

REVIEW

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# Searching for the proverbial needle in a haystack: advances in mosquito-borne arbovirus surveillance

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## Abstract

Surveillance is critical for the prevention and control of mosquito-borne arboviruses. Detection of elevated or emergent virus activity serves as a warning system to implement appropriate actions to reduce outbreaks. Traditionally, surveillance of arboviruses has relied on the detection of specific antibodies in sentinel animals and/or detection of viruses in pools of mosquitoes collected using a variety of sampling methods. These methods, although immensely useful, have limitations, including the need for a cold chain for sample transport, cross-reactivity between related viruses in serological assays, the requirement for specialized equipment or infrastructure, and overall expense. Advances have recently been made on developing new strategies for arbovirus surveillance. These strategies include sugar-based surveillance, whereby mosquitoes are collected in purpose-built traps and allowed to expectorate on nucleic acid preservation cards which are submitted for virus detection. New diagnostic approaches, such as next-generation sequencing, have the potential to expand the genetic information obtained from samples and aid in virus discovery. Here, we review the advancement of arbovirus surveillance systems over the past decade. Some of the novel approaches presented here have already been validated and are currently being integrated into surveillance programs. Other strategies are still at the experimental stage, and their feasibility in the field is yet to be evaluated.

**Keywords:** Arboviruses, Surveillance, Mosquito, Sentinel animals, Honey-based surveillance, Next-generation sequencing

## Background

Arthropod-borne viruses (arboviruses) transmitted by mosquitoes are of public health and veterinary importance globally causing disease syndromes including encephalitis, viral haemorrhagic disease and arthritis. Dengue viruses (DENVs) alone cause an estimated 96 million clinical cases a year, especially in the tropics and sub-tropics [1]. The flaviviruses, Japanese encephalitis virus (JEV) and West Nile virus (WNV), are major causes of viral encephalitis throughout their geographical range. Recently, the expansion of chikungunya (CHIKV) [2] and Zika (ZIKV) [3] viruses in the Western Hemisphere, and the yellow fever (YFV) outbreaks in

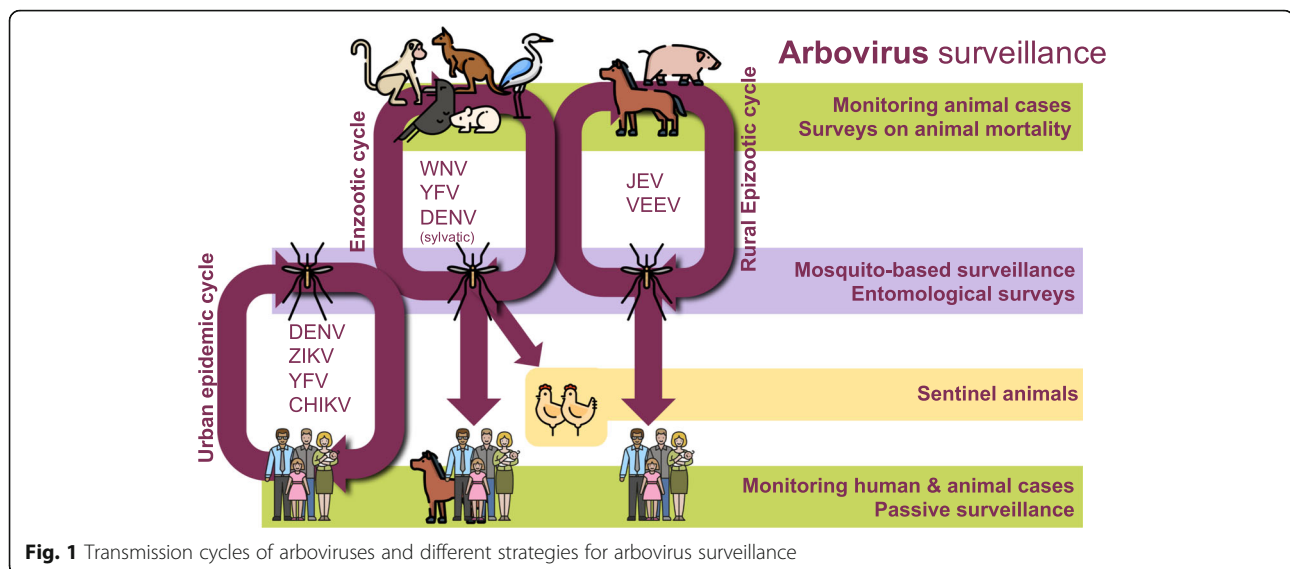
Africa [4] and Brazil [5] have highlighted the continuing threat emerging and re-emerging arboviruses pose.

