Minireview

Arbovirus surveillance in mosquitoes: Historical methods, emerging technologies, and challenges ahead

Luis Janssen Maia[1*], Cirilo Henrique de Oliveira[2*], Arthur Batista Silva[3], Pedro Augusto Almeida Souza[2], Nicolas Felipe Drumm Müller[4], Jader da Cruz Cardoso[5], Bergmann Morais Ribeiro[1], Filipe Vieira Santos de Abreu[2] and Fabrício Souza Campos[3,4]

[1]Instituto de Ciências Biológicas, Departamento de Biologia Celular, Laboratório de Baculovírus, Universidade de Brasília, Brasília 70910-900, Brasil; [2]Laboratório de Comportamento de Insetos, Instituto Federal do Norte de Minas Gerais, Salinas 39560-000, Brasil; [3]Laboratório de Bioinformática e Biotecnologia, Universidade Federal do Tocantins, Gurupi 77402-970, Brasil; [4]Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre 90035-003, Brasil; [5]Divisão de Vigilância Ambiental em Saúde, Centro Estadual de Vigilância em Saúde, Secretaria Estadual de Saúde do Rio Grande do Sul, Porto Alegre 90610-000, Brasil
*These authors contributed equally to this article.
Corresponding authors: Fabrício Souza Campos. Email: camposvet@gmail.com; Filipe Vieira Santos de Abreu.
Email: filipe.vieira@ifnmg.edu.br

Impact Statement

Mosquitoes are important indicators for predicting, monitoring, and controlling the spread of arboviruses and pose a significant threat to public health worldwide. However, the effectiveness of arbovirus surveillance has been affected by various challenges including climate change, insecticide resistance, and human activity. To address these issues, collaborative efforts between different areas of expertise and the integration of emerging technologies, such as next-generation sequencing (NGS), paper-based devices, spectroscopic detectors, and synthetic biosensors, present promising opportunities for improving surveillance and control efforts, leading to a faster response to outbreaks. The successful surveillance of arboviruses in mosquitoes requires human and material resources to collect mosquitoes, identify their taxonomy, detect arboviruses, and employ sequencing techniques whenever possible. Mapping at-risk areas and implementing appropriate prevention and control measures are crucial for this process. By leveraging this valuable information, health systems worldwide can promptly prepare, respond, and make informed decisions to mitigate or prevent the transmission of arboviruses to humans.

Abstract

Arboviruses cause millions of infections each year; however, only limited options are available for treatment and pharmacological prevention. Mosquitoes are among the most important vectors for the transmission of several pathogens to humans. Despite advances, the sampling, viral detection, and control methods for these insects remain ineffective. Challenges arise with the increase in mosquito populations due to climate change, insecticide resistance, and human interference affecting natural habitats, which contribute to the increasing difficulty in controlling the spread of arboviruses. Therefore, prioritizing arbovirus surveillance is essential for effective epidemic preparedness. In this review, we offer a concise historical account of the discovery and monitoring of arboviruses in mosquitoes, from mosquito capture to viral detection. We then analyzed the advantages and limitations of these traditional methods. Furthermore, we investigated the potential of emerging technologies to address these limitations, including the implementation of next-generation sequencing, paper-based devices, spectroscopic detectors, and synthetic biosensors. We also provide perspectives on recurring issues and areas of interest such as insect-specific viruses.

Keywords: Insect-specific viruses, arbovirus, mosquito, NGS, surveillance, molecular methods

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Introduction

Until the end of the 19th century, the role of insects as disease vectors was not known and the transmission of several infectious diseases was attributed to "miasmas," which were supposedly fluid carriers of disease. However, this paradigm changed at the end of the century. In 1881, the Cuban physician Carlos Finlay postulated that a different component, commonly present in the air, was the actual culprit for the spread of the yellow fever (YF) disease: mosquitoes. However, this postulate did not have a definitive proof. In 1901, Reed *et al*. demonstrated that these insects were the actual transmitters of the disease, disproving the notion of

