

Wastewater and Environmental Surveillance Summary for Arboviruses of Human Significance

Pilot version, 1 December 2025



This document provides information on wastewater and environmental surveillance (WES) for arboviruses relevant to human health. It should be used together with the accompanying *WES Guidance for one or more pathogens* which includes general and cross-cutting information, and the target sheets for SARS-CoV-2 and influenza viruses (available [here](#)).

WES for Arboviruses of human significance at a glance

Arboviruses transmitted by mosquitoes of public health significance include dengue virus (DENV), Zika virus (ZIKV), chikungunya virus (CHIKV), West Nile virus (WNV), Japanese encephalitis virus (JEV), yellow fever virus (YFV), and Oropouche virus (OROV). Of these, dengue, Zika, chikungunya, West Nile and Japanese encephalitis have at least preliminary evidence or rationale for wastewater or environmental surveillance. Yellow fever and Oropouche viruses are included for completeness; however evidence for these is currently lacking. Further review will be warranted if such studies emerge.

Table 1: At a glance assessment of key WES criteria for arboviruses given current evidence^{a b}

Setting	Categorical Assessment (CA)	Public Health Significance	Actionability / Relative value	Technical Feasibility	Operational Feasibility	Acceptability ^c	Optimisation	
	Strength of Evidence (SoE)						Integrated disease response	Multitarget WES
Sewered	CA	DENV, JEV, YFV	DENV, ZIKV, CHIKV, JEV, WNV	DENV, ZIKV, CHIKV, WNV	DENV, ZIKV, CHIKV, WNV	DENV, ZIKV, JEV, WNV, CHIKV, YFV, OROV	DENV, ZIKV, JEV, WNV, CHIKV, YFV, OROV	DENV, ZIKV, CHIKV, WNV
		WNV, ZIKV, CHIKV, OROV	YFV, OROV	JEV, YFV, OROV	JEV, YFV, OROV	DENV, ZIKV, JEV, WNV, CHIKV, YFV, OROV	DENV, ZIKV, JEV, WNV, CHIKV, YFV, OROV	JEV, WNV, CHIKV, YFV, OROV
	SoE		DENV, ZIKV, CHIKV, JEV, WNV	DENV, ZIKV, CHIKV, WNV	DENV, ZIKV, CHIKV, WNV	DENV, ZIKV, JEV, WNV, CHIKV, YFV, OROV	DENV, ZIKV, JEV, WNV, CHIKV, YFV, OROV	DENV, ZIKV, CHIKV, WNV
			YFV, OROV	JEV, YFV, OROV	JEV, YFV, OROV	DENV, ZIKV, JEV, WNV, CHIKV, YFV, OROV	DENV, ZIKV, JEV, WNV, CHIKV, YFV, OROV	JEV, YFV, OROV

Key:

1. Categorical Assessment (CA) of criteria

Category	Code	Description
High		Criteria is evaluated as met at the highest level
Intermediate		Criteria is evaluated as met at an intermediate level (it may be that not all sub-components of the criteria are met)
Low		Criteria is evaluated as low
Not-supported		Criteria is evaluated as not supported
Not applicable		Criteria is not applicable OR cannot be assessed due to inadequate evidence

2. Strength of evidence (SOE)

Evidence level	Code	Description
Strong		High quality consistent evidence, including from multiple relevant studies/settings, at scale, over a prolonged period, with evidence from program settings, not only from research studies or short projects.
Moderate		Relevant evidence is available but does not meet criteria for 'Strong' classification. ^d
Inadequate evidence		Evidence is inadequate and further study/evaluation is needed

^a Further description of the criteria used to assess the applicability of WES for a specific pathogen, as well as the methods used to evaluate them, is included in *WES Guidance for one or more pathogens*. The assessment in Table 1 provides a snapshot at the global level, but country level assessment may differ.

^b Sewered settings refers to closed reticulated sewage systems. Non-sewered settings refers to the diverse settings which are not 'sewered', including open drains and community sampling points. Individual small septic tanks at residential or building level are not viable to sample individually and are not considered here separately. Most WES evidence to date is reported from reticulated sewer settings, often from high-income settings. Yet much of the global population is on heterogeneous non-sewered systems and this has implications for assessment of various WES categories.

^c Experts did not achieve consensus on the assessment of these criteria. The majority view is shown here, with others evaluating both higher and lower.

^d Evidence classified as 'Moderate' meets one or more of the following criteria: not from numerous settings, for a short period, without program-level evidence, and/or where findings are not consistent or of high quality.

Summary

Arboviral WES has been demonstrated for ZIKV (in one setting), there is limited operational evidence for DENV, CHIKV and WNV, early proof-of-concept evidence for JEV and none yet for YFV, or OROV. Current evidence supports cautious optimism of the potential of WES to strengthen existing surveillance and underscores the need to evaluate and optimise methods as part of integrated arboviral initiatives at the local, regional and global levels.

- **Public health importance:** Arboviruses of greatest concern for human health include DENV, ZIKV, CHIKV, WNV, JEV, YFV and OROV. Together they cause millions of cases annually, with varying severity and global/regional impact.
- **Drivers of risk:** Climate change, urbanisation, mobility, and vector expansion increase populations at risk and outbreak frequency, scale, and pandemic potential, particularly for *Aedes*-mosquito borne viruses (e.g. DENV, ZIKV, CHIKV).
- **Human shedding:** Low-level, short-duration RNA shedding in urine following acute infection (+/- upper respiratory or genital secretions), no evidence for chronic shedding (with exception of WNV) - which supports biological plausibility for WES.
- **Feasibility:** Arboviruses are shed at much lower levels than established enteric and respiratory targets used in WES programs; detection likely requires larger sample volumes, and use of sensitive concentration and molecular assays. Sequencing use may be limited given its success depends on adequate viral levels.
- **WES implementation evidence:**
 - **DENV:** Detections in various contexts (outbreak/non-endemic and endemic).
 - **CHIKV:** Detections in high and low prevalence non-outbreak settings.
 - **ZIKV:** Detections in Singapore outbreaks with public health utility.
 - **JEV:** Proof-of-concept detection in Australia during small outbreak.
 - **WNV:** Proof-of-concept detection in USA in outbreak.
 - **YFV, OROV:** No published WES evidence to date
- **Optimization:** Integration into multi-pathogen WES workflows (e.g. with polio, SARS-CoV-2 and for multiple arboviruses) is practical at low marginal cost. Compared with case-based and entomological surveillance, WES can provide a relatively low-cost, population-level signal when embedded in existing systems.
- **Public health use cases / Applications:** Not currently recommended for arboviral surveillance. However early adopters exist (eg ZIKV in Singapore). Given gaps in current surveillance methods, pilot results suggest there may be potential value of WES, including for early warning and in enhanced surveillance in response to outbreaks. Multi-target WES has potential to identify multiple circulating arboviruses (eg DENV and CHIKV). A key caveat with low viral levels is that absence of detection does not equate to no circulating virus.
- **Key research questions**
 - In which contexts and how could WES add value to arboviral surveillance?
 - What sampling, concentration, and analytical workflows maximise sensitivity and specificity for RNA detection of targeted arboviruses?
 - How to interpret a positive signal (differentiating imported and local transmission)?