With the exception of YFV [6] and JEV [7], there are currently few vaccines or antiviral drugs available against most of these viruses. Thus, prevention and control of most arboviruses is almost solely reliant on effective mosquito management. This can be enhanced by surveillance, where detection of elevated or emergent virus activity serves as a warning system to implement appropriate actions to reduce the severity and duration of outbreaks. However, designing an appropriate arbovirus surveillance system is challenging. Arboviruses have complex transmission cycles with dual-host tropism: they replicate in vertebrate hosts (such as birds or mammals) and arthropod hematophagous vectors (such as mosquitoes or ticks) [8]. This complexity needs to be accounted for, and an ideal surveillance system should rely on different sources of information (Fig. 1), and can include

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meteorological data, evidence of virus infection in vertebrate hosts, entomological surveys, virus detection in vectors, and reports of human or animal disease. The scale of surveillance can vary regionally [9] and is particularly challenging in remote locations, or in areas with limited resources and infrastructure.

Given the broadness of this subject, few attempts have been made to provide a synthesis of arbovirus surveillance methods. The objective of this review is to describe the development and implementation of mosquito-borne arbovirus surveillance strategies. First, we evaluate traditional methods that have been commonly used where arboviruses are a public health threat, then outline and assess recently developed methodologies, before identifying future research needs.

## Methods for arbovirus surveillance

### Monitoring human and animal disease

Human or animal case surveillance relies on hospitals, laboratories and health practitioners notifying public health authorities of confirmed or suspected cases of arbovirus infection that occur in the population. Almost every state in the United States conducts surveillance of human WNV cases as a part of the national arbovirus surveillance system, ArboNET [10], whilst in Australia, human arbovirus disease notifications are monitored using the National Notifiable Disease Surveillance System (NNDSS) [11]. These surveillance systems require strict case definitions and laboratory diagnostic testing criteria, as well as demographic, clinical, laboratory and epidemiological information [12]. In the summer and autumn of 1999, reports of dead crows played a critical role in identifying the outbreak of WNV in New York [13]. With bird cases often preceding human cases by up to 3 months, it served as an ideal early warning system

for WNV [14]. In Argentina [15] and Brazil [16], dead howler monkeys acted as an early warning for sylvatic transmission of YFV and prompted vaccination campaigns in the human population in 2008 and 2017, respectively.

A major limitation of monitoring human and animal cases is that confirmatory laboratory testing is not available in many limited resource countries, so arboviral disease is diagnosed on clinical symptoms. However, symptoms can overlap between arboviruses, as well as with non-arbovirus pathogens, complicating their clinical diagnosis. Furthermore, most arbovirus infections are mild, or sub-clinical, which may lead to them being under-reported. Ultimately, using human and animal case data is not ideal, since it indicates that active transmission is already occurring.

### Vertebrate host arbovirus surveillance: sentinel animals

Sentinel animals provide evidence of virus activity and increased risk to the target animal or human population [17]. For this, immunologically naïve animals are deployed in a specific location, bled on a defined schedule, and tested for the presence of virus-specific antibodies as an indication of exposure. Virus isolation or molecular detection on pre-seroconversion blood samples can provide an isolate and/or a sequence for genotypic analysis of circulating virus strains [18]. A suitable sentinel animal should: (i) be susceptible to the monitored virus; (ii) develop an antibody response that can be detected in serological assays; (iii) have low morbidity and mortality; (iv) be attractive to the vector; (v) be easy to handle; and (vi) allow for multiple sampling [12].

Different vertebrate species are used as sentinels (Table 1) and choice of animal is dependent on the target virus. In terms of WNV surveillance, some studies

**Table 1** Animal species that have been used as sentinels for arbovirus surveillance

Animal	Virus	Example location	References
Chickens	WNV	USA, UK	[119, 120]
	SLEV	USA	[121]
	MVEV, WNV <sub>KUN</sub>	Australia	[122]
Pheasants	WNV, SLEV, EEEV	USA	[123, 124]
Pigs	JEV	Japan, Australia, Thailand	[125–127]
Dogs	WNV	USA, Africa	[128, 129]
	JEV	Japan, Thailand	[130, 131]
Sheep and goats	RVFV	Africa, Saudi Arabia	[132–134]
Cattle	BTV, Akabane	Australia, Papua New Guinea, Japan	[135–137]
Horses	EEEV, WEEV	Argentina	[138]
	WNV, SLE	Colombia	[139]
Hamsters	EEEV, VEEV	USA, Central and South America	[140–143]
Non-human primates	YFV	Brazil, Argentina	[144–147]

