIKV may be the etiological agent of severe disease in sub-Saharan Africa, where similar reports have not been published, mostly due to lack of a surveillance systems for CHIKV.

## 2.4 First Report of Dengue-3 in Mozambique

Dengue is the most-widespread arbovirus worldwide and historical data on its epidemiology is important to understand current and future trends of the virus. The epidemiology of Dengue is complex due to serological and genetic diversity. Mozambique has played an important role in the global epidemiology of Dengue, as the first time that Dengue serotype 3 was described in Africa was during a 1984/5 outbreak of febrile illness in Pemba city, the capital of Cabo Delgado Province in northern Mozambique [4]. This further suggests that northern Mozambique was in the past a hotspot of arboviral activity. Since Mozambique has intense trade with countries in the region as well as in Asia and South America, we believe that the country played a relevant role in the spread of this serotype. Of the two deaths reported during this outbreak, one was a Chinese traveler, and the strain circulating in Mozambique was later shown to be similar to that circulating in southeast Asia, suggesting that trade with China and other countries in Asia could have played an important role in the import of dengue-3 into Africa in the early 1980s. An entomological investigation conducted during this outbreak showed a high density of *Aedes aegypti* [4] suggesting that Mozambique had ecological conditions favorable for *Aedes* breeding and arbovirus transmission.

## 2.5 Summary

The presentation revisits history of arboviruses in Mozambique and highlights that the country may have played an important role in spread of several arboviruses such as Dengue and Zika in sub Saharan Africa and other regions. Despite of this, research and investigation on arbovirus in Mozambique was abandoned for several decades due to a changed focus to the spread of HIV/AIDS and the continuing challenges of parasitic

diseases like malaria. For this reason, there is a current lack of knowledge of arboviral activity in Mozambique. The TASW meeting highlights the past and current importance of arboviruses and the need to implement surveillance similar to the work done by [9] to better understand the current risk of arbovirus in the country and region.

**Acknowledgement** The authors thank the library of the National Institute of Health (Mozambique) for providing old literature about arbovirus in Mozambique.

## Discussion of Chapter 2 in *Dengue and Zika: Control and Antiviral Treatment Strategies*

*This discussion was held at the 2nd Advanced Study Week on Emerging Viral Diseases at Praia do Tofo, Mozambique.*
*Transcribed by Hilgenfeld R and Vasudevan SG (Eds); approved by Dr. Eduardo Samo Gudo.*

**Aravinda de Silva**: Just a quick comment about those Dengue 3 strains from the mid 1980s and their importance for Mozambique, because we showed subsequently that those strains were first described in Mozambique. I think some of those isolates came from Chinese workers in Mozambique and they spread it to the Indian subcontinent. In fact, the first major epidemic of severe Dengue caused by serotype 3 in the Indian subcontinent was very closely related to those strains from Mozambique. And then subsequently, those same strains were introduced into Panama and Nicaragua. And the severe Dengue epidemics in Latin America were also caused by a very close relative of the Mozambique viruses and so this highlights the importance of the circulating strains here in Mozambique. What kind of work is going on in terms of looking at enzootic cycles? For Dengue 3, we still do not know the sylvatic cycle – the non-primate cycle. Also for Chikungunya virus, you indicated that very early isolates came from this part of the world. Is there any work being done in this area?

**Eduardo Samo Gudo**: Not yet. This is the first and only description of Dengue 3 in Mozambique as you can see in the map in my presentation. So nothing happened after that and it's unclear and we do not know if we eliminated DENV3 from Mozambique.

**Aravinda de Silva**: In terms of understanding the enzootic cycles of Dengue and Chickungunya – it may be an important area to focus in terms of non-human primate studies.

**Gao George**: So where did the strains in Mozambique come from?

**Aravinda de Silva**: That strain was first described in East Africa in Mozambique. It was subsequently described in Kenya, it went up to Somalia. It moved into the Indian subcontinent, where the very first description of the 1985 isolates came from.

**Subhash Vasudevan**: So you said it came from China?

**Aravinda de Silva**: No, I thought the Mozambican isolates came from Chinese workers in Mozambique, who got very sick, who got something like severe Dengue. But they were here working in Mozambique.

**Maurice Demanou**: It is incredible, because the figures you presented are similar to the picture in Cameroon. But in the 60's there was a lot of research on arboviruses done by medical research institutes. Later on in the 80's, probably because of the outbreak of HIV, the arbovirus surveillance ceased. Even though there were some surveillances on yellow fever, nothing at all was done for other arbovirus research. In the year 2000, with the spread of epidemic arboviruses worldwide, people started to be interested. I think we have the same problem in Cameroon, but I do not know how you explain the 40 years' gap in the attention.

**Félix Rey**: Is there a political will now to change the situation and focus more on arboviruses?

**Eduardo Samo Gudo**: Arboviruses are seriously neglected in Mozambique, as malaria, tuberculosis and HIV are the leading cause of morbi-mortality in the country and will for sure be the focus of intervention for many

decades to come. Not just in Mozambique, this is similar in many countries in Africa. When we raise the issue of arboviruses, we are simply asked the question "How many people die per year because of Dengue and other arboviruses and how many from malaria, tuberculosis and HIV?" The fact is that it is much less than malaria, tuberculosis and HIV, so that is the end of he conversation.

**Subhash Vasudevan**: What about co-infections of malaria with Chickungunya or Dengue?

**Eduardo Samo Gudo**: There is high coinfection rates between malaria and arboviruses. This actually is something that we are very interested in, but I did not show any data here in the talk. If you are familiar with asymptomatic malaria infection – it's a problem that is mostly being ignored. We did some research in Mozambique, back in 2005, where we went to our primary schools in areas of high transmission for malaria. We took blood samples from the children – otherwise healthy looking children – and we tested for malaria not using rapid tests, but by using blood smear. We found that 55% of the children showed parasitemia in the blood and then there were some with high parasitemia in their blood. So coming back to your question of coinfection: People that have immunity against malaria, because of the continous exposure, can have parasitemia and not be sick. Imagine now if they got fever and they go to the hospital. The hospital will test first for malaria and 55% of the children that go to the hospital will be positive but mainly have other causes of fever. So that means that they will never be picked for other possible infections. It is not bad that the patients are treated for malaria because of their parasitemia, because at some point they can develop malaria or they can transmit. But other diseases would be ignored and arbovirus problems will expand. It is amazing that in several parts of Mozambique where we have carried our vector survellence studies, the population of *Aedes* is high, but the control is based on Anopheles. So arborviral diseases are ignored and have been ignored for very long because of these reasons.

**Félix Rey**: Is there any interference between the circulating parasites and arboviruses? Nobody knows?

**Eduardo Samo Gudo**: That's right, nobody knows.

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