

Review Article

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Evolution and emergence of mosquito-borne viruses of medical importance: towards a routine metagenomic surveillance approach

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Abstract

During the last two decades, the world has witnessed the emergence and re-emergence of arthropod-borne viruses, better known as arboviruses. The close contact between sylvatic, rural and peri-urban vector species and humans has been mainly determined by the environment-modifying human activity. The resulting interactions have led to multiple dead-end host infections and have allowed sylvatic arboviruses to eventually adapt to new vectors and hosts, contributing to the establishment of urban transmission cycles of some viruses with enormous epidemiologic impact. The metagenomic next-generation sequencing (NGS) approach has allowed obtaining unbiased sequence information of millions of DNA and RNA molecules from clinical and environmental samples. Robust bioinformatics tools have enabled the assembly of individual sequence reads into contigs and scaffolds partially or completely representing the genomes of the microorganisms and viruses being present in biological samples of clinical relevance. In this review, we describe the different ecological scenarios for the emergence of viral diseases, the virus adaptation process required for the establishment of a new transmission cycle and the usefulness of NGS and computational methods for the discovery and routine genomic surveillance of mosquito-borne viruses in their ecosystems.

Introduction

According to the World Health Organisation (WHO), vector-borne diseases (VBDs) cause more than 700,000 deaths annually, representing 17% of all infectious diseases around the world. During the last two decades, the emergence, re-emergence and prevalence of some arthropod-borne viruses (arboviruses) have increased worldwide, constituting a major threat to public health (Grubaugh *et al.* 2019) (Figure 1). Arboviruses are naturally maintained in transmission cycles through the arthropod vector–virus–vertebrate host triad, in which haematophagous arthropods biologically participate in virus transmission between susceptible hosts (Figure 2). Arboviruses, such as *Chikungunya virus* (CHIKV) and *Zika virus* (ZIKV), historically restricted to sylvatic regions in Africa are now widely distributed in the tropical zone, causing mild-to-severe disease in humans due to well-established urban transmission cycles (Gould *et al.* 2017). Although there are several determinants of arbovirus emergence and transmission to humans, the close contact between sylvatic vector species from several genera (e.g. *Aedes* (*Ae.*), *Anopheles*, *Culex*, *Haemagogus*, *Sabethes*, *Ochlerotatus*, *Psorophora*, *Coquillettidia*, *Ixodes*, *Amblyomma*, *Dermacentor*, *Lutzomyia*, *Phlebotomus*, *Culicoides*, etc.) and humans in the tropical zone affected by ecological changes associated with the environment-modifying human activity could be of major importance (Echeverry *et al.* 2012). A more mobile population and migration caused by political and economic instability facilitate the spread of VBDs. New infectious disease agents are introduced into areas where vector populations are not controlled, while massive influxes of susceptible people are coming to unplanned urban and deforested areas. Strengthening current control programmes is therefore fundamental to protect against these risks (Parra-Henao *et al.* 2021).

The vectors become infected by ingesting blood from a viremic vertebrate host or through transovarial or even venereal transmission (Campos *et al.* 2017; Heath *et al.* 2020). Commonly, despite changes in the transcriptomic profile of vectors associated with viral infection (Bonizzoni *et al.* 2012), arboviruses do not exert a deleterious effect on its vector, which could