**Abbreviations:** WNV, West Nile virus; SLEV, St. Louis encephalitis virus; MVEV, Murray Valley encephalitis virus; WNV<sub>KUN</sub>, West Nile virus (Kunjin subtype); EEEV, eastern equine encephalitis virus; JEV, Japanese encephalitis virus; RVFV, Rift Valley fever virus; BTV, bluetongue virus; WEEV, western equine encephalitis virus; VEEV, Venezuelan equine encephalitis virus; YFV, yellow fever virus

have suggested that the use of sentinel chickens is the most sensitive indicator of virus activity, when compared with other methods, such as detection of seroconversion in wild birds and virus isolation from mosquito pools [19]. Whilst they can undoubtedly serve as an early warning system, in some areas of the USA, sentinel chickens to monitor WNV have proven unsuccessful, since seroconversions were detected only after the onset of human cases [20, 21].

Even though sentinel animal surveillance enables the timely detection of circulating arboviruses, it also comes with limitations. In many cases, the locations of enzootic arbovirus foci are unknown or difficult to access. Thus, animals are placed near towns, which may be too far from virus foci to detect elevated activity [22]. Furthermore, some animals serve as amplifying hosts (i.e. pigs for JEV) increasing the risk of transmission to humans [23]. Additionally, the cost of rearing and replacing sentinel animals, especially in remote locations, can be prohibitive [24, 25], and bleeding large animals presents a workplace health and safety hazard [26]. There are also ethical considerations associated with the use of sentinel animals [27]. Finally, closely related viruses (i.e. JEV, WNV and Murray Valley encephalitis virus (MVEV)) can cross-react in some serological assays, requiring confirmation by other methods to obtain unequivocal results [28].

Another approach to vertebrate host surveillance relies on monitoring wild vertebrates or livestock, which are captured, sampled and released [12]. However, one of the biggest issues with surveillance of these animals is the cross-reaction between antibodies and the interpretation of the results. Given that many of these animals

are mobile, it is difficult to determine exactly when and where an animal acquired the infection, especially since IgG antibodies are present for the life of the animal.

#### Mosquito-based arbovirus surveillance

Mosquito-based arbovirus surveillance monitors vector populations and virus infection prevalence within them. Mosquitoes are collected, identified, pooled by species or other taxonomic grouping, and sent to the laboratory where they are tested for virus infection status. There are different strategies for mosquito collection. In areas with low-level mosquito infections or early in the transmission season, efforts should be directed towards performing targeted surveillance at “hotspots” where a high likelihood of arbovirus presence is suspected; as vector populations increase later in the season, the number of sampling sites should be expanded for broader monitoring [29]. There are a variety of commercial traps designed to collect mosquitoes, the design of which and application have been comprehensively reviewed elsewhere [30, 31]. It is essential that the selection of the collection method takes into consideration the physiological and behavioural characteristics of the studied vector [32] (Table 2).

A variety of methods have been utilized for detection of arboviruses in captured mosquitoes. Historically, arbovirus isolations were conducted in animals, such as suckling mice and chickens. With the development and establishment of cell lines, virus isolation in cell culture became the gold standard for arbovirus detection from pools of mosquitoes. This method can only detect viable viruses, so a cold chain keeping samples at ultralow temperatures during transport needs to be maintained to

**Table 2** Collection methods commonly used for mosquito-based arbovirus surveillance

Mosquito behaviour	Collection method	Advantages	Disadvantages	References
Host seeking	Human-landing catches <sup>a</sup>	Larger collections than resting or oviposition collections. Collections can be increased by using CO <sub>2</sub> or chemical lures	Most traps require batteries or AC power to operate. Depending on environmental conditions, the fan components are prone to malfunction. Require CO <sub>2</sub> as the primary attractant	[148]
	BG Sentinel			[149]
	CDC-light trap			[150]
	EVS-trap			[151]
	Mosquito Magnet™			[152]
	Animal baited traps			[153–155]
Resting	CDC-backpack aspirator	More blood fed mosquitoes collected, ideal for blood meal analysis	Labour intensive and inefficient mosquito capture	[156]
	Prokopack			[157]
	Resting boxes			[158–160]
Oviposition	Sticky ovitraps	Mosquitoes have bloodfed and thus a higher probability of detecting positive mosquitoes. Targets <i>Aedes</i> -borne viruses such as DENV and CHIKV	Smaller collections than other methods, thus all mosquitoes can be easily processed	[161–163]
	Gravid <i>Aedes</i> trap (GAT)			[66]
	CDC-gravid trap			[164]