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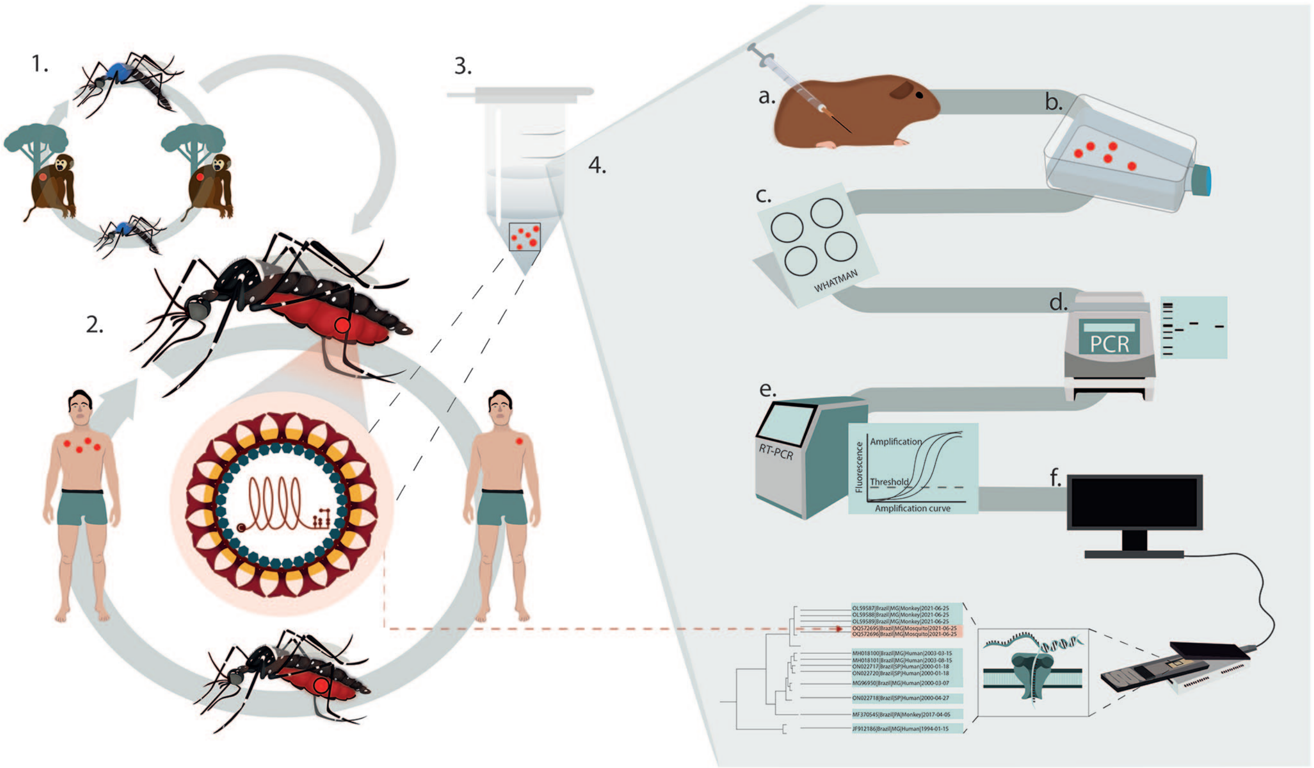

Figure 1. Overview of arbovirus wild and urban cycles and evolving detection technologies.
(1) Wild mosquito cycle; (2) urban mosquito cycle; (3) mosquito pool sample after ultracentrifugation; and (4) evolution of technologies: (a) animal inoculation; (b) cell culture inoculation; (c) use of Whatman paper for sample collection and transportation; (d) polymerase chain reaction (PCR); (e) real-time PCR; and (f) third-generation sequencing using Nanopore's portable MinION sequencer, with details on nucleotide sequence acquisition followed by viruses characterization through phylogenetic analysis.

fomites or infected human blood as the primary means of transmission. Based on this understanding, measures were implemented to isolate patients with YF and initiate mosquito control.[1] Remarkably, after 6 months, YF was eradicated from Havana for the first time in 150 years.[2]

Nevertheless, the complexity of the circulation of mosquito-borne diseases has increased recently. According to the Mosquito Taxonomy Inventory,[3] over 3700 mosquito species are found worldwide, with the majority being generalists who consume blood meals based on host availability, including humans, contributing to the transmission of several zoonoses in sylvatic and/or rural cycles.

However, some species of two distantly related mosquito genera, *Aedes* and *Anopheles*, have evolved into human specialists. Among them, *Aedes aegypti* is the primary vector for diseases such as YF, chikungunya (CHIKV), dengue, and Zika (ZIKV) in urban cycles.[4] Genomic studies on *Ae. aegypti aegypti* subspecies indicate that this specialization toward biting humans occurred approximately 5000 years ago, following the end of the African humid period and a shift from savannah to desert conditions. Consequently, these mosquitoes increasingly rely on water sources associated with humans rather than natural sources.[5] After becoming human specialists, the potential for disease spread by mosquitoes has increased with the increase in human migration. Over the centuries, the transmission of arboviruses within and between continents has undergone significant changes, often affected by human navigation (e.g., the introduction of YFV in the Americas during the slave trade) and more recently by aviation (e.g., the recent introduction of ZIKV in the Americas through the flow of tourists).[5]

Over the last few decades, the incidence of arboviral diseases in humans has remarkably changed. These changes range from fluctuations in low-level, year-long, continuous transmission in endemic areas, to continental epidemics of previously known viruses. For example, ZIKV caused outbreaks throughout the Pacific region in 2007 and reached the American continent in 2013.[6] Another example is CHIKV, which spread from Kenya to Southeast Asia between 2004 and 2010 and then to the Americas, first in St. Martin Island in 2013, and then in 2014 to the rest of the continent, comprising approximately 1.5 million cases. Moreover, newly emergent arboviruses, such as Mayaro (MAYV) and Oropouche (OROV), have been spreading on a smaller geographic scale but are becoming increasingly concerning for public health.[7] The rapid global spread of these previously obscure pathogens underscores the importance of mosquito-borne diseases and the need to understand the underlying factors driving their emergence.