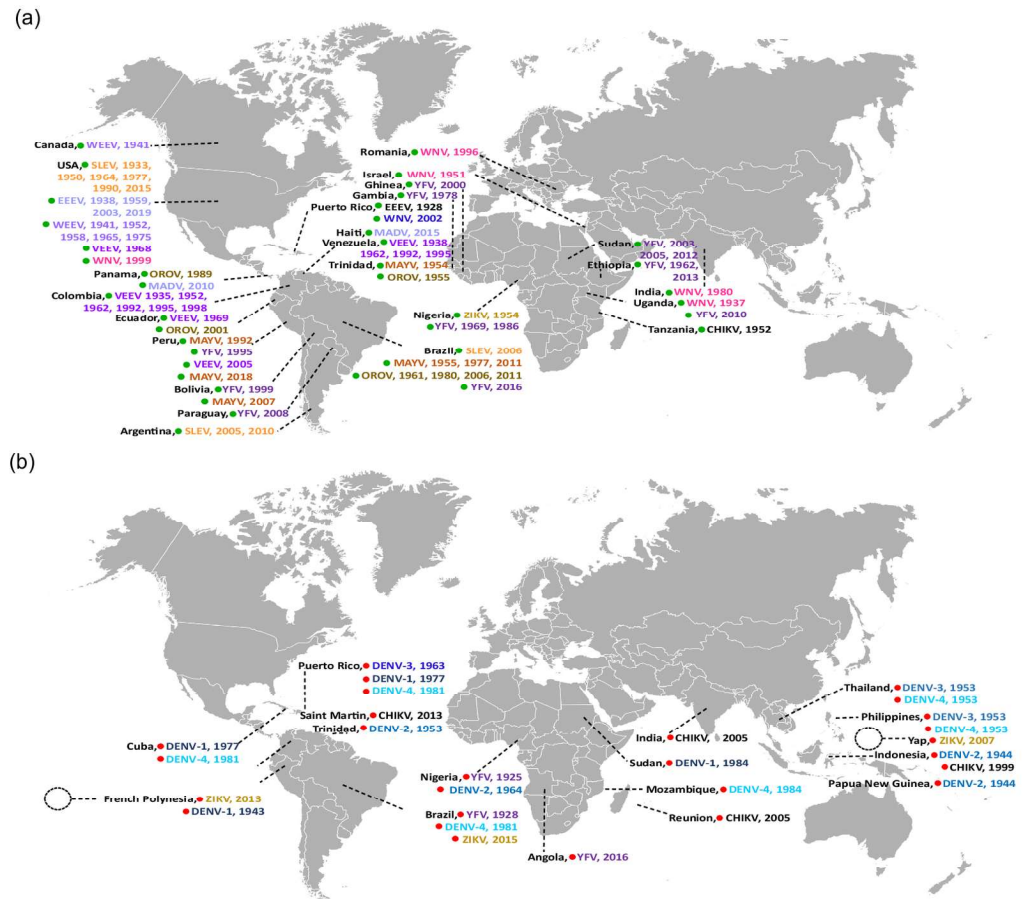


Figure 1. Emergence and re-emergence of major arboviral diseases in humans. (a) Emergence and major arbovirus outbreaks in the human population linked to the enzootic cycles. (b) Re-emergence and establishment of relevant arboviruses in urban cycles.



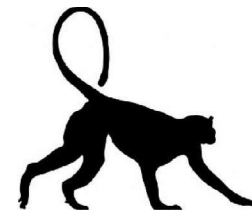
Vector determinants:

- Presence of arthropod vector species
- Vector population density
- Flying distance
- Vectorial capacity
- Other environmental factors affecting the vector life cycle



Virus determinants:

- Presence of arbovirus species
- Virus adaptation to at least an arthropod species (allowing vector competence).
- Virus adaptation to at least a vertebrate species (ability to attach and entry into a vertebrate host cell)



Vertebrate determinants:

- Presence of vertebrate host species
- Host population density
- Migration rate
- Host susceptibility
- Viremia titer
- Other environmental factors affecting the host life cycle

Figure 2. Determinants for arbovirus establishment and dynamics in different transmission settings. The establishment of a virus in a transmission cycle depends on several vector, virus and vertebrate host determinants, including the presence and abundance of the species and the ability of the virus to productively infect every vector and host species.

be crucial for long-term virus persistence. In contrast, the same virus could be from mild to extremely pathogenic in vertebrate hosts, where it is usually associated with acute infections (Weaver & Reisen 2010).

From an evolutionary ecological perspective, the resulting interactions, if sustained through time, can allow sylvatic

arboviruses to explore the fitness landscape and adapt several features including the virus tropism to a new host cell environment, leading to the host range expansion (Coffey *et al.* 2013).

If the virus is successfully amplified in the new host and blood viral loads are high enough for a second round of arthropod infection, a new transmission cycle can be potentially established with