<sup>a</sup>Although this method has been used for arbovirus studies in the past, it has considerable drawbacks, including the risk of infection to the collector, which is considered unethical even illegal in some countries

**Abbreviations:** CDC, Centers for Disease Control and Prevention; EVS, Encephalitis virus surveillance

preserve virus infectivity [33]. Maintenance of a cold chain requires the use of dry ice or liquid nitrogen shippers in the field, which can be logistically challenging. Virus isolation is time consuming and obtaining definitive results can take weeks, which defeats the purpose of using it for early warning. Some viruses do not replicate on common cell lines used in the laboratory. This can be the case for previously unrecognized or unknown viruses, such as insect-specific flaviviruses (ISF) that do not grow in vertebrate cells [34]. Virus isolation can be expensive and requires special infrastructure and trained personnel. However, even with these limitations, virus isolation is still an important method for arbovirus diagnostics, as it increases viral titer, which allows for full genome sequencing and provides viruses for phenotypic characterization.

Nucleic acid detection using RT-PCR has become one of the most popular methods of virus detection and has potentially displaced virus isolation as the new gold standard. Real time quantitative RT-PCR (qRT-PCR) platforms, such as TaqMan®, are ideal for routine testing of mosquitoes, since they reduce processing time significantly (sometimes to less than an hour), allowing for high throughput screening [35, 36]. Since these assays detect both infectious virus and RNA, they have comparable or better sensitivity than virus isolation [37]. Depending on the protocol or application, these techniques enable the detection of one infected individual from a pool of up to 5000 non-infected mosquitoes [38, 39]. Additionally, although a cold chain is still recommended, it has been possible to detect viral RNA from dead mosquitoes kept for several weeks in hot and humid conditions by qRT-PCR [33, 39, 40]. Currently, a variety of qRT-PCR assays exist for the detection of almost every

arbovirus of human (and veterinary) importance, with some even available in multiplex format [41]. In spite of this, it is important to note that RT-PCR and qRT-PCR will only pick up RNA from viruses that the primers and probes were designed to detect [42]. Historically, one of the main drawbacks of this method has been its high installation and reagent costs, limiting its use in low-resource settings. However, recently, costs associated with qRT-PCR have dropped considerably making it an accessible alternative for routine screening.

Rapid antigen detection assays were initially developed to test clinical samples but have proven to be a useful tool to test mosquito pools in the field [43]. These assays allow for qualitative detection of arboviruses, and have the advantage of being rapid, without the need for specialized equipment. Currently, there are tests commercially available for a variety of viruses including CHIKV [44], DENV [45] and WNV [43], among others. In Singapore [46] and Malaysia [47], a dengue NS1 rapid test has been used to detect infected mosquitoes as part of a routine surveillance programme. These tests have shown high specificity for the target virus, although some assays have reduced sensitivity when compared with molecular methods [48, 49]. However, although they may provide an underestimate of infection rate, they provide a first screen and have applicability in regions without access to more resource intensive or expensive diagnostic capacity.

Traditional mosquito-based surveillance systems that target processing of pools of mosquitoes come with inherent limitations. Mosquito populations often have very low carriage rates, whereby only one in 1000 mosquitoes is actually infected [50]. To increase the probability of detection, large numbers of mosquitoes are required,

resulting in numerous mosquitoes to identify, pool and test, increasing laboratory costs and turnaround time. Additionally, many traps require attractants, such as CO<sub>2</sub>, to increase collections. This comes in the form of dry ice or pressurized cylinders, which may not be readily available, or only allow overnight deployment of the trap. A cold chain of storage at < -50 °C is required to preserve the integrity of the virus for detection, which can be a challenge in remote locations. Finally, specialized laboratory equipment and infrastructure is required for diagnostics, which might not be available in developing countries.