In this review, we summarize the complexities of viral surveillance in mosquitoes based on historical events as well as how scientific advancements have evolved to address the challenges in detecting and tracking arboviruses (Figure 1).

Table 1. Discovery sites and main vectors for some of the most important arboviruses in circulation worldwide.

Virus	Discovery	Urban vectors	Sylvatic vectors	References
YFV	1900, Cuba	*Ae. aegypti, Ae. africanus, Ae. simpsoni, Ae. Albopictus*	*Haemagogus* spp., *Sabethes* spp.	Kuno and Chang,[20] Xu *et al*.,[45] and Zaim and Guillet[46]
DENV	1940s, Southeast Asia and Pacific Islands	*Ae. aegypti*, *Ae. albopictus, Ae. polynesiensis*	*Aedes luteocephalus, Ae. furcifer, Ae. niveus*	Dusfour *et al*.[47]
ZIKV	1947, Zika Forest, Uganda	*Ae. aegypti* and *Ae. albopictus*	*Aedes* spp., *Mansonia uniformis, Eratmapondites*	Vontas *et al*.[48]
CHIKV	1952, Tanzania	*Ae. aegypti* and *Ae. albopictus*	*Aedes* spp.	Carpenter and Clem[49]
WNV	1937, West Nile District, Uganda	*Culex. pipiens, Cx. restuans, Cx. tarsalis*, *Cx. quinquefasciatus*	*Culex (melanoconion)* spp.	Holmes *et al*.,[50] Abreu *et al*.[51] and Andrade *et al*.[52]
MAYV	1950s, Trinidad and Tobago	*Ae. aegypti* and *Ae. albopictus*[a]	*Haemagogus janthinomys*, *Hg. leucocelaenus*, *Hg. equinus*	Almeida *et al*.,[53] Romano *et al*.,[54] Andrade *et al*.[55] and Artika *et al*.[56]

[a] Shows vector competence for MAYV under laboratory conditions.

Mosquito vectors of arboviruses

Emerging and re-emerging arboviruses are major public health problems worldwide. The main medically important arboviruses causing morbidity and mortality in humans worldwide belong to the families *Flaviviridae*, *Togaviridae*, and *Bunyaviridae*, which are transmitted by Culicine mosquitoes, such as *Aedes*, *Haemagogus*, and *Culex* spp.[8] Transmission can occur in urban, wild, and rural areas.

In the urban arbovirus cycle (i.e., Dengue, ZIKV, and CHIKV), humans act as primary vertebrate hosts and anthropized mosquitoes such as *Ae. aegypti* and *Ae. albopictus* act as vectors.[9]

Before establishing themselves in human hosts during urban cycles, arboviruses typically circulate in sylvatic or enzootic cycles between sylvatic mosquitoes and wild or domestic animals, such as rodents, birds, horses, and non-human primates.[10,11] Previously, the sylvatic cycles were unknown.[11,12] The pivotal discovery of the role of wild mosquitoes in arbovirus transmission occurred in the 1930s during an investigation of a YFV outbreak in the absence of the urban mosquito *Ae. aegypti*.[13]

In recent years, the incidence of arbovirus infections has significantly increased in humans.[14–16] Since their discovery, several other mosquito species have been implicated in the transmission of arboviruses (Table 1).[17,18]

Importantly, there is a complex relationship between viruses and their vectors, including genetic, immunological, and environmental aspects, and transmission only occurs when viruses are capable of survival, amplification, and dissemination from the mosquito midgut to various other tissues, particularly the salivary glands.[19] Once transmitted to a vertebrate host, for example, humans, the virus replicates and may lead to a variety of symptoms, including fever, joint pain, and rash, evolving into cure or death.[15]

Recent studies on the transmission of arboviruses by mosquitoes have focused on understanding the factors that influence transmission, such as the abundance and behavior of the mosquito population, and the role of human behavior in promoting the spread of viruses.[16,20,21] Most studies have focused on developing new control strategies aimed at reducing the population of mosquito vectors, such as the development of genetically modified mosquitoes and the use of the endosymbiont *Wolbachia* which can reduce or block the transmission of arboviruses.[22–24] It is crucial to acknowledge the significant role of vaccination in combating these diseases. One of the most effective vaccines against arboviruses is the YFV-17D live-attenuated vaccine developed in 1937 to combat YF.[25] More recently, phase III trial results for CYD-TDV (commercially known as Dengvaxia) were initially promising, indicating its effectiveness against all serotypes.[26] While this minireview does not focus on vaccination, it is important to briefly mention that these vaccines play a critical role in preventing and controlling arbovirus infections. Understanding mosquito control strategies and vaccination efforts can contribute to a comprehensive approach to decreasing the incidence and effect of arboviral diseases.