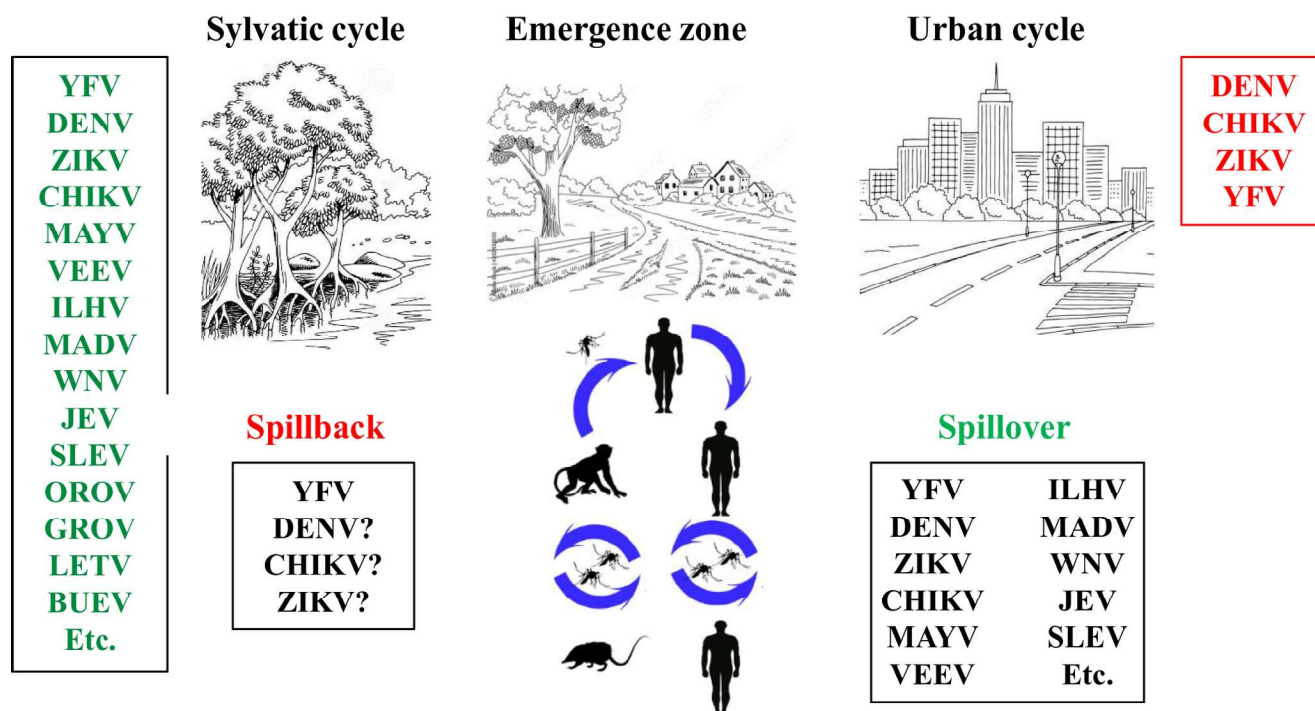


Figure 3. Arbovirus dynamics in different transmission cycles. Arboviruses have been tracked to natural environments where they have evolved to efficiently replicate and transmit from the available vectors and hosts. Ecological factors are involved in the contact between enzootic viruses and humans (spillover), sometimes leading to virus emergence. Eventually, an arbovirus from the urban cycle can again reach the species from natural environments (spillback) and become established in new enzootic cycles.

unpredictable clinical and epidemiologic consequences (Go *et al.* 2014). Arbovirus emergence is not a unidirectional spillover process; indeed, we have witnessed the spillback of *Yellow fever virus* (YFV) and serious concerns exist about the potential establishment of enzootic cycles of urban arboviruses, involving new vectors and hosts/reservoirs (Guth *et al.* 2020) (Figure 3).

Several mosquito-borne viruses with a potential impact in public health have been historically identified in enzootic cycles, mainly in the tropical zone (Vasilakis *et al.* 2019). They represent different phylogenetic origins and are the source for present and future viral emergence threats.

The implementation of vector surveillance and control programmes and massive vaccination, when possible, have helped to mitigate the impact of arbovirus in public health; however, with the increase in vector resistance to insecticides, the extensive tropical urbanisation and the expansion of the habitat of invasive and highly anthropophilic mosquito vectors, such as *Ae. aegypti* and *Ae. albopictus* (Weaver & Reisen 2010), including rural and temperate areas, the elimination and potential eradication of arboviral diseases become challenging. For these reasons, the application of robust tools that allow the identification and discovery of viruses with emergence potential, the surveillance of circulating arboviruses and the real-time monitoring are essential to identify, mitigate and predict future outbreaks and epidemics. With the development of next-generation sequencing (NGS) technologies and the metagenomic approach for the unbiased characterisation of nucleic acid sources in complex biological samples (Shi *et al.* 2016; Zhang *et al.* 2018), a new early-warning system for virus discovery and surveillance was reached, indeed imposing an enormous challenge for the current virus taxonomy assignments (Simmonds *et al.* 2017). One of the main tasks to reduce the public health impact of arboviral diseases is to expand the tools and

methods for virus identification and discovery, which is also fundamental to unravel transmission dynamics and to an adequate global genomic surveillance strategy and response to eventual emergence and re-emergence episodes, as it has been recently encouraged by the WHO (WHO 2022).