### Novel methods for arbovirus surveillance

The majority of mosquito species feed on carbohydrates (i.e. flower nectar, honeydew or rotting fruit) which are the primary energy source of their diet [51]. One exception is *Ae. aegypti*, which appears to obtain enough energy from blood and rarely feeds on sugar in domestic environments [52]. The ingestion of carbohydrates is important for the survival of the mosquito, and plays an indirect role in disease transmission, allowing an infected female to live long enough to become infective [53]. It was hypothesized by Doggett et al. [54] and confirmed by van den Hurk et al. [55], that infected mosquitoes expectorate virus while sugar feeding, which can be detected using molecular assays. This finding led to the development of novel sugar-based approaches for the detection of arboviruses in mosquitoes in the field. This system integrates purpose-built CO<sub>2</sub>-baited box traps, which house nucleic acid preservation cards (Flinders Technology Associates, FTA® cards) soaked in honey and on which mosquitoes feed and expectorate onto [56]. The FTA® cards inactivate any expectorated viruses and preserve the liberated RNA. The cards are then sent to the laboratory in the post without requirement of a cold-chain, where they are screened for viruses using molecular assays.

Commonly used traps employed to collect mosquitoes (i.e. CDC-light trap and Encephalitis Virus Surveillance, EVS, trap) require batteries to operate which can be logistically challenging. To circumvent this limitation, a non-powered CO<sub>2</sub>-baited passive box trap (PBT) was developed by Ritchie et al. [57] to collect and house mosquitoes. A variation of the PBT, the sentinel mosquito arbovirus capture kit (SMACK) was developed to increase mosquito survivorship and consequently increase the probability of infected mosquitoes feeding on the FTA® card [58]. Although designed for weekly or fortnightly servicing, the SMACK has demonstrated similar trap efficacy to the CDC-light trap and EVS trap in overnight collections, making it an alternative to traps that require batteries to operate.

Free-standing sugar bait stations have the potential to be used instead of CO<sub>2</sub>-baited traps [59]. These stations consist of a dental wick soaked in sucrose solution and a floral lure, such as phenyl acetaldehyde. Mosquitoes lured to the station feed on the wick, which is tested for expectorated viral RNA. The sugar bait stations do not require CO<sub>2</sub> or electricity, so a number of stations can be deployed simultaneously, thus increasing geographical coverage. In a proof of concept, the sugar bait stations detected WNV before sentinel animals seroconverted in California. However, this method appears more efficacious in arid habitats, probably because of lack of competition with other sucrose sources, such as floral nectars. As sugar bait stations facilitate increased geographical coverage, they may have higher costs associated with analysing an increased number of samples, although this would be offset by savings by not having to use CO<sub>2</sub> baited light traps.

Sugar-based surveillance has several advantages over traditional methods. When mosquito populations are elevated, sorting becomes time consuming, and a high number of pools can overwhelm laboratory capacity. When combined, these issues can reduce the ability to provide results in a timely manner. Sugar-based methods potentially overcome these issues, since only 1-2 FTA® cards per trap are tested, compared to a variable number of mosquito pools. As only transmitting mosquitoes will yield positive results, the presence of virus in saliva expectorate is a better estimate of transmission risk. FTA® cards can preserve viral RNA for up to 28 days [56], making this an ideal alternative for surveillance in remote or difficult to access locations, where regular servicing of traps is not feasible. Results suggest that sugar-based surveillance is a more sensitive indicator of arbovirus activity than sentinel animals. In northern Australia, it has been possible to detect WNV<sub>KUN</sub> before sentinel animal seroconversions [60]. However, a comparison of the sugar-based surveillance system with existing strategies still needs to be thoroughly evaluated. Sugar-based surveillance, using either SMACK or EVS traps, has been successfully incorporated into existing surveillance programs in Australia, with multiple detections of MVEV, WNV<sub>KUNV</sub>, RRV, BFV, Edge Hill virus and Stratford virus [61–64].

Honey-soaked FTA® cards have the potential to be integrated into surveillance of *Ae. aegypti*-borne arboviruses. The cards have been used in Biogents sentinel traps (BGS traps) and modified double sticky ovitraps for the detection of CHIKV in French Guiana [65]. The approach appeared time consuming with only one CHIKV positive FTA® card out of 234 analysed. Traps that are more efficient at collecting *Ae. aegypti* may be able to increase trap collections, thus increasing the likelihood of detecting virus. For instance, the Gravid *Aedes*

Trap (GAT) [66] collects 2.4 times more *Ae. aegypti* and significantly more gravid females than double sticky ovi-traps [67], which could increase the chances of finding positive mosquitoes. However, *Ae. aegypti* collections are usually small, and in many cases, it would be easier to pool the mosquitoes (or alternatively, squash them into FTA® cards [68]) and process them by molecular methods.