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1. General Information on Arboviruses

1.1. The pathogens and associated diseases

Arboviruses transmitted by mosquitoes and other vectors are among the leading causes of epidemic viral disease globally. Of more than 500 arboviruses described, over 100 are known to infect humans. This summary focuses on seven of greatest (human) public health importance: dengue (DENV), Zika (ZIKV), chikungunya (CHIKV), Japanese encephalitis virus (JEV), West Nile virus (WNV), yellow fever virus (YFV), and Oropouche virus (OROV) (1). Table 2 (next page) describes key features of the seven priority arboviruses including diseases, geographic distribution and principal vectors and hosts.

Clinical presentation varies from acute febrile illness (DENV, CHIKV, OROV), to neuroinvasive disease (WNV, JEV), to severe viscerotropic disease (YFV). ZIKV infection is mostly asymptomatic but its congenital and neurological complications are of particular concern and sexual transmission is known to occur. In addition to acute disease, several arboviruses are associated with chronic or long-term sequelae, including persistent arthralgia (CHIKV), neurological impairment (ZIKV), neurocognitive deficits (JEV, WNV) and renal disease (WNV) (1).

While this document focusses on the seven arboviruses listed above, there are also other arboviruses of regional or emerging public health significance. These include : Crimean–Congo hemorrhagic fever virus (CCHFV) (tick-borne; Africa, Asia, Eastern Europe), Rift Valley fever virus (RVFV) (mosquito-borne; zoonotic with major outbreaks in Africa and the Middle East), Tick-borne encephalitis virus (TBEV) (tick-borne; endemic in parts of Europe and Asia), Eastern equine encephalitis virus (EEEV) (mosquito-borne; rare but highly fatal in the Americas), Venezuelan equine encephalitis virus (VEEV) (mosquito-borne; Central and South America and noted as a pandemic risk), Mayaro virus (MAYV) (mosquito-borne; emerging in South America), and Ross River virus (RRV) (mosquito-borne; regionally important in Australia and the Pacific).

1.2. Global burden, geographic distribution & risk factors

The burden of arboviruses varies widely by pathogen and geography, but together they account for millions of infections annually, significant disability-adjusted life years and social and economic costs.

As shown in Table 2, DENV is the most widespread, causing an estimated 100–400 million infections annually across the tropics and subtropics (2). ZIKV (3) and CHIKV (4) have shown explosive epidemic spread in the Americas, Asia, and Africa. JEV (5) remains restricted to Asia and the Pacific with spread to more temperate zones. While YFV remains restricted to Africa and South America. WNV has expanded widely across North America, Europe, and parts of Asia and Africa (6). OROV emerged in South and Central America in 2024 after decades of more limited geographic circulation in the Amazon basin (7).

Table 2. Overview of selected arboviruses of public health importance

Pathogen	Main disease(s)	Geographic distribution	Burden of disease	Principal vectors	Animal reservoirs
Dengue virus (2,8)	Dengue fever, severe dengue	Tropical and subtropical regions; widespread Asia, Latin America, Africa	100–400 million infections annually; leading arboviral burden	<i>Aedes aegypti</i> , <i>A. albopictus</i>	Humans (amplifying host)
Zika virus (3,9,10)	Mostly asymptomatic; Zika fever; congenital Zika syndrome; neurological disease in adults and children	Tropical/subtropical; Americas, Asia, Africa, Pacific	Major outbreak in the Americas (PHEIC 2016); ongoing sporadic cases and limited outbreaks	<i>Aedes aegypti</i> , <i>A. albopictus</i>	Humans (amplifying host)
Chikungunya virus (4,7)	More commonly symptomatic; chikungunya fever; chronic arthralgia	Tropical/subtropical; outbreaks in Africa, Asia, Americas	Millions infected in large outbreaks; chronic disability burden	<i>Aedes aegypti</i> , <i>A. albopictus</i>	Humans (amplifying host)
West Nile virus (6,11)	Mostly asymptomatic; febrile illness; neuroinvasive disease	Africa, Europe, Middle East, North America, Asia	Most infections asymptomatic; thousands neuroinvasive cases annually	<i>Culex</i> spp.	Birds (primary reservoir); horses, humans (dead-end)
Japanese Encephalitis virus (5,12)	Mostly asymptomatic; encephalitis; neurological sequelae	Asia-Pacific; risk in rural rice-growing regions	~68,000 cases/year; high fatality and disability	<i>Culex</i> spp. (principal: <i>Cx. tritaeniorhynchus</i>)	Pigs (amplifying), wading birds (reservoirs); humans (dead-end)
Yellow Fever virus (13)	Yellow fever; viscerotropic disease	Sub-Saharan Africa, tropical South America	Est. 200,000 cases and 30,000 deaths annually; vaccine-preventable	<i>Aedes aegypti</i> (urban); <i>Haemagogus</i> , <i>Sabethes</i> (sylvatic)	Non-human primates (reservoirs); humans (urban cycle)
Oropouche virus (7,14)	Oropouche fever	South and Central America (esp. Brazil, Peru, Panama)	Thousands of cases in outbreaks; emerging concern	<i>Culicoides</i> midges; possible <i>Culex</i> spp.	Sloths, primates, other mammals (reservoirs)

Arboviral epidemics have increased in frequency, scale, and geographic extent in recent decades, shaped by climate change, rapid urbanization, and global travel. Between 50–80% of arboviral infections are asymptomatic, complicating burden estimates and delaying outbreak recognition through case-based surveillance. These features highlight the substantial and evolving global burden of arboviruses.

1.3. Pandemic potential

Only a subset of arboviruses pose a realistic pandemic threat. Those transmitted by urban *Aedes* mosquitoes — particularly DENV, ZIKV and CHIKV — combine high global distribution, human amplification, explosive outbreak potential, and sensitivity to climate and urbanization, suggesting they have the greatest pandemic potential. These as well as VEEV are identified with risk of causing public health emergencies of international concern (15).

1.4. Hosts, vectors, and routes of transmission

Transmission of arboviruses is primarily vector-borne, but the dynamics vary depending on the pathogen (16).

- DENV, ZIKV, and CHIKV are maintained predominantly in human–mosquito–human cycles, with humans serving as the main amplifying hosts and *Aedes aegypti* and *A. albopictus* as the principal epidemic vectors.
- In contrast, JEV and WNV depend on zoonotic reservoirs: pigs and ardeid birds for JEV, and birds for WNV, with humans and horses acting as incidental dead-end hosts.
- YFV circulates in both sylvatic cycles involving non-human primates and sylvatic mosquitoes (*Haemagogus* or *Sabethes*), and in urban cycles transmitted by *A. aegypti*.
- OROV is transmitted primarily by *Culicoides* midges, with a range of mammalian reservoirs suspected, including sloths and primates.