Sylvatic origin and emergence of mosquito-borne viruses

In general, mosquito-borne viruses are confined to sylvatic areas and naturally circulate between vertebrate reservoirs or hosts and permissive and competent arthropod vectors. Most of the public health relevant arboviruses originally circulated in non-human primates (NHPs) in sylvatic areas (Braack *et al.* 2018), where several mosquito species or other haematophagous arthropods would have allowed the transmission from viremic to susceptible vertebrates, enabling a transmission dynamic known as the enzootic, jungle or sylvatic cycle (Valentine *et al.* 2019). NHPs rarely show signs of disease despite their important role in virus amplification and maintenance in nature (Valentine *et al.* 2019), which could be interpreted as an evolutionarily stable strategy warranting low virulence and host survival. Phylogenetic analysis of most mosquito-borne viruses suggests an ancestral origin in the sylvatic area of the old world (Gould *et al.* 2003) with several emergence attempts and a few successful establishments in the human population. A notorious example of a successful emergence as a common process is the establishment of *Dengue virus* (DENV) in the urban cycle, with phylogenetic evidence (closely related sequences obtained from strains circulating in mosquitoes and NHPs in forests in Asia and Africa) of independent origin from sylvatic ancestors for at least three of the four DENV serotypes (Vasilakis *et al.* 2011; Wang *et al.* 2000).

As a spillover event, humans could be exposed to an arbovirus by contact with vectors entering areas of human settlements (e.g. rural or peri-urban areas of populated centres and indigenous communities living far from the cities) in search of blood meals, mainly through bridge vectors that could move and feed on several vertebrate hosts in transition zones (Hoyos *et al.* 2021); by the direct incursion of people in sylvatic settings for several activities, such as hunting, deforestation and mining (Valentine *et al.* 2019); by the contact of a vertebrate host or reservoir from the enzootic cycle with vectors from the urban area that could lead to virus adaptation to a new potential vector and host; and finally, by the initial adaptation to domestic vertebrates; the virus could reach the human host. The result of the interaction of a new viral entity with humans could be unpredictable as the introducing virus might not find a suitable or permissive host to replicate and reach enough viral titers to establish a second round of infection (Weaver *et al.* 2018); therefore, humans act as a dead-end host, impeding new rounds of transmission from this source (Ellwanger & Chies 2021). For instance, it has been reported that *Japanese encephalitis virus* (JEV) infects swine, *Rift Valley fever virus* (RVFV) infects cattle and sheep, *West Nile virus* (WNV) infects birds and *Venezuelan equine encephalitis virus* (VEEV) infects equids, with eventual or sometimes frequent spillover events to humans (Guzmán-Terán *et al.* 2020).

Under certain conditions, arboviruses might find an appropriate environment to establish a transmission cycle in a new environment for a prolonged time (Vasilakis *et al.* 2011). First, vector competence is crucial for the success in the process. Several features are required for a productive viral infection of the vector, including the presence of appropriate receptors and cell factors in the cells at the midgut and salivary glands to allow virus entry and successful replication and to overcome the different mosquito barriers to become competent for transmission (Kuno & Chang 2005). In addition, the infection should not negatively affect the vector's life cycle. Second, the arbovirus should reach a naïve or significantly susceptible host population without history of previous immunological challenges by the infecting arbovirus or other viruses belonging to the same serocomplexes. Third, in the fact of virus inoculation through haematophagous arthropod bite, viraemia levels should be high enough to sustain secondary rounds of vector–host transmission (Pereira dos Santos *et al.* 2018; Valentine *et al.* 2019).

Mosquito-borne viruses with major public health impact

During the last decades, different arboviruses have successfully emerged or re-emerged in urban settlements, such as YFV, DENV, CHIKV, ZIKV, WNV, VEEV and JEV, causing small outbreaks or even epidemics of major importance (Weaver & Reisen 2010).

YFV is the representative species of the family *Flaviviridae* and genus *Flavivirus*. Sailing from Africa to the Americas during the colonisation period, YFV became established in urban transmission cycle during the 15th and 16th centuries, involving *Ae. aegypti* (Barrett & Monath 2003; Reed *et al.* 2001). In 1937, the use of the 17D vaccine was implemented for the first time by attenuating an isolated strain of an asymptomatic individual. This vaccine was used in population older than 9 years and is still administered in endemic countries (Frierson 2010; Theiler & Smith 1937). In 1961, most of the American countries were considered free of *Ae. aegypti*, by the intense and efficient vector control and eradication campaigns led by WHO, and the efforts of immunisation

through administration of the vaccine achieved the successful interruption of the urban cycle (Soper 1963). However, YFV managed to infect sylvatic mosquitoes, such as *Sabethes* spp. and *Haemagogus* spp., and new world NHPs, establishing a successful sylvatic transmission cycle in different countries in the Americas. While only sporadic epizootics and human cases were since annually reported from endemic countries in the Americas, a major outbreak was registered in the municipalities of Minas Gerais, Sao Paulo and Rio de Janeiro, Brazil during 2016–2019, with at least 2,240 human cases and 760 deaths (Sacchetto *et al.* 2020).