Like any system, sugar-based surveillance has some limitations. Perhaps its main limitation is that the cycle threshold ( $C_t$ ) values obtained by real time RT-PCR are high (> 30 cycles), reflecting the relatively small amount of saliva expectorated by mosquitoes [69]. Additionally, this method will only detect positive mosquitoes after the extrinsic incubation period which, depending on the virus, can last from two to 14 days. Thus, the proportion of mosquitoes in a population that survive to transmit the virus can be quite low. In order to increase mosquitoes feeding on the FTA® cards, trapped mosquitoes must be kept alive in the trap for as long as possible. The SMACK was developed to include a water reservoir in the trap to increase humidity, the lack of which can be a problem in remote and arid locations. To save on reagent costs, some agencies will wait until they have sufficient samples to batch together, which can extend the turnaround time. Finally, sugar-based surveillance does not provide data on the mosquito species that expectorated the virus. Instead, detection of virus on a FTA® removed from a trap could be used to trigger intensive trapping to collect mosquitoes for pooling and processing to provide information on potential vectors at a given time point or location.

A potential way to increase sensitivity of sugar-based surveillance systems is through the collection and analysis of mosquito excreta. When mosquitoes feed on a sucrose solution it takes approximately 30 min for it to reach the midgut, after which excreta is ejected from the anus [70]. In terms of pathogen detection, the focus has mainly been on the detection of filarial nematodes, such as *Brugia malayi* [71] and *Plasmodium vivax* [72]. In the late 1920s, de Bearepaire Aragão and da Costa Lima performed a series of experiments in which they infected rhesus macaques with the excreta collected from YFV infected *Ae. aegypti* [73–75]. Laboratory-based experiments have recently demonstrated that *Ae. aegypti* with a disseminated infection excrete DENV RNA, which can be detected through qRT-PCR [76]. The rate of detection was higher in excreta samples, 89%, compared with 33% for saliva samples. This suggests that collection of excreta from trapped mosquitoes could enhance the sensitivity of current sugar-based surveillance systems. This is not surprising, given that mosquitoes excrete considerably more fluid than they salivate (~1.5  $\mu$ l [77] vs 4.7 nl [69]). Integration of excreta collection into current surveillance

systems would require modification of current trap designs to selectively capture mosquito excreta.

## Advances in arbovirus detection, characterization and data interpretation

### Next-generation sequencing for the detection of arboviruses

Traditionally, diagnostic assays utilised in arbovirus surveillance programs only screen for characterised endemic and enzootic viruses. Because virus specific primers and probes are used for molecular diagnostics, it is likely that many other viruses, whether pathogenic or not, remain undetected. Metagenomic analysis using next-generation sequencing (NGS), allows for the simultaneous identification of viruses, mosquito species, and endosymbionts, such as *Wolbachia*, from a single mosquito in a single reaction [78] without prior sequence knowledge. This approach relies on bioinformatics tools to analyse the millions of sequence reads [79–81] and the availability of high-quality sequence databases to analyse the large and complex datasets generated. In Australia, viral metagenomics has been used for the identification of multiple arboviruses, including novel rhabdoviruses, bunyaviruses [82] and mesoniviruses [83] from field collected mosquitoes.

At this stage, NGS methods have some disadvantages compared with other molecular methods of virus detection. NGS is less sensitive than qRT-PCR for the detection of samples with low virus titres [84]. At present, the costs associated with NGS are higher than the cost of qRT-PCR, and its associated equipment has a relatively large laboratory footprint. It also requires intimate bioinformatics knowledge and reference sequence databases to analyse the data produced. Over the past years, there has been advancement in the hardware used for NGS, with equipment getting smaller and cheaper. The first hand-held portable sequencer (MinION) is already available on the market. This platform reduces processing time significantly (e.g. < 6 hours for detection of CHIKV from blood samples [85]). Even with operational challenges, the MinION's high portability and low energy requirements have enabled its use in extreme field conditions [86] and it has been used to investigate outbreaks of Ebola [87] and *Salmonella* [88]. It has recently been demonstrated that the MinION can be used for metagenomic arbovirus detection from infected mosquitoes [89], so it could be used during arbovirus outbreaks. Although the MinION still has limitations, such as high error rates and requirement for an internet connection for base calling, technologies like this, together with lower reagent costs, will be crucial in making sequencing accessible in the field in the near future.