Although clinical infection arises from vector-borne transmission, viral RNA may enter wastewater indirectly via human shedding or animal waste, and these pathways are explored in subsequent sections.

2. Information Related to Arboviruses and wastewater

2.1. Potential inputs to wastewater and environmental waters

Arboviruses and genetic fragments can enter wastewater systems through a range of human and non-human inputs which vary by pathogen. Human shedding of viral RNA has been reported in urine and, less frequently, in saliva, genital secretions and stool. Human shedding is typically at low levels (much lower than those seen in enteric pathogens and SARS-CoV-2 or influenza) with considerable variability or shedding (and of available evidence) between arboviruses. Zoonotic reservoirs may indirectly contribute RNA to wastewater and environmental waters through run-off or animal waste, if there is overlap with human sanitation systems.

Table 3. Summary of available shedding evidence by major arboviral pathogen (relevant to inputs into wastewater and environmental waters)

Pathogen	Urine	Faeces	Vaginal secretion / semen	Upper respiratory specimens ¹	Zoonotic source	Prolonged shedding	Vaccine derived
Dengue virus	✓✓ (17–26)	✗	✓ (19)	✓ (17–20,23,25,27)	✓ (28)	✗	✓ Saliva/urine (29)
Zika virus	✓✓ (30–38)	✓ (30)	✓ (31,39)	✓ (33,35–37)	✓ (40,41)	✓ Semen (31)	✗
Chikungunya virus	✓✓ (42–46)	✗	✓ (44)	✗	Humans main	✗	
West Nile virus	✓ (47–52)	✗	✗	✗	Birds (enzootic), Horses (dead-end)	✓ Urine (49)	
Japanese Encephalitis virus	✓ (53,54)	✗	✗	✓ (55)	Pigs, Wading birds	✗	
Yellow Fever virus	✓✓ (56–59)	✗	✓ (56)	✗	Non-human primates	✓ Urine (57,59)	✓ Urine (60,61)
Oropouche virus	✓ (62,63)	✗	✓ (63)	✗	Humans (urban), animal cycle unclear	✗	

¹ Upper respiratory specimens include saliva, throat swab, and nasopharyngeal swab.

Overall, the strongest evidence for human urinary shedding comes from DENV, ZIKV, WNV and YFV with multiple studies documenting RT-PCR detections of urinary shedding and occasional detection in other bodily fluids following acute infection. Stool results are rarely reported, although animal and laboratory studies suggest this is likely with ZIKV (64). JEV and OROV have more limited evidence demonstrating urine and throat detection can occur but is not a typical feature. A study from China involving 52 acute JE cases and urine collected 3 to 9 days from onset reported no urine RT-PCR JEV RNA detections (65).

Non-human contributions, such as pigs (JEV), birds (WNV), and primates (YFV), may provide additional pathways for RNA entry into wastewater and environmental samples, complicating interpretation of detections. Finally, live attenuated vaccines may result in vaccine-associated shedding as documented for polio, measles and YFV (60). There is evidence for rare and low level shedding of the tetravalent dengue vaccine CYD-TDV (29). There is no evidence of JEV vaccine associated shedding. These findings highlight the importance of interpreting arbovirus WES signals cautiously, considering human, zoonotic and vector sources and their potential contribution to WES samples.

2.2. Target persistence and degradation in water

Arboviruses are enveloped RNA viruses (as are SARS-CoV-2 and influenza viruses) and are generally less environmentally stable than non-enveloped viruses. Nevertheless, experimental and field studies demonstrate that viral RNA can persist long enough in wastewater and related matrices to be detected, particularly during outbreaks. Experimental persistence studies demonstrate that DENV and other arboviral RNA can persist in wastewater influent for several days, longer at cooler temperatures (66–68). Decay rates are strongly influenced by temperature, pH, organic load, and matrix composition, with faster degradation observed at higher temperatures and in aqueous fractions. These persistence characteristics are broadly comparable to those reported for SARS-CoV-2 and other enveloped respiratory viruses, reinforcing the biological plausibility of WES for arboviruses (69). Another study showed that ZIKV persisted long enough in aquatic environments to be picked up by *Aedes* mosquitoes breeding in that environment and be transmitted to a new host (70).

A key consideration is that arboviruses are shed into wastewater at much lower concentrations compared to classical WES targets such as enteric pathogens and SARS-CoV-2. This necessitates modifications such as the processing of larger sample volumes, preferential concentration of the solid fraction, and highly sensitive molecular assays (e.g. droplet digital PCR) to optimise detection and reduce false negative results (66–68, 71, 72).

The total, liquid and solid fraction of wastewater have all been applied successfully in WES studies (66, 67, 73–78). A recovery study showed good recovery of arboviruses from the liquid fraction (75). In the DENV outbreak investigation in Italy, both solid and liquid fractions were investigated and only the method used for the solid fraction yielded DENV detections. The partitioning study (52) showed arbovirus were less favorably attached to the solid fraction than respiratory viruses tested. Freeze–thaw cycles and delayed processing likely accelerate

degradation, limiting the utility (specifically the sensitivity) of archived samples for retrospective analysis.

Beyond wastewater influent, limited investigations have examined arboviral RNA persistence in surface waters and animal effluent. For example, piggery effluent has been studied for JEV (79), and environmental water bodies may plausibly receive contributions from sylvatic cycles of YFV or OROV. Persistence in such matrices is typically lower than in influent, but may be detectable under outbreak conditions, raising relevance for One Health interfaces at the human–animal–environment boundary.

Overall, arboviral RNA persistence in wastewater and environmental waters is limited but sufficient for detection in outbreak settings when sensitive sampling and analytic methods are used. These constraints emphasize the importance of optimized workflows for sampling, concentration, and RNA preservation, and the need to interpret negative results with caution. Further standardisation of experimental protocols and field studies will help to refine estimates of arboviral RNA stability and improve the reliability of WBS for this pathogen group.

2.3. WES experience

Arboviruses are not classically enteric pathogens, yet a growing body of work has established the biological plausibility and early feasibility of WES for selected arboviruses. Viral RNA has been detected in urine and, less frequently, in upper respiratory and genital secretions following acute arboviral infection, providing potential human source inputs detectable through WES.

Experimental studies confirm persistence of DENV, ZIKV, and CHIKV RNA in wastewater matrices, with preferential partitioning into solids. Pilot field studies and outbreak investigations have reported detection of arboviral RNA in wastewater influent, particularly for DENV and ZIKV, while exploratory studies have examined CHIKV, WNV and JEV. Evidence for YFV and OROV have not been reported. These findings are summarized in Table 4 and expanded in selected case studies in Annex 1.