DENV is the flavivirus of major epidemiologic impact, with at least 390 million infections worldwide each year, including 96 million symptomatic cases and around 50,000 deaths (Bhatt *et al.* 2013). Four serotypes comprise the DENV serocomplex, all of them currently circulating in the urban cycle involving the human host and *Aedes* spp. as vectors, mainly *Ae. aegypti*. There is phylogenetic evidence for the independent emergence of at least three of the four DENV serotypes, from forest areas in Africa and Asia (Wang *et al.* 2000). Despite efforts to develop a vaccine, its use is currently limited to people with previous DENV exposure, and the intensified control of the mosquito vector has shown to be unfruitful, leading to virus maintenance with DENV epidemic periods happening every 3–5 years in hyperendemic countries where different serotypes are co-circulating (Laiton-Donato *et al.* 2019).

CHIKV belongs to the family *Togaviridae* and genus *Alphavirus*. It was first isolated in Tanzania in 1952 (Robinson 1955) and was confined to Asia and Africa until the 1960s–1970s, with sporadic reports (De Ranitz *et al.* 1965). The virus re-emerged in epidemic form in East Africa in 2004 and quickly spread to tropical and subtropical countries in Africa, Asia and Oceania. In 2013, CHIKV reached the Caribbean through Saint Martin island and in short time it spread to several continental countries of the Americas (Morrison 2014; Mowatt & Jackson 2014; Tsetsarkin *et al.* 2007; Weaver 2014). CHIKV Asian genotype was successfully disseminated through the Americas owing to the widespread and high densities of *Ae. Aegypti*, especially in urban areas, while the ECSA (East-Central-South African) genotype spread mainly in Europe where the A226V amino acid substitution in the E1 protein conferred the virus the ability to successfully replicate in the vector, *Ae. albopictus* (Tsetsarkin *et al.* 2007).

ZIKV belongs to the family *Flaviviridae*, genus *Flavivirus*. It was isolated for the first time in Uganda in 1947 (Dick 1952). The first human cases were reported in Nigeria during 1954 (MacNamara 1954) and ZIKV distribution was limited to Africa and Asia until 2007, when it spread to different Pacific Islands (Attar 2016; Hayes 2009). In 2015, ZIKV was introduced to Brazil, presumably from French Polynesia (Musso 2015; Zanluca *et al.* 2015) and subsequently dispersed to several countries of the Americas (European Centre for Disease Prevention and Control 2018; Fauci & Morens 2016). ZIKV has been involved in economic and social burden for public health systems for the atypical disease manifestations, among them, microcephaly in neonates (Pan American Health Organisation 2015; Ventura *et al.* 2016) and Guillain Barré syndrome (Oehler *et al.* 2014). Moreover, it has been described the possibility of sexual transmission of ZIKV (Moreira *et al.* 2017; Musso *et al.* 2015). The high density of *Ae. aegypti* in urban settings and ineffective vector control measures have sustained endemic/epidemic DENV transmission from 1970s and have been determinant for the rapid dissemination of CHIKV and ZIKV in the Americas.

WNV is a member of the genus *Flavivirus*, family *Flaviviridae*. It was first isolated in Uganda in 1937 and since then, it has spread extensively to the Middle East, Europe, South Asia and Australia. The first urban epidemic was reported in 1996 in Bucharest, Romania, and the first report in America was in 1999 after an arboviral encephalitis outbreak in New York City (Nash *et al.* 2001). WNV has migratory birds as the main amplifying hosts that occasionally transmit the virus to domestic birds and mammals, including horses and humans through the bite of mosquitoes from the genus *Culex*. Mammals act as dead-end hosts as they are accidental hosts that do not play an essential role in maintaining the transmission cycle (Johnston & Conly 2000). Just before WNV was detected in human population in the Americas, an unusual increase in deaths of crows was observed in New York city and the virus responsible for the outbreak was demonstrated to be closely related to the virus that caused an outbreak of disease in geese in Israel in 1998 (Weaver & Reisen 2010). This zoonosis underscores the importance of the One Health approach to establish stronger links between animal and human disease and the potential use of animals as sentinels for outbreak prediction and surveillance.