### Xenosurveillance

Mosquitoes have the potential to act as environmental samplers (“biological syringes”) that feed on the blood of a variety of vertebrate hosts. Xenosurveillance offers an alternative to directly sampling hosts, a process that is time consuming and requires individual informed consent in the case of humans or animal ethics approval, in the case of veterinary pathogens. Mosquitoes can be used as a proxy for syringe sampling of small animals for virus titer determination [90]. This approach has mainly been used to study vector-borne pathogens, such as filarial parasites [91] or apicomplexans [92]. For example, in Sri Lanka, xenosurveillance has been successfully used to map areas with persistent *Wuchereria bancrofti* after mass drug administration programmes [93]. Furthermore, it has been possible to detect DENV from (non-competent) *Anopheles stephensi* mosquitoes 24 h after ingestion [94]. In addition to viruses that actively replicate in them, engorged mosquitoes potentially possess viruses or other pathogens that do not replicate in them but might be present in hosts they feed upon [95]. Xenosurveillance monitors these potential non-vector borne human and animal pathogens [96] by performing nucleic acid detection or vector enabled metagenomics [97] on mosquito samples. Mosquitoes have been successfully used to monitor non-mosquito borne pathogens such H5N1 influenza virus [98], Epstein-Barr virus, canine distemper virus [96], human herpesvirus, human papillomaviruses, anelloviruses and circoviruses, among others [95].

One of the main limitations of xenosurveillance is the difficulty in collecting sufficient blood engorged mosquitoes for analysis. Some of the methods to collect engorged mosquitoes (i.e. use of an aspirator) are labour intensive and can be intrusive, especially when sampling inside houses and villages [99]. To circumvent this issue, mosquito excreta could be used to provide the template for xenosurveillance. Indeed, hepatitis B virus, which does not replicate in the vector, has been detected in mosquito excreta by RT-PCR and Southern Blot up to 7 days after the ingestion of an infectious blood meal [100].

### Emerging technology

Integration of data acquisition, storage and sharing methodologies, such as cloud networks and geographic information systems, will form an integral component of surveillance and control programmes. An example of this is the Intelligent Dengue Monitoring technology (MI-Dengue) developed in Brazil [101]. MI-Dengue consists of an array of tools to collect gravid *Ae. aegypti* females, collect field data, detect virus and create georeferenced infestation maps that are available in real time, providing information to optimize vector control.

This system has been successful at reducing dengue in the municipalities that have adopted it.

In the age of mobile phones, social media and internet, citizen science will undoubtedly play an important role in disease surveillance in general. In Spain, Mosquito Alert was implemented as a system to collect reports of invasive *Ae. albopictus*. To date, it has more than 30,000 registered participants [102]. As a part of the GLOBE project sponsored by NASA, Mosquito Habitat Mapper merges data generated by citizens with satellite-based research [103]. Interestingly, with minimal training, the data generated by programmes like these is considered as reliable as data collected by experts [104]. Mobile phones, even low-end ones, can also be used as acoustic sensors to identify mosquito species [105]. All these initiatives will allow large-scale data acquisition, which is critical for adequate mosquito control.

Over the past 20 years, single device detection platforms for clinical and environmental analyses have been rapidly evolving. A promising technique for integration into surveillance programmes is the use of microfluidic devices [106] and biosensors [107] which are designed to process very small volumes of liquid, requiring minimal amount of sample and reagents to yield results in minutes [108, 109]. Some applications of these devices include diagnosis of infections caused by DENV [110–112] and CHIKV [113] from clinical samples, detection of DENV NS1 antigen from pools of mosquitoes [114] and genotyping of closely related *Anopheles* species [115].