Challenges in arbovirus surveillance in mosquitoes

Contemporary changes in geography have indicated an increase in arbovirus transmission. Modeling techniques have shown that the temperature of temperate zones is generally too low for the transmission of mosquito-borne infectious diseases. However, warmer climates due to climate change may favor shorter developmental times of mosquito larvae and the feeding frequency of adult females. Thus, they can accelerate the transmission and spread of infectious diseases to new areas.[27–29] Rose *et al*.[30] showed that intense dry seasons and increasing human population are two crucial factors affecting the biting behavior of *Ae. aegypti* mosquitoes toward humans as opposed to non-human hosts in Africa. While dry season changes are expected to change modestly until 2050 in Africa, the human population density in different urban zones is expected to change considerably. Thus, *Ae. aegypti* mosquito bites are expected to transition from non-human to human hosts more frequently in these urban zones. Lee *et al*.[31] used climate data and *Aedes* mosquito distribution models to predict the risk of dengue transmission in Brazil. Their findings indicated that the geographic range of *Aedes* mosquitoes is expanding in Brazil and that climate change is likely to increase the risk of dengue transmission in many regions of the country.[31] Increases in temperature at equatorial latitudes have been associated with increased malaria epidemics in Kenya, Rwanda, and Tanzania due to the faster mosquito developmental times and the expansion of mosquito populations to higher altitudes.[29] Another climatic variable that plays an important role in the transmission of arboviruses by mosquitoes, in addition to temperature, is rainfall, supplying suitable larval habitats for the proliferation of these insects.[32] Some changes in traits, such as daylight meals and life cycles within indoor spaces, have helped mosquitoes become highly effective vectors.[33] These changes, along with other factors, such as air and sea travel, facilitate the transmission of arboviruses by mosquitoes, leading to recent arboviral outbreaks, such as the transmission of ZIKV from Africa to Oceania and America and CHIKV from Southeast Asia to the Americas.[34,35]

In addition to climatic changes, many other factors affect the epidemiology of arboviral transmission, such as environmental changes, international travel, and urbanization. Kraemer *et al*.[36] discussed the global distribution of the arbovirus vectors *Ae. aegypti* and *Ae. albopictus* are associated with trade and travel. Global connectedness through international trade and human movement facilitates the spread of these vectors and their associated pathogens, such as DENV and CHIKV, making it imperative to address the risk of importation and subsequent autochthonous transmission in an increasingly connected and urbanized world.[36]

Anthropogenic land use has also been identified as a potential driver of arboviral transmission[37] and deforestation has often been studied in this regard.[38] Deforestation enables contact among hosts, competent vectors, and susceptible human hosts, thereby facilitating pathogen transmission.[39] Several mosquito species that serve as vectors for multiple human pathogens are associated with deforested habitats, indicating high frequency and increased abundance in different regions of the world.[40] Among the taxa most favored by deforestation are *Aedes* spp.[41,42] and *Anopheles* spp.[43,44]

In addition to climatic, geographic, and human behavioral changes, insecticide resistance is another emerging variable of concern. Insecticide resistance has been proposed to be an adaptive phenomenon because before the exposure of organisms to a stressor (in this case the exposure of mosquitoes to an insecticide), rare individuals may carry one or more resistance alleles (such as polymorphisms in the resistance allele sequence or increased expression of the resistance allele) that allow them to survive exposure to the stressor.[45] The proportion of individuals carrying resistance polymorphisms or alleles is expected to increase following insecticide-selective pressure.[45] The widespread development of mosquito resistance to the most commonly used insecticides (pyrethroids and organophosphates) has resulted in serious problems in many regions.[46] Examples of insecticide resistance include *Anopheles*[47] and *Aedes* urban mosquitoes,[48] which have become increasingly resistant to insecticides, posing significant challenges to vector control programs aimed at combating malaria and arboviruses.

Carpenter and Clem[49] discussed how known factors, including temperature, exposure to pesticides, and larval density/competition, contribute to physiological and biochemical processes, such as the ability of viruses to escape from the midgut barrier and virus-vector specificity.[49] They also identified mosquito factors such as physical characteristics, such as basal lamina thickness and structure, and immune-related gene expression that affect viral circulation within these insects. Huang *et al*.[15] provided insights into arthropod vectors that actively mount immune and antiviral responses to limit arbovirus infection. These vectors provide a unique intracellular environment that selects and promotes the transmission of specific viral populations, ultimately influencing the arbovirus transmission patterns. Furthermore, vector–host interactions during feeding on vertebrate hosts have been shown to play a critical role in disease pathogenesis and can create alternative transmission routes.[15]

Although changes in geography and insecticide resistance are known to contribute to the spread of viral diseases, spillover events are rare and depend on many variables such as host distribution, mutation events, and selection of viruses. Therefore, it is nearly impossible to predict the timing and location of an epidemic. In the dynamic context of changes in mosquito and arboviral prevalence, it is essential to prioritize surveillance to prepare for and mitigate epidemics.[50]

In Brazil, significant progress has been achieved in eco-epidemiological surveillance through both passive and active methods owing to the efforts of research institutions and health services. A noteworthy example of integrated surveillance is the monitoring of wild YF, where arbovirus detection in non-human primate hosts and mosquitoes was conducted even before identifying cases in humans. This proactive approach has allowed timely preventive and control measures to be implemented in unvaccinated populations.[51–55]

Arbovirus surveillance in mosquitoes: Viral detection methods

Early viral detection techniques play an important role in the identification and characterization of arboviruses, laying the foundation for the development of more advanced diagnostic methods.[56,57] These techniques include tissue maceration, animal inoculation, cell culture inoculation, and molecular techniques such as polymerase chain reaction (PCR).[58–60] Because these methods were developed chronologically, each method has its advantages and disadvantages. Currently, these techniques complement each other in characterizing viruses, as detailed in the following discussion.