VEEV belongs to the genus *Alphavirus*, family *Togaviridae*. The virus was first isolated in 1935 after equine outbreaks in Venezuela (Lundberg *et al.* 2017). It is mainly distributed in South and Central America. The principal host of the epizootic cycle is horses and main vectors are *Ae. taeniorhynchus* and *Culex* species, while the human is an accidental host (Zacks & Paessler 2010). During the epizootic cycle, the equine fatality rate was 19–83% and 14% of encephalitis incidence in humans has been estimated (Zacks & Paessler 2010). There are six antigenic subtypes (I–VI), and only the subtype I strains have been associated with human disease (Forrester *et al.* 2017; Kumar *et al.* 2018).

JEV belongs to the family *Flaviviridae*, genus *Flavivirus*, and the virus was isolated from a human brain in the 1930s (Solomon 2006). There is an estimation of 30,000–50,000 cases of Japanese encephalitis each year with a fatality rate as high as 30% and peaks each 2–15 years (WHO 2019). Japanese encephalitis is found especially in Asia, Western Pacific and Australia (Erlanger *et al.* 2009). JEV has been isolated from humans and farm animals, and the principal vectors are *Culex tritaeniorhynchus* and *Culex vishnui* (Solomon 2006). At the phylogenetic level, JEV has been classified into four genotypes, the genotype 1 being the more frequent during the last 30 years (Gao *et al.* 2013).

Worldwide, there is a plethora of arboviruses that could have the potential to emerge, but our knowledge of their ecological dynamics and adaptive potential is limited. Among them, *Mayaro virus* (MAYV), *Usutu virus* (USUV), *Oropouche virus* (OROV), *RVFV* and *Saint Louis encephalitis virus* (SLEV) have reached some interest (Esposito & Fonseca 2017; Gould *et al.* 2017) and justify further surveillance.

Arbovirus spillover from the urban cycle

While spillover events have been widely reported, evidence of the establishment of sylvatic cycles by arboviruses circulating in urban areas, known as spillover, is scarce and only represented by the new word sylvatic YFV cycle, despite being plausible for other arboviruses due to the proximity of peri-urban, rural and sylvatic areas in several scenarios of the tropical world that allow the interaction between arboviruses and potential new vectors and vertebrate hosts (Figure 3).

Despite YFV being officially eradicated from the urban cycle in the Americas, epizootics and human cases have been periodically reported ever since (Cunha *et al.* 2019), involving new world NHPs from the genera *Aotus*, *Alouatta*, *Cebus*, *Ateles*, *Callithrix* and *Saimiri* and mosquito vectors from the genera *Haemagogus* and *Sabethes* (Domingo *et al.* 2018). While it is uncertain if the circulating sylvatic YFV in the Americas has conserved its ancestral biological ability to infect the urban vector, *Ae. aegypti*, vaccination coverages in areas of demonstrated natural transmission have been the major measure to avoid its re-emergence in the urban cycle, being used massively during the 2016–2017 outbreak in Brazil (WHO 2017).

Ecological factors, and the viral, vector and vertebrate features discussed above as determinants for the spillover event and viral emergence could be critical for the success of the arbovirus spillover, mainly those related to the close and sustained contact between original and potentially new hosts leading to the gradual and successful virus adaptation (Dennehy *et al.* 2006), and to the ability of the virus to reach virus loads enough to warrant transmission through the mosquito bite.

The historical circulation of DENV, and the recent re-emergence and establishment of CHIKV and ZIKV in the urban cycle in the Americas represent a potential risk for a new sylvatic cycle to be established (Althouse *et al.* 2016; Figueiredo 2019). Human outbreaks, as well as the natural infection of urban and sylvatic mosquitoes in peri-urban and rural settings have been documented (Alencar *et al.* 2021; Catenacci *et al.* 2021; Hoyos-López *et al.* 2019; Lee *et al.* 2021).

Recently, vector competence of the sylvatic mosquitoes *Haemagogus leucocelaenus* and *Ae. terreus* for CHIKV transmission was experimentally demonstrated (Lourenço-de-Oliveira & Failloux 2017), and although these species are mainly confined to the tropical rainforest, their populations can establish in human-modified rural areas (Cardoso *et al.* 2010). Seroprevalence studies have failed to show evidence of CHIKV circulation in NHPs in the Americas (Moreira-Soto *et al.* 2018), while in Africa, it was determined that NHPs are amplifiers (Althouse *et al.* 2018), and in Asia, a low seroprevalence of CHIKV in NHPs was described (Rougeron *et al.* 2015). Mathematical models have predicted that ZIKV could establish sylvatic transmission cycles in South America (Althouse *et al.* 2016) near to urban regions where it is endemic (Vorou 2016), due to the presence of the YFV vectors, and abundant NHP species suitable for acting as sylvatic hosts, as it has been experimentally supported (Li *et al.* 2022).