### Conclusions

Over the past decade, there have been key scientific advances in arbovirus surveillance, particularly with regard to sample collection, virus detection and data analysis. Table 3 summarises the relative advantages and disadvantages of current and emerging surveillance methodologies. Alternative samples for virus detection, such as mosquito excreta, may enable more sensitive detection of arboviruses than existing methodologies. It has been proposed that we are on the cusp of a revolution in genomic epidemiology [116]. With NGS technologies becoming more accessible in the near future, they will enable the collection of real-time in-depth genetic information on circulating arboviruses before or during an outbreak. There is still room for improvement of surveillance systems used in remote locations where surveillance coverage is limited by cost and limited access to sites. Use of other sources of CO<sub>2</sub> in mosquito traps (such as fermentation using yeast) [117] or CO<sub>2</sub>-free systems could provide an alternative in areas where dry ice or pressurized gas cylinders are not available. Deployment of in-field portable molecular laboratories or point of care assays could provide same-day assessment of arbovirus circulation and rapid response in these locations [118]. In the future, other technologies,

**Table 3** Summary of traditional and novel arbovirus surveillance methods

Method	Advantages	Disadvantages	Application
Monitoring human and animal disease	Uses data that is already being collected by hospitals, health practitioners, and animal health personnel	Overlap of clinical symptoms within arboviruses and other pathogens. Not ideal for early warning since active transmission will be already occurring	National disease surveillance databases
Sentinel animals	Can act as an early warning system	Animals can be amplifying hosts. High costs associated with animal rearing. Cross reactivity between closely related arboviruses when using serological assays	Routine surveillance, inform control strategies
Virus isolation from pools of mosquitoes	Increases virus titer allowing for genotypic and phenotypic characterization	Time consuming. Requires special infrastructure (biological containment). Requires a cold chain	Routine surveillance, virus identification, inform control strategies
Virus detection in pools of mosquitoes using molecular assays	Allows high throughput screening. High sensitivity	Will only detect RNA from viruses that the assays were designed to detect. Requires special infrastructure	Routine surveillance, research, inform control strategies
Virus detection in pools of mosquitoes using rapid antigen detection assays	Rapid. Does not require specialized equipment. Lower cost	Lower sensitivity than molecular methods	Routine surveillance in low resource settings
Sugar-based surveillance	Does not require a cold chain. Only 1-2 samples per trap are tested potentially compared with 1000s of mosquitoes using other methods of surveillance. Better estimation of transmission risk	Relies on a nanoliter amounts of expectorate. Mosquitoes need to be kept alive for as long as possible to increase feeding on cards. Cannot be used to incriminate mosquito species as vectors. Requires special infrastructure	Routine surveillance, ideal for remote locations
Next-generation sequencing of mosquito samples	Does not require prior information (will detect any arbovirus present in the sample)	High cost. Requires bioinformatics knowledge. Requires special infrastructure	Research, virus discovery
Xenosurveillance	Mosquito acts as an environmental sampler. Allows detection of viruses that do not replicate in the mosquito	Blood engorged mosquitoes are difficult to collect	Research and surveillance of arboviruses and other pathogens

such as unmanned aerial vehicles (UAVs) could be used to automate sample collection in difficult to access locations increasing the coverage of surveillance. Regardless of the surveillance system, there are always going to be issues and limitations, which can vary between jurisdictions. Currently, the extent of arbovirus surveillance varies between countries and even states with many jurisdictions lacking any form of monitoring. There is a need for sharing of arbovirus surveillance intelligence between public health agencies at regional level as a means to apply better control measures. Moreover, the implementation issues that might arise from new approaches cannot be underestimated. Agencies that are familiar with set methodologies may be reluctant to adopt new technologies or not have the capacity to implement change. Because of this, when designing new arbovirus surveillance methodologies, there should be a clear understanding of the needs and limitations of field, laboratory and public health personnel.

#### Abbreviations

DENV: Dengue virus; JEV: Japanese encephalitis virus; WNV: West Nile virus; CHIKV: Chikungunya virus; ZIKV: Zika virus; YFV: Yellow fever virus; SLEV: St. Louis encephalitis virus; RRV: Ross River virus; BFV: Barmah Forest virus; MVEV: Murray Valley encephalitis virus; BTV: Blue tongue virus; RVFV: Rift Valley fever virus; EEEV: Eastern equine encephalitis virus; WEEV: Western equine encephalitis virus; ISF: insect-specific flaviviruses; IgG: immunoglobulin G; PCR: polymerase chain reaction; RT-PCR: reverse-transcription polymerase chain reaction; RNA: ribonucleic acid; FTA\*: Flinders Associate Technologies; PBT: passive box trap; SMACK: sentinel mosquito arbovirus capture kit; C<sub>t</sub>: cycle threshold; GAT: gravid *Aedes* trap; NGS: next-generation sequencing; UAV: unmanned aerial vehicle

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**Authors' contributions**

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