Table 4. Summary of wastewater and environmental surveillance (WES) evidence for arboviruses

Virus	Location	Matrix and method	Detection results	Contribution	Reference
Zika virus	Singapore	Sewage via manholes, WWTP influent; RT-qPCR and hybrid capture sequencing	RNA detected in multiple samples; aligned with epidemic peaks	Facilitated outbreak situational awareness	(77) Case Study 1
	Brazil (Belo Horizonte)	Hospital and municipal sewage, compared three molecular methods	1 of 63 samples positive with WGS in absence of clinical cases. RT-PCR resulted in high (65%) rate of false positives.	Highlights limitations of clinical RT-PCR. Incidental finding together with dengue and chikungunya WS	(71) Case Study 3
Dengue virus	China (Guangzhou)	Sewage from manholes, compared PEG and magnetic bead concentration, RT-qPCR and WGS	14 of 618 (2.3%) grab samples. Early warning hours before clinical case diagnosis. Matched wastewater sequence to case (low case/outbreak prone)	Agile localized WS - enhanced surveillance in outbreak response; Method optimization insights for sampling, concentration and WGS.	(72)
	Italy	WWTP influent; RT-qPCR/ddPCR/hybrid capture sequencing	Viral RNA detected during an outbreak (non-endemic setting)	Early warning, outbreak corroboration	(67) Case Study 2
	Portugal (3 regions)	WWTP influent; RT-qPCR quantification, crassphage normalization	69 of 273 (25%) 24-h composite samples positive DENV.	Exploratory study, method development and insights on seasonal trends – cocirculation with CHIKV	(75)
	USA (Florida)	WWTP influent; dd-RT-PCR for DENV types 1,2,3 and 4.	24 of 112 (21%) of settled solids from composite samples. DENV-3 detected corroborated with reported cases. (low case/outbreak prone setting)	Demonstrates DENV-3 RNA detectable in low case setting. Method contribution with use of solids and quantification dd-RT-PCR	(66)
	Brazil (Belo Horizonte)	Hospital and municipal sewage, compared three molecular methods	20/30 (67%) samples positive by WGS. None by RT-qPCR. (high case endemic setting)	Highlights importance of methods specific to pathogen target/context.	(71) Case Study 3

	<i>Nepal (Kathmandu Valley)</i>	<i>Hospital and municipal sewage, EN membrane vortex concentration. RT- qPCR and RT-dPCR.</i>	<i>0/34 grab samples - No detections</i>	<i>Informs further method development</i>	<i>(76)</i>
Japanese Encephalitis virus	<i>Australia (South Australia)</i>	<i>WWTP influent; RT-PCR</i>	<i>JEV RNA detected in municipal sewage aligned with reported cases.</i>	<i>First proof of concept – demonstrates technical feasibility in field</i>	<i>(78)</i> Case Study 4
West Nile virus	<i>USA (Oklahoma)</i>	<i>WWTP solids; dd-RT-PCR</i>	<i>WNV RNA only detected in WES in counties with reported cases. Conversely not detected in all counties with reported cases.</i>	<i>First proof of concept – demonstrates technical feasibility in field</i>	<i>(74)</i>
	<i>USA (California, Nebraska)</i>	<i>WWTP solids; dd-RT-PCR multiplex with SARS-CoV-2</i>	<i>Detections at 3 of 5 WWTPs aligned to reported cases; where positive : detection rates 3.3 – 13.0%.</i>	<i>Further demonstrates technical feasibility; with use of solids and multiplex approach.</i>	<i>(80)</i>
Chikungunya virus	<i>Brazil (Belo Horizonte)</i>	<i>WWTP influent; RT- qPCR/hybrid capture sequencing</i>	<i>12/14 (86%) samples positive by MinION. None by RT-qPCR. (endemic setting)</i>	<i>First proof of concept – demonstrates technical feasibility in field</i>	<i>(71)</i> Case Study 3
	<i>Portugal (3 regions)</i>	<i>WWTP influent; RT-qPCR quantification, crassphage normalization</i>	<i>30 of 273 (11%) 24-h composite samples positive CHIKV.</i>	<i>Exploratory study, method development and insights on seasonal trends – cocirculation with DENV</i>	<i>(75)</i>
Yellow Fever virus	<i>None published</i>	<i>n/a</i>	<i>n/a</i>	<i>n/a</i>	<i>nil (81)</i>
Oropouche virus	<i>None published</i>	<i>n/a</i>	<i>n/a</i>	<i>n/a</i>	<i>nil</i>

Overall, published evidence for use of wastewater and environmental surveillance for arboviruses have been demonstrated for ZIKV (in one setting), there is limited operational evidence for DENV, CHIKV and WNV, early proof-of-concept evidence for JEV and none yet for YFV, or OROV.

- ZIKV WES experience has been reported from Singapore with rigorous study design and highlighted added value with clinical and entomological correlations linked to public health actions on multiple occasions. In Singapore, WES is now an integrated part of national multimodal Zika surveillance. An incidental single ZIKV detection is reported in hospital wastewater in Brazil (71).
- DENV : multiple outbreak studies showing wastewater detection aligned with clinical trends (China and Italy), as well as detections and quantification in settings with low (Florida USA) to very low reported case numbers (Portugal). Wastewater detection reported in settings with high numbers of reported cases (Brazil). One other outbreak study (Nepal) did not detect DENV in wastewater attributed to be likely to methods used. Noting these studies used various sampling, concentration and analytic methods.
- CHIKV: Wastewater detections reported in a setting with relatively high prevalence (Brazil), and also in settings with few cases and including cocirculation with DENV (Portugal).
- The JEV report from Australia provides a proof of concept for JEV WES detections in a low case/non-endemic outbreak setting. This is particularly relevant given the sparse JEV shedding data suggests urinary shedding is low level and infrequent; this study supports technical feasibility of detection at low viral levels.
- The two WNV reports from USA provide evidence of feasibility using wastewater solids, with WES detections corroborated with reported cases and mosquito evidence.
- There are no WES field reports for YFV or OROV to date.

These findings highlight that while arboviruses can be detected in wastewater, routine application will require further validation, optimisation of sampling, concentration and analytic methods, and integration with clinical and entomological surveillance to ensure actionable public health value. In line with the shedding evidence they also reinforce that a negative WES result does not mean absence of cases. Its utility should also be weighed against the cost and feasibility of enhancing other surveillance methods in the epidemiological context.

3. Global Strategies for Surveillance and Control

3.1. Global strategies for control

Arboviruses are a rapidly growing global public health threat, with more than 3.9 billion people already at risk of dengue alone and projections suggesting that climate change and urbanization could expose over a billion additional people to arboviral transmission zones in coming decades (82). Reported dengue incidence has reached record levels in recent years, with 2024 marking the highest global total recorded by WHO (8). Some arboviruses also pose a threat to animal health: e.g. JEV causes reproductive losses in pigs (12) and WNV causes neuroinvasive disease in horses (11), and YFV affects non-human primates(13), serving as sentinels for spillover risk.