Metagenomic NGS for the identification and discovery of new mosquito-borne viruses with potential public health implications

Conventional methods have been used to detect the viral agent through viral isolation in permissive cell cultures and virus characterisation via serologic and sequence-based molecular methods, and several arboviruses were detected or discovered, and associated with animal and human health through this classic virologic approach during the last seven decades (Kauffman *et al.* 2010). However, only a fraction of viruses can be detected in this way due to the low sensitivity of cell culture to allow replication of all possible species of arboviruses that can be present in a biological sample, and the fact that conventional nucleic acid amplification techniques require *a priori* knowledge of the virus genetic background, limiting the whole description of the virodiversity and

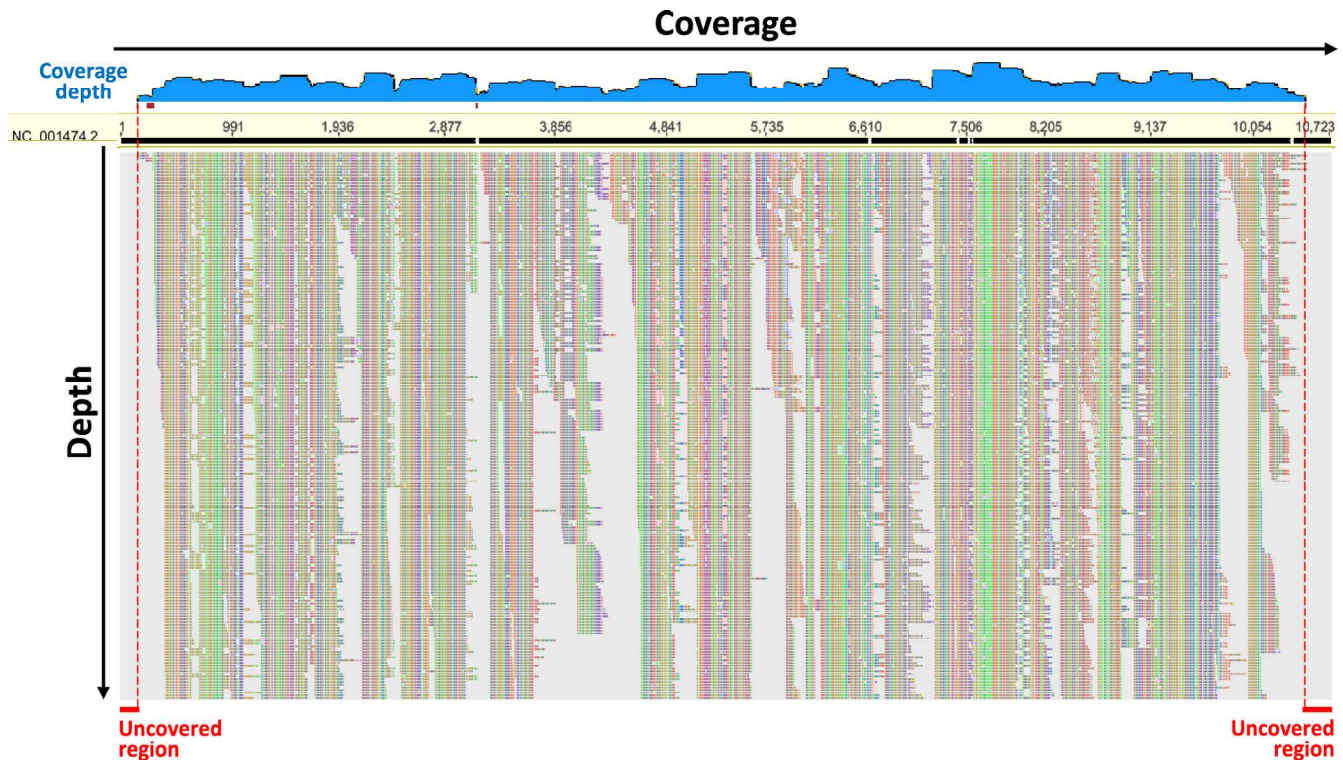


Figure 4. Genomic characterisation through next-generation sequencing (NGS). Bioinformatic tools allow the successful assembly of a nearly complete genome of an arbovirus based on a reference sequence. The coverage is represented horizontally by the regions for which genetic information was obtained, in this case only excluding the 5' and 3' ends of the viral genome. The depth is represented vertically by the number of reads that contribute genetic information to every nucleotide position.

the discovery of new arbovirus species with emerging potential (Zhang *et al.* 2018).