Tissue maceration and animal inoculation: Tissue maceration involves grinding or homogenizing a sample of infected or suspected tissue (from vertebrates or mosquitoes) in a buffer, followed by inoculating in a laboratory animal, such as a guinea pig.[61,62] The specimens were monitored for signs of disease, such as fever, weight loss, and hemorrhagic lesions. This method was widely used in the early 20th century to detect and identify viruses such as YFV,[63] DENV,[64] and West Nile virus (WNV).[65] Although novel viruses are typically cultured in cell lines before being inoculated into animals, their use remains crucial for assessing viral virulence.[66] In addition, guinea pigs can be used directly for the detection of arboviruses in specific environments. Sentinel specimens can be strategically placed in areas suspected of harboring mosquito vectors carrying arboviruses. This assessment involves evaluating animals for viral circulation after blood meals, followed by seroconversion, virus isolation, or molecular detection. This sentinel guinea pig approach has been successfully used to study several alphaviruses and YFV.[67] For instance, ZIKV was first isolated from a febrile rhesus monkey, which was placed as a sentinel in the Zika forest in 1947. The isolated virus was subsequently found to be infectious in other rhesus monkeys and mice in a laboratory setting.[68] Even today, sentinels continue to play a critical role in arboviral surveillance, particularly in urban areas where animals live near humans and resource-challenged environments. For example, domestic dogs have been used as sentinels for WNV signaling.[69]

Cell culture inoculation: In the 1940s and 1950s, the development of tissue culture techniques enabled the *in vitro* growth and propagation of viruses.[70] Infected tissue samples were inoculated into cultured cells, such as clone C6/36 cells (from *Ae. albopictus*),[71] which allows the isolation and identification of a wide range of viruses, including DENV. The progenitor cell line, C6/36, was successfully established from male larvae in 1967. C6/36 cells have a short replication time, consistently high viral titers, and null mutations in their *dicer* genes, which result in defective RNA interference pathways. Therefore, this cell line has become instrumental in the propagation of several RNA viruses from different families such as *Flaviviridae*, *Togaviridae*, and *Bunyaviridae*.[72] Alternatively, human viruses can also be propagated through vero cells. These cells belong to a continuous cell line with indefinite division potential, unlike primary cell lineages. This cell line was first established in a female African green monkey in 1962. One notable characteristic is their inability to produce interferons (IFNs), which makes them highly susceptible to various human viruses. Interestingly, even at high viral titers, vero cells did not undergo IFN-mediated cell death.[73] However, certain other factors must also be considered. To maintain viral viability, a cold chain involving low temperatures is necessary for viral sample transport. In addition, the isolation of viruses from cell lines may be a time-consuming process, making it less suitable for rapid epidemiological responses and requiring specialized laboratories and significant material resources. Notably, not all viruses can infect established cell lines.[67] New arboviruses have been characterized and evaluated for vertebrate infectivity using cell culture inoculation.[74]

Molecular techniques: PCR is a molecular technique that allows for the amplification and detection of viral nucleic acid in a sample. This technique has revolutionized viral detection and diagnosis because it is highly sensitive and can detect a single positive mosquito in a pool of hundreds or thousands. The use of specific primers improves the specificity of the assay. Unlike cell culture or animal tests, PCR can detect viral nucleic acids even in the absence of viable viruses, irrespective of cell infectivity.[67] This approach has significantly improved viral detection and diagnosis, making PCR an indispensable tool in the field of virology. This method, in contrast to the previous two methods, can also be performed in hours, instead of weeks or months. PCR can detect a single pathogen, such as ZIKV, or a higher taxonomic level, such as members of the *Flaviviridae* family. These assays can be combined with multiplex assays and are highly reproducible. Therefore, PCRs are particularly useful in mosquito-based surveillance (entomovirological surveillance) and have been revolutionary for the diagnosis of arboviruses and the tracking of outbreaks.[56,67,75,76] However, they require prior knowledge of arboviral genetics and cannot detect highly divergent pathogens. In addition, they require infrastructure resources, such as cold chains and electricity, and trained personnel to perform the assays properly.[76]