Despite this rising One Health burden, current control strategies are limited, underscoring the need for innovation and coordination (1,83,84). The WHO Global Arbovirus Initiative provides the overarching framework for arbovirus control, built on four pillars: (i) vector control, (ii) vaccination where available, (iii) surveillance and early warning, and (iv) community engagement and risk communication (1).

Vector control relies on integrated vector management (IVM), combining source reduction, insecticide use, Wolbachia-infected mosquitoes, resistance monitoring, and environmental management through intersectoral action on water, sanitation, and housing (16).

Vaccination plays a major role for JEV and YFV, both of which have effective vaccines (85,86). The JEV vaccine is widely deployed across Asia, while the YF vaccine is central to outbreak prevention and response in Africa and South America. DENV vaccines remain limited: CYD-TDV is recommended only for seropositive individuals in certain settings, restricting its utility (2). TAK-003 (Qdenga) has recently been licensed in several countries and recommended in high dengue transmission settings. It is under WHO prequalification review, offering expanded potential use (87). Two CHIKV vaccines have received regulatory approval and/or have been recommended for use in populations at risk in several countries, but the vaccines are not yet widely available nor in widespread use (4). For other arboviruses including ZIKV, WNV, and OROV, no licensed vaccines are available, though several candidates are under development (88,89).

Global drivers of arboviral risk—including climate change, urbanization, international travel, fragile health systems, and conflict and political instability—underscore the need for coordinated multisectoral and One Health approaches(1,90–92) .

3.2. Surveillance and early warning

The third pillar of the WHO Global Arbovirus Initiative is surveillance and early warning. Routine case-based surveillance (syndromic reporting, clinical diagnosis, laboratory confirmation) is complemented by entomological surveillance (larval indices, adult trap counts, insecticide resistance), sero-surveys in some settings, and animal or sentinel surveillance (e.g., pigs for Japanese encephalitis virus, chickens or horses for West Nile virus) (1).

Surveillance approaches differ across endemic countries, outbreak-prone settings, and those at risk due to vector presence (83):

- Endemic areas (e.g., large parts of Asia-Pacific, the Americas) often have established dengue, Zika, or chikungunya surveillance. However, under-reporting, inconsistent case definitions, and limited laboratory confirmation reduce accuracy and timeliness.
- Outbreak-prone settings (such as regions with recent incursions of ZIKV, CHIKV, or JEV) often have strained systems that are rapidly overwhelmed when cases surge.
- At-risk areas (where competent vectors are present but transmission is not yet established, e.g., parts of Europe, Africa, EMR) often have limited preparedness, highlighting the need for early warning systems and importation monitoring.

Key limitations include:

- Asymptomatic transmission (especially DENV and ZIKV) and syndromic overlap with other febrile illnesses (e.g., malaria, influenza, COVID-19) that reduce sensitivity and specificity of detection.
- Diagnostic gaps: limited access to molecular and serological testing, shortages of reagents, long turn-around times, and cross-reactivity among flaviviruses that complicates case confirmation.
- Inconsistent case definitions and reporting frameworks, with some arboviruses not classified as notifiable diseases even in endemic countries.
- Shortages of human resources, particularly entomologists and field epidemiologists, and a reliance on donor-funded projects for surveillance operations.
- Animal and sentinel surveillance, while valuable for early warning, remain under-developed and poorly integrated with human and vector systems.
- Regional disparities: surveillance networks are comparatively stronger in the Americas, South-East Asia, and the Western Pacific, while Africa and the Eastern Mediterranean Region report substantial gaps in diagnostic capacity, entomological monitoring, and timely reporting (83).

Overall, current surveillance systems provide essential data for outbreak response but are fragmented, inconsistently implemented, and prone to under-reporting. These limitations underscore the need for complementary approaches, improved diagnostic capacity, and integrated multi-sectoral systems, as highlighted in both the WHO Global Arbovirus Initiative and recent systematic reviews(1, 93, 94).

4. Potential value of adding WES to current surveillance

WES is not currently part of global arboviral surveillance recommendations.

Current evidence including from pilot studies indicate it could complement and strengthen existing surveillance systems, addressing one or more of their known substantial limitations (81, 94). Its application can be considered in two modes: routine (ongoing likely as part of multi-pathogen WES) and agile (responsive, time-limited). Relative to case-based and entomological surveillance, WES offers a comparatively low-cost, population-level signal when incorporated into existing multi-pathogen workflows. Table 5 summarizes how WES might complement existing surveillance across different epidemiological contexts - as part of ongoing routine surveillance or agile surveillance which is responsive and time-limited.

Table 5. Potential use cases of WES for arboviruses of human significance

Context	Routine WES (ongoing)	Agile WES (responsive, time-limited)
<i>Endemic areas</i>	<i>Monitor seasonal trends; support long-term evaluation of interventions.</i>	<i>Surge monitoring during peak seasons</i>
<i>Outbreak-prone areas and at-risk areas (vectors present, no transmission)</i>	<i>Limited role due to resource intensity. Baseline monitoring only in high-risk hubs.</i>	<i>Early detection during periods of heightened risk.</i> <i>Early warning during mass gatherings, importation events, or post-disaster settings.</i>
<i>Outbreak (non-endemic)</i>	<i>n/a</i>	<i>Monitoring outbreak trends (geographic and temporal) and impact of interventions.</i>
<i>Cross-cutting</i>	<i>Multi-pathogen integration (e.g., with polio, SARS-CoV-2 and with other arboviruses).</i>	<i>Targeted, rapid deployment for situational awareness – including of multiple concurrently circulating arboviruses (e.g. DENV, CHIKV).</i>
<i>Limitations</i>	<i>Low shedding, host variability, environmental instability.</i> <i>Non-detection ≠ absence.</i>	<i>Same limitations (as for routine) apply; agility requires preparedness, strong laboratory and governance capacity.</i>

These applications illustrate how WES could enhance arbovirus preparedness and response when triangulated with existing surveillance, including case-based, entomological, and animal surveillance systems. However, given low viral shedding and biological variability, non-detection cannot be interpreted as absence. Further pilots are needed to clarify feasibility, cost-effectiveness, and public health impact in relevant endemic, outbreak prone and at-risk settings.