The enormous diversity of viruses, as an essential component for the equilibrium in natural ecosystems, is yet waiting to be unraveled (French & Holmes 2020), as it can be inferred from recent metagenomic studies (Roux *et al.* 2021; Shi *et al.* 2016). In the new wave of the genomics era, high-throughput sequencing technologies have been made available for genomic surveillance and monitoring of known mosquito-borne viruses (Andrade *et al.* 2021; Edgerton *et al.* 2021; Giovanetti *et al.* 2020). When combined to robust unbiased molecular approaches for experimental data collection (Conceição-Neto *et al.* 2018; Lewandowska *et al.* 2017; López-Labrador *et al.* 2021) and computational analysis (Ajami *et al.* 2018; de Vries *et al.* 2021; Kalantar *et al.* 2020; Vilsker *et al.* 2019; Ye *et al.* 2019), the metagenomic NGS (mNGS) emerges as a powerful approach for molecular characterisation of the 'infectome' of cases of clinical relevance (Holmes *et al.* 2018).

Through the mNGS approach, it is now possible to obtain millions of sequence reads from RNA or DNA molecules present in a biological sample, which constitutes an unprecedented amount of information for a full characterisation of the presence of biological entities, without previous knowledge of the suspected agents (Vasilakis *et al.* 2019). By using robust bioinformatic tools and directed pipelines, sequence reads are submitted to quality control, and then, rationally trimmed to eliminate nucleotide sequences at the ends, corresponding to the indexes, adapters or primers that were used during the Polymerase Chain Reaction (PCR)-based enrichment and library preparation for NGS (Hess *et al.* 2020). There are two main strategies for metagenomic assembly: first, with previous knowledge of the infectious agent, single reads are

mapped to a reference genome. On the other hand, a robust and unbiased approach for *de novo* assembly of single reads into contigs or scaffolds generates a high number of assembled sequences that can be confronted to genome databases in the search for sequence homology and identity (Bahassi & Stambrook 2014) (Figure 4).

Several viruses (including arboviruses) have been identified as aetiological agents of human disease, by using the mNGS approach (Grard *et al.* 2012; Huang *et al.* 2021; Phan *et al.* 2018; Piantadosi *et al.* 2021; Quintão *et al.* 2022; Reyes *et al.* 2021; Tang & Chiu 2010; Xie & Zhu 2021; Yozwiak *et al.* 2012), including the pandemic SARS-CoV-2 (Wu *et al.* 2020). This approach can also be used for the rapid identification of new vector species, the feeding sources and the screening for arboviruses, allowing the surveillance of potential emerging viruses and the prevention and control of VBDs (Batovska *et al.* 2018). When applied to the whole body, midgut, salivary glands or expectorated saliva samples of haematophagous arthropods that feed on the blood of several vertebrate hosts, including mammals and birds, this strategy contributes to vector incrimination in enzootic, endemic and epidemic transmission cycles and to the assessment of the risk for the human population (Batson *et al.* 2021; Birnberg *et al.* 2020; Brinkmann *et al.* 2016; Charles *et al.* 2018; Grubaugh *et al.* 2015; Hameed *et al.* 2021; Russell *et al.* 2018; Xiao *et al.* 2018; Yang *et al.* 2015).

More recently, the whole genome characterisation of viruses has been proposed as a powerful tool for the identification of changes that contribute to viral fitness, virulence of strains, transmission capacity and epidemic potential. This characterisation also provides information for other research approaches, including the detection of epidemiological changes through genotype surveillance, phylogeographic analysis, interaction with host receptors

and vector competence (Geoghegan & Holmes 2018). In the clinical and veterinary fields, mNGS is a current and future trend, as it allows the rapid identification of viruses and microbial pathogens causing diseases of unknown aetiology and the surveillance of new potential zoonotic threats. Perhaps the most important application of this technology is the possibility of integrating the mNGS approach to the clinical, epidemiological and ecological information from humans, domestic animals, wild vertebrates and vectors, as a One Health framework to establish early-warning public health systems for a rapid response (Bohl *et al.* 2022; Botti-Lodovico *et al.* 2021; Gardy & Loman 2018; Vasilakis *et al.* 2019; WHO 2022).

Conclusions

Determination of the spatial and temporal distribution of emerging and re-emerging arboviruses of public health interest has been a challenge historically. The biological characteristics of arboviruses and their intrinsic ability to infect different vector and host species leading to dramatic changes in their transmission cycles have forced the health systems to establish a multidisciplinary approach to prevent and mitigate their impact in animal and human health. The mNGS approach has demonstrated its robustness for arbovirus discovery and monitoring in a wide spectrum of biological samples and ecological settings. Its integration to the recently proposed global genomic surveillance strategy with a holistic One Health approach will warrant the early-warning, preparedness and public health response to future biological threats.

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Conflicts of interest. The authors declare none.

Ethical statement. None.

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