Arbovirus surveillance in mosquitoes: Challenges and innovations

Prompt response for the detection of viral circulation, including human cases and epizootics, is a critical factor for the successful implementation of arbovirus surveillance.[55] Establishing an epidemiological link between laboratory results and the investigated events is essential. Entomological surveillance focusing on mosquitoes has proven to be an excellent tool.[67] Figure 2 summarizes the main stages of the entomological surveillance. Although the primary mosquito species responsible for arbovirus transmission are well known, there remains a knowledge gap concerning the biology and ecology of other species, such as wild mosquitoes. Identifying these species and understanding their behavior can significantly improve the entomological surveillance of arboviruses.[77] One of the main challenges in entomological surveillance is the proper conditioning, storage, and transport of mosquitoes after capture in the field. Collected samples must reach the laboratory under optimal conditions to facilitate taxonomic identification and reliable diagnoses. However, this process is often slow, expensive, and requires skilled professionals to handle the specimens, and the training and hiring of new taxonomists is a problem worldwide.

In recent decades, research has shown the potential of using near-infrared spectroscopy (NIRS) for detecting arboviruses in mosquitoes.[78] It is also possible to measure the backscattered light of mosquitoes to identify the sex and species of mosquitoes and gravid female mosquitoes. This identification is achieved through the analysis of the harmonic content and/or optical properties.[79–83] Although the current identification accuracy of such systems may be lower than that of traditional laboratory analyses of captured specimens, they offer the advantage of observing a larger number of insects in real time. This real-time observation capability can significantly improve surveillance efforts and provide valuable insights into mosquito populations and their dynamics. The incorporation of machine learning (ML) algorithms has significantly improved the interpretation of spectroscopic results in recent years.[84,85] In addition, an effective alternative approach involves using software to detect and classify mosquito wingbeat sounds, leading to species identification.[86] These two methods play a vital role in the arbovirus surveillance of mosquitoes, as they help reduce costs, speed up the diagnostic process, and promote their widespread adoption. Other techniques for storage, preservation, and detection of genetic material are discussed in this section.

Recently, more deployable methods that require less infrastructure and resources but still require some knowledge of the viral pathogen have been developed.[87] Among these, loop-mediated isothermal amplification (LAMP) assays are