5. WES Methodological Considerations

5.1. General considerations

Arboviruses are enveloped RNA viruses and are typically less environmentally stable than non-enveloped or DNA viruses. Persistence studies showed that the arbovirus RNA signal is reduced in wastewater at 25°C by 1 log in 4-6 days, suggesting the arboviral RNA signal is sufficiently stable for WES (68). Similar persistence was found by Zhu et al for ZIKV in wastewater (95). Shedding into wastewater is generally low-level and short-lived, principally via urine and, in some cases, saliva or genital secretions. There is little documentation of extent or absence of faecal shedding (which represents a knowledge gap). These shedding characteristics make arboviruses more challenging targets for WES than classical enteric viruses or SARS-CoV-2, necessitating methodological adaptations and cautious interpretation, particularly of non-detects (94).

5.2. Sampling approaches

The few studies on arboviruses in wastewater used grab, composite or settled solid samples at the intake of the WWTP, from manholes in the outbreak area or from hospitals. The JEV study in piggery waste used grab samples of the effluent. Composite sampling is preferable to grab sampling for improved representativeness while grab samples have operational advantages given lower resource requirements. Passive/trap and on-site ultrafiltration approaches have not yet been evaluated. The studies conducted to date were partially proof-of-concept and partially outbreak response. For proof-of-concept, sampling was conducted at the intake of WWTP, generally in different areas and WES signals were compared to reported cases (if available) and in some cases to mosquito surveillance, recognizing the limitations of each of the surveillance methods. In the outbreak studies, efforts were made to sample the wastewater of the population where the outbreak was reported, generally via the WWTP that served this population. In the ZIKV outbreak in Singapore (77) and the DENV outbreak in Guangdong, China (72) manholes were sampled in the area of the reported cases to create better spatial resolution and also higher probability of WES detection, given lower dilution in smaller catchments.

Optimal sample collection methods are use case specific. Several studies showed a connection between the WES signal and other surveillance signals, but in some studies there was no clear link and studies typically reported detection/non-detection rather than quantitative levels. While clinical surveillance signals may suffer from under-reporting, the absence of a clear correlation makes the WES signal difficult to interpret in these studies. In addition, studies have reported not to be able to detect arboviral RNA in wastewater in the setting of an outbreak or consistently in presence of reported cases (66, 67, 74). More information on correlation between WES signal and reported (and underreported) cases is needed for each of the specific pathogens (with expected low case numbers and shedding levels), sanitation contexts and epidemiologic setting.

Even though the currently applied WES methods are based largely on the methods for viruses with high shedding rates, sampling and concentration approaches to increase sensitivity are likely to be beneficial: these could include larger volume sampling, ultrafiltration at point of collection,

concentration of solids and/or other approaches. Because these approaches generally produce also more matrix background and potential for inhibition of the RNA extraction and RT-PCR, it is important to balance sensitivity and recovery of the sampling and processing methods (96).

5.3. Laboratory methods

These need to be assessed for each target pathogen considering likely viral levels and surveillance objectives. Given the potential value of WES for surveillance for multiple arboviruses, or febrile-rash clusters, multi-pathogen panels also need to be assessed, particularly those which allow efficient use of existing workflows. Further, known limitations and challenges for arboviral clinical diagnostics will likely be relevant to WES as well.

- **Recovery methods:** The arbovirus WES studies all used different methods for processing wastewater (liquid or solid) samples. Most methods were based on methods described for enveloped respiratory virus recovery from wastewater. There are a few published assessments comparing recovery methods for arboviruses including (PEG, ultrafiltration, membrane filtration) (71, 72, 94, 97). Use of nanotrap particles has been shown to improve capture and enrichment for ZIKV, CHIKV and DENV in human samples (urine) (98). Some studies compared and reported concentration methods; for DENV, Mancini et al found solid extraction superior to the other 3 methods in Italy - (67), Ma et al found magnetic beads superior to PEG in China (72), while de Araujo et al found WGS superior for DENV and MinION sequencing superior for CHIKV compared to RT-qPCR (71). All emphasized the importance of method development and optimization specific to pathogen target, viral levels and context. .
- **Molecular detection:** RT-qPCR and ddPCR are most commonly used for low-abundance targets for WES. Increasing the number of technical replicates also increases sensitivity but there are trade-offs due to increased cost. The primers and probes that are used in WES assays of arboviruses are derived from clinical assays. De Araujo et al report that the clinical kits they tested were not directly applicable to WES, as the primer/probe-sets were either not detecting arboviral RNA that was present (false negatives), or where frequently detecting non-arboviral RNA (false-positives) (71). The specificity of clinical assays has been confirmed in clinical samples, but needs to be validated in wastewater/environmental samples to be reliable for WES interpretation. The primers/probe-sets need to be tailored to generate sufficient specificity and sensitivity for WES. The WES signal interpretation can be strengthened by using multiple PCR target sequences/genes for the same virus and/or to conduct amplicon sequencing (55). Target enrichment or hybrid capture sequencing with a commercial virus panel was reported to be sensitive enough to detect DENV, CHIKV and ZIKV in wastewater samples simultaneously (99). Target enrichment panels are increasingly available and could support multitarget WES. This method increases specificity and would have the potential for molecular typing and epidemiology, but this has not been reported for arboviruses in WES. This method does not provide quantitative information.
- **Controls:** Standard controls are blanks and positive controls, and controls for PCR inhibition. The use of RNA fecal strength controls (such as PMMoV) is essential for quality assurance and to benchmark recovery efficiency.
- **Sequencing:** Full genome sequencing is rarely achievable due to low viral loads, but amplicon sequencing is more sensitive and may provide genotype confirmation in outbreak settings.

Target enrichment sequencing has been shown to successfully detect DENV, CHIKV and ZIKV in one WES study (71). Another study compared sequence data from clinical and wastewater samples for DENV and noted degradation-related challenges including mitigation opportunities for collection, transport and storage (72).

5.4. Reporting and communication

- Community engagement and risk communication is the 4th pillar of the global arboviral initiative framework (1).
- WES results for arboviruses should always be interpreted in conjunction with case-based, entomological and other available surveillance information.
- Timely reporting is critical but must emphasise caveats, particularly the: limited sensitivity given low viral levels of arboviruses in wastewater; that absence of wastewater detection does not exclude presence of cases; and that WES provides population-level signals, not individual diagnosis.
- Technical reports should include detection thresholds, assay performance parameters and details of the sampling, laboratory and analytic methods used (or reference to published protocols).
- Public facing communication strategies should avoid over-interpretation of single detections, instead integrating available data and stressing the value of triangulating WES with case-based, entomological and other data.
- Information about the presence of an arbovirus +/- other pathogens should be provided together with a clear and understandable call to action relevant to the target audience (e.g. reduce mosquito exposure, note symptoms, access vaccines etc)
- For zoonotic arboviruses such as JEV and WNV, WES detections may derive from both human and animal sources, requiring a One Health interpretation involving health, veterinary, and environmental sectors. This complexity must be addressed in communication frameworks to ensure appropriate and proportionate responses.

5.5. Acceptability and implementation context

- WES for infectious diseases in large catchments generally has high acceptability when framed as complementary to other surveillance. Sampling from smaller or targeted catchments may raise specific ethical and other acceptability issues.
- One Health considerations are important: signals potentially reflecting animal reservoirs or vector habitats need clear interpretive frameworks to prevent misattribution to human sources and miscommunication.
- Industry and community trust are essential, particularly where detections could affect travel, trade, or raise concerns about pathogens with established vaccines (e.g., yellow fever), where misinterpretation could undermine confidence in immunization programs.

6. Integrated surveillance and multi-target considerations

Arbovirus WES should be interpreted alongside clinical, entomological and animal surveillance, vector indices, climate and other data. Triangulation enhances public health decision-making but requires predefined and context specific action thresholds.

6.1. Integration of arboviral WES into existing arboviral surveillance and response

- As WES for arboviral pathogens is relatively recent and its place in multimodal arboviral surveillance is evolving, there has not yet been substantial integration at global or local levels (with some frontrunner exceptions such as Singapore (77).
- There is potential for improved integration at the national and subnational levels, including at the planning stage to optimize complementary multimodal surveillance, as well as at the analysis and reporting stage to better visualize and enable use of combined information including from WES to inform public health policy and practice decisions (94).
- There is also potential for strengthening cross-border, regional and global surveillance and strengthening genotypic surveillance (93).

6.2. Integration of targeted WES into existing fever-rash surveillance and response

- Noting there are multiple fever-rash or arthralgia diseases with similar presentations, multi-pathogen WES may also have potential to complement syndromic and laboratory confirmed case surveillance and assist to identify which pathogens are circulating in a community. For fever-rash diseases ; these may include chikungunya, dengue, zika as well as measles, rubella, varicella (chickenpox), mpox, coxsackie and others. WES has been shown to be feasible for multiple of these targets (100).

6.3. Integration of arboviral targets as part of multi-target WES surveillance

- Existing polio, SARS-CoV-2 or other multi-pathogen WES activities allows the integration of additional targets at low marginal cost with substantial alignment with multiple existing work-flows. Trade-offs may need to be considered between optimal methods for individual pathogen sensitivity and resource allocations.
- Likewise, routine WES activities for arboviral pathogens would provide local capability to which agile WES (for the same pathogen) can be initiated in response to an outbreak.
- In many high-income settings, multitarget WES surveillance already combines multiple targets from the same samples with publicly accessible dashboards – and with a design which allows additional targets to be added (eg the US National Wastewater Surveillance Program (101)

7. Key knowledge gaps and applied research priorities

Arboviral WES is moving from proof-of-concept toward potential public health application, but key knowledge gaps remain (see table 6 below). Coordinated efforts are needed to:

- standardise methods,
- generate pathogen-specific data on shedding and persistence, and
- evaluate integration with existing surveillance systems.

Research guided by the WHO Global Arbovirus Initiative and regional networks will be critical to define whether and how WES can strengthen preparedness for arbovirus outbreaks and future pandemic threats.

Table 6. Key questions in relation to WES for arboviruses of interest

Domain	Question
Biological plausibility and shedding	• What is the prevalence, quantity and duration of viral RNA shedding in urine, saliva, stool or other excretions/secretions? • Do immunocompromised or other individuals contribute meaningfully to persistent shedding? • To what extent could animal reservoirs (e.g., pigs for JEV, birds for WNV) or vectors introduce RNA into wastewater and environmental waters and confound interpretation? • To what extent could vaccinated individuals (or animals) introduce RNA into wastewater and environmental waters and confound interpretation?
Persistence and stability	• How long does arboviral RNA persist in wastewater and surface waters under different temperatures and other environmental conditions? • How does the stability of specific arboviruses compare to other enveloped RNA viruses with well-established WES use?
Methods and validation	• What sampling volumes, frequencies, and matrices (solids vs aqueous fractions) maximize detection sensitivity? • Which concentration and extraction methods work best for low-abundance, enveloped RNA viruses including arboviruses specifically? • How can molecular assays (RT/digital PCR) be validated to ensure both high sensitivity and minimal cross-reactivity? • Can partial or target enrichment sequencing methods reliably identify genotypes or distinguish imported from locally transmitted cases?
Integration with existing surveillance systems	• What is the arboviral epidemiological situation of the area/country? • How can arboviral WES results complement existing surveillance? • In what settings could WES provide the greatest added value? • What is the operational feasibility and cost-effectiveness of embedding arboviruses into existing multi-pathogen WES platforms?
Public health actionability	• What are the clearest use cases where arboviral WES has supported timely interventions or outbreak management? • How should negative or sporadic detections be interpreted and communicated to avoid misperception or loss of trust? • What ethical and community acceptability issues arise when monitoring arboviruses in non-endemic or low-incidence settings?

Pathogen-specific priorities

- *Dengue and Zika: Can wastewater signals be validated against case incidence and entomological indices, and do they provide predictive value? (noting issues with case ascertainment – no gold standard)*
- *Chikungunya and West Nile: What sensitivity and utility can be demonstrated through pilot studies in at-risk regions?*
- *Japanese Encephalitis: Beyond outbreak investigations, is wastewater or environmental sampling feasible and useful?*
- *Yellow Fever and Oropouche: Is there biological plausibility for WES detection, given the lack of field evidence to date?*

8. Conclusions: Advancing Arboviral WES within the Global Arbovirus Initiative

Arboviral WES is at a proof-of-concept stage, with the strongest evidence for DENV and ZIKV, proof of concept for JEV, and limited operational insights for WNV. No field evidence yet exists for CHIKV, YFV or OROV. While detections are biologically plausible and technically feasible, broad implementation is premature. However, the comparatively low cost of embedding arboviral targets into multi-pathogen WES platforms, coupled with gaps in current surveillance, underscores the importance of further pilots, standardised methods, and alignment with the WHO Global Arbovirus Initiative to inform preparedness and response.

Arboviral WES may not have adequate evidence for recommended broad adoption, but gaps in existing surveillance systems coupled with its relatively low cost and potential complementary value make it a strategic priority for piloting and further assessment under the WHO Global Arbovirus Initiative.

Annex 1. Case Studies

Case Study 1: Zika Virus as part of multimodal agile surveillance in Singapore (77)²

Background

Singapore remains vulnerable to Zika outbreaks, due to presence of competent vectors and low-level immunity in the human population (102). The first local Zika outbreak in 2016 saw 458 reported cases. Since then, sporadic infections have occurred, including a cluster of 15 cases in 2023.

Intervention

In response to the cluster in 2023, Singapore swiftly deployed wastewater and entomological surveillance to enhance situational monitoring. Signals detected in wastewater and mosquito samples corroborated with the peak in reported cases, demonstrating the potential value of WES. Integrated surveillance combining case, mosquito and wastewater surveillance has since been implemented in areas with reported Zika cases. Public alerts are issued when persistent signals are detected in wastewater and mosquito samples, indicating potential transmission.