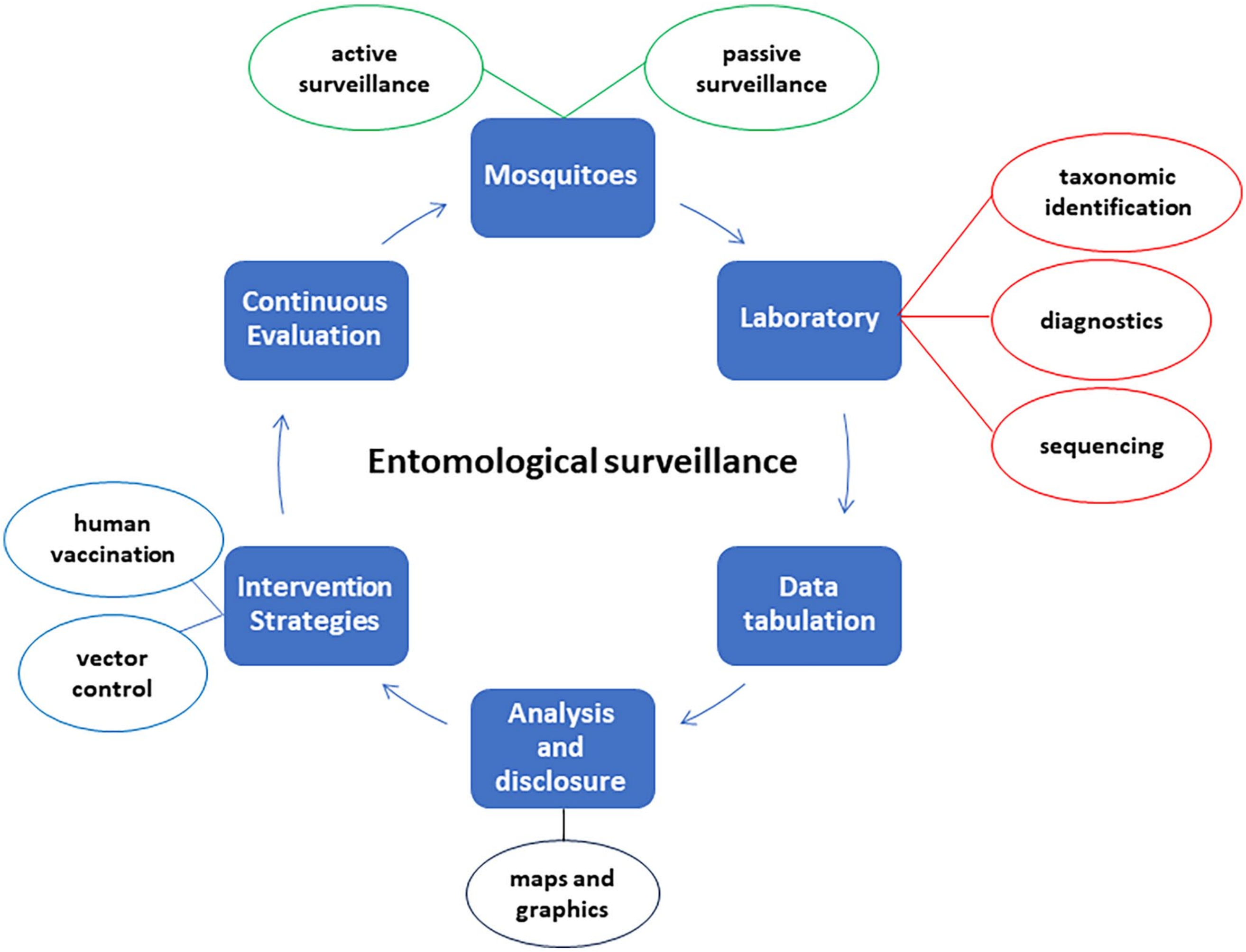

Figure 2. Cycle of entomological surveillance for arbovirus control.

promising tools for pathogen detection. One of their key advantages is that they eliminate the requirement of expensive thermocyclers and help to obtain the presence/absence results within minutes.[87] Moreover, other isothermal methods such as recombinase polymerase amplification (RPA), rolling circle amplification (RCA), helicase-dependent amplification, and nucleic acid sequence-based amplification (NASBA) also offer viable alternatives with reduced resource requirements.[88] Furthermore, paper-based devices show promise as low-cost and portable solutions for pathogen detection. These devices have the potential to facilitate pathogen testing in a variety of settings. For instance, Whatman paper cards are a reliable method for transporting nucleic acids, enabling their detection without requiring cold chains. This has been successfully used for YFV, DENV, and ZIKV.[89,90] In addition, low-cost and dependable equipment for sample preparation was designed using manual centrifuges made from paper.[91] Toehold RNA biosensors represent another innovative approach capable of activation in the presence of target sequences, which then express markers such as GFP or beta-galactosidase. These sensors can be freeze-dried on paper and activated upon rehydration, making them highly portable and useful in low-resource settings. Notably, they have been successfully applied in conjunction with NASBA for clinical surveillance of ZIKV and CHIKV.[88,92] To enhance the specificity of isothermal amplification methods and RNA toehold sensors, these can be combined with the CRISPR-Cas systems.[88] Moreover, the integration of isothermal amplification methods with NGS allows differentiation between different DENV serotypes.[93]

NGS technologies have revolutionized virus discovery and surveillance by allowing the analysis of entire viral genomes without the need for cell line cultures or observation of cytopathic effects in infected cells. In principle, NGS strategies do not require prior genetic information regarding the viruses, though nucleic acid amplification strategies may be required to increase the input of viral genetic material.[76,94,95] Moreover, genome-based epidemiology has proven invaluable for providing critical insights into various aspects of an epidemic, including its location, timing, causative viral agent, viral evolution, and other pertinent questions, without the need for prior pathogen knowledge.[76] Oxford Nanopore MinION systems, in particular, have played a crucial role in enabling on-site sequencing of viral strains, significantly facilitating viral surveillance and discovery in local communities and environments.[96,97] Our group, along with other researchers,[52,98,99] has effectively used NGS to track the spread of YFV across several

Table 2. Summary characteristics of the five most common ISV and some arboviral families found in mosquitoes.

Viruses family/(reference)	*Flaviviridae* (107)	*Mesoniviridae* (108)	*Rhabdoviridae* (109)	*Reoviridae* (110)	*Peribunyaviridae* (111)
Genome type	ssRNA+	ssRNA+	ssRNA–	dsRNA	ssRNA–
Genome segmentation	Monopartite or segmented	Monopartite	Monopartite or segmented	9–12 segments	3 segments
Genome size	9–13 kb	20 kb	Varied	Varied	10–12 kb

Brazilian states, allowing us to observe changes in viral transmission during the most recent epidemic (2016–2021) compared with historical records. This approach offers valuable insights into the dynamics of the epidemic and has contributed to a deeper understanding of YFV transmission patterns. In addition to their role in the surveillance of known arboviruses, NGS methods offer a powerful tool for detecting rarely observed viruses or even discovering entirely new ones by utilizing metagenomic analysis of both short and long reads. For instance, Nastri *et al.*[100] employed long-read metagenomic sequencing to detect two Sabiá virus infections in humans who were initially suspected of having YFV.[100] Before their study, only four other Sabiá virus cases were registered in Brazil for two decades, despite its potential to cause hemorrhagic fever. However, these metagenomic techniques have mainly focused on animals of human interest, because the highest known diversity of animal viruses is found in vertebrates, followed by arthropods. Among arthropods, mosquito-associated viruses are the most extensively studied group, with the majority of novel viral species found in mosquitoes belonging to the *Aedes*, *Anopheles*, *Culex*, and *Mansonia*, and are insect-specific, that is, they do not have vertebrate hosts, such as arboviruses.[101,102]

In addition to the discovery and characterization of arboviruses, the study of insect-specific viruses (ISVs), including virome studies and their interactions with invertebrate hosts, is a growing area of interest. Although there is still much to learn about these viruses and their relationship with arboviruses, mounting evidence suggests that ISVs can modulate their transmission capacity.[103,104] Therefore, monitoring specific ISVs that affect vector competence, in addition to arboviruses, may be crucial for conducting accurate risk assessments in different geographical locations. From 2007 to 2017, 187 novel mosquito-associated viruses have been described, with the most representative viral families being *Flaviviridae*, *Rhabdoviridae*, and *Peribunyaviridae*[101] (Table 2). While most of the novel viruses reviewed in Atoni *et al.*[101] were described in Africa and Asia, it is important to note that the majority of RNA-dependent RNA polymerases, which are typical of RNA viruses found in the Sequence Read Archive, come from datasets from the Global North,[105] despite some of the largest biodiversity hotspots being located in the Global South.[106] Disparities in sequencing efforts between the global northern and southern regions may partly explain this difference. Specifically, regarding the study of viral communities and viromes in mosquitoes, Brazil, the United States, China, and Australia have the highest number of articles.[102] However, more extensive sequencing of diverse mosquitoes in diverse habitats, as well as the mining of public sequence read databases, have the potential to discover and characterize novel arboviruses.

According to Moonen *et al.*,[102] mosquito virome studies have witnessed a significant increase in frequency over the last two decades. The number of such studies has increased from just two between 2000 and 2008 to an impressive 27 studies in recent years. A total of 175 articles published during this period were identified, indicating growing interest in this field. Regarding the methods used in these studies, NGS-based strategies and PCR-based approaches employing sequences of previously known viral families were commonly employed in 70 and 67 studies, respectively. In addition, a smaller proportion of studies used cell cultivation before NGS or PCR (38 and 21 studies, respectively). The most studied mosquito genera in virome studies are *Aedes*, *Culex*, and *Anopheles*, given their importance as vectors of known arboviruses, with Anopheles being the main vector of the O'Nyong-Nyong virus. Altogether, these 3 genera represent nearly 90% (278 of 312) of the virome records, as a given article can present multiple virome records published until January 2022. In contrast, sylvatic mosquitoes, such as *Haemagogus*, *Sabethes*, and *Psorophora*, have only a single record published for the same period.[102] Due to the importance of changes in land use, particularly deforestation, in promoting pathogen emergence and spillover events,[107,108] we believe that it is necessary to characterize the viromes in sylvatic mosquitoes to improve outbreak preparedness.

Studies on viromes have led to the inclusion of arthropods as hosts for previously known viral families, expanded the clade diversity within taxa, such as orders and families, and led to important evolutionary discoveries, such as the detection of the Nam Dihn virus in mosquitoes. This has led to the establishment of the *Mesoniviridae* family with intermediate genome sizes between smaller *Arteriviridae* and larger *Coronaviridae*.[102]

Conclusions

In conclusion, the surveillance of arboviruses in mosquitoes should be integrated with other strategies, such as the surveillance of human cases and animal reservoirs and the vaccination of the human population. Entomological surveillance is an important domain of research requiring ongoing attention and innovation. With increasing challenges posed by climate change, insecticide resistance, and human activity, it is important to develop effective strategies to mitigate the spread of arboviruses. While traditional methods have provided valuable insights into mosquito-borne disease transmission, emerging technologies promise to increase the current vigilance capabilities. NGS allows for near real-time tracking of outbreaks and the discovery of new or rarely observed viruses, spectroscopic devices accelerate mosquito identification, and paper-based devices allow for the tracking of viruses even in resource-challenged conditions, thus democratizing vigilance efforts and helping to fill epidemiological gaps.

Altogether, these technologies offer exciting new possibilities for improving surveillance and control. Through continued exploration of these innovative technologies and approaches, researchers can play a crucial role in safeguarding public health and mitigating the effect of arbovirus outbreaks worldwide. Effective surveillance of arboviruses in mosquitoes relies on a combination of human and material resources and involves the following key steps: (1) continuous collection of mosquitoes in different areas; (2) taxonomic identification and preparation of the mosquito pools; (3) diagnostic techniques for detecting arboviruses in mosquitoes; (4) sequencing whenever possible to gain deeper insights into the viral strains; (5) mapping risk areas based on the surveillance results; (6) initiation of targeted prevention and control measures, when indicated; and (7) swift dissemination of results for rapid public health interventions followed by comprehensive communication in the scientific literature. Using this comprehensive information, health systems can better prepare for and respond to potential arboviral outbreaks. Informed decision-making is possible, enabling effective measures to mitigate or prevent the transmission of arboviruses to humans. Thus, a proactive approach should be taken to protect communities against the threat of arboviral diseases.

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ORCID IDS

Pedro Augusto Almeida Souza https://orcid.org/0000-0002-3969-9264
Filipe Vieira Santos de Abreu https://orcid.org/0000-0001-9768-4688
Fabrício Souza Campos https://orcid.org/0000-0002-5948-472X

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