Findings

Detection of ZIKV RNA in wastewater supported outbreak mapping and supplemented clinical and entomological surveillance. Rapid response to early signals was facilitated by established wastewater and vector surveillance teams.

Public health significance

Demonstrated feasibility of non-intrusive WES in high-density urban settings with largely asymptomatic infection profiles. This complements traditional case testing, providing non-intrusive, population-level monitoring of Zika. This is particularly valuable given that Zika infections are generally mild and underreported yet can occasionally lead to severe sequelae.

Lessons learned

Importance of combining WES with vector and case data to maximise interpretability.

- **Triangulation:** Integrating WES with vector and clinical data enhances interpretability and operational decision-making.
- **Rapid deployment:** Prompt activation of agile wastewater and mosquito surveillance when Zika cases/clusters are reported enabled timely public health responses
- **Public communication:** Issuing alerts based on environmental signals helped raise awareness and community vigilance, even before case numbers escalated

Conclusion

The addition of WES has strengthened Zika surveillance in Singapore. Its integration with case and vector data enabled a holistic understanding of Zika transmission and supported proactive, evidence-based public health action.

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Case Study 2: Dengue virus wastewater detections in Italy (2024)(67) ³

Background

In August–October 2024, Italy experienced its largest recorded dengue outbreak, with 216 confirmed and probable cases in the Marche region, centred in the city of Fano. This outbreak highlighted the growing risk of dengue transmission in temperate regions due to climate change and the spread of *Aedes* mosquitoes.

Intervention

To explore the feasibility of wastewater surveillance (WS) in the context of a dengue viral outbreak, 27 wastewater samples (24-hour composite) were collected in October 2024 from three sites in treatment plants in two cities (Fano and Pesaro). Four viral concentration methods were tested: polyethylene glycol (PEG), electropositive membrane filtration, Nanotrap® Magnetic virus and solid fraction analysis. Digital RT-PCR was compared to real-time RT-PCR to assess relative sensitivity.

Findings

- Dengue virus serotype 2 RNA was detected in 9 of 27 samples (33%), exclusively from the solid fraction consistent with the circulating outbreak serotype.
- Digital RT-PCR identified more positives than real-time RT-PCR.
- Viral concentrations were low; approx. 10^2 – 10^3 genome copies per gram of solids.
- Detections corresponded to both Fano (outbreak epicentre with 18 confirmed and 12 suspected cases in the same month) and Pesaro (with only two reported cases).

Public Health Significance

The study demonstrated proof-of-concept that dengue RNA can be detected in wastewater during outbreaks, when using solid fractions and sensitive molecular methods. Despite sampling beginning after the epidemic peak, results indicate WES could complement case-based and entomological surveillance by capturing community transmission signals including unreported as well as known symptomatic cases.

Lessons Learned

- Larger sample volumes and targeting solids appeared helpful to detect dengue
- Digital PCR may enhance sensitivity at low viral loads
- Pre-emptive WES systems are essential for early outbreak detection; in this study opportunistic late sampling limited correlation with epidemic curves.

Conclusion

This study shows that WS for dengue is technically feasible and offers potential as a complementary public health tool for outbreak monitoring and preparedness in regions at risk of arboviral emergence. Further research is needed to optimise methods.

³ Contact: Giuseppina La Rosa: giuseppina.larosa@iss.it This work was partially supported by EU funding within the NextGeneration EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, PE13 INFANT), and partially by the EU-WISH Joint Action (EU Wastewater Integrated Surveillance for Public Health), co-funded by the European Union under Grant Agreement No 101140460.

Case Study 3: Arboviral detection in Brazil's hospital and community wastewater (71)⁴

Background

Brazil faces recurrent arbovirus epidemics, with millions of dengue cases annually as well as substantial chikungunya, and Zika burdens. There are known limitations in existing surveillance systems. To assess feasibility of wastewater surveillance (WS) for these arboviruses, an 11-month study (July 2022–May 2023) was conducted in Belo Horizonte across hospitals and three wastewater treatment plants.

Intervention

Sixty-three samples were collected from hospital and municipal WWTP sites. Three molecular methods were compared:

- Hybrid-capture whole-genome sequencing (WGS)
- MinION nanopore sequencing
- Clinical RT-qPCR kits (CDC Trioplex; IBMP ZDC)

Findings

- DENV-1 detected in 23.8% of samples; early detections in hospital wastewater preceded community peaks.
- WGS detected 66.6% of DENV-positive samples; MinION 13.3%; RT-qPCR detected none.
- CHIKV detected in 19% of samples; MinION outperformed WGS (85.7% vs 6.7%).
- RT-qPCR appeared to produce false positives for CHIKV and ZIKV.
- Hospital wastewater yielded earlier and more frequent detections than WWTPs.

Public health significance

Demonstrates first multi-virus genomic confirmation of DENV, CHIKV and ZIKV in Brazilian wastewater. WS provided early warning and complemented traditional surveillance, especially where asymptomatic infection is common.

Lessons learned

- WS at hospitals appears to have potential as an early-warning tool.
- Sequencing methods outperformed clinical RT-qPCR in environmental matrices.
- Virus-specific differences: WGS better for DENV; MinION better for CHIKV.
- Need for wastewater-validated assays to reduce false positives.

Conclusion

WS is feasible and informative in Brazil for multiple key arboviruses including dengue, chikungunya and Zika viruses. Genomic methods can strengthen early detection and outbreak preparedness when integrated with clinical and vector surveillance.

⁴ Contact: Juliana Calabria de Araujo juliana@desa.ufmg.br Contribution from UFMG, Fundação Ezequiel Dias, FIOCRUZ-MG, COPASA, and partners. With appreciation to CNPq, FAPEMIG, CAPES and the CLIMADE Global Consortium (L.C.J.A., V.F., M.G., J.C.d.A.: Principal Investigators from Latin America) (<https://climade.health/>) for supporting the study.

Case Study 4: Wastewater detections of JEV in a southern Australia outbreak (78)⁵

Background

An outbreak of Japanese Encephalitis Virus (JEV) was detected in temperate South-Eastern Australia for the first time in 2022, with human cases linked to piggeries and widespread mosquito activity during extensive flooding (103). Most JEV infections are not symptomatic, contributing to under-ascertainment and limiting the sensitivity of traditional surveillance approaches such as clinical reporting as well as vector and animal monitoring.

Intervention

In 2022/2023, real-time surveillance in affected regions included mosquito trapping, sentinel chicken serology, animal health notifications, and clinical case reporting. After the acute outbreak, retrospective testing was done using archived samples extracts in South Australia (SA). These were collected for SARS-CoV-2 WS using Torpedo passive sampling devices from December 2021 – February 2022, collected weekly from wastewater treatment plant influent for periods ranging from 2-8 days (104). Two RT-qPCR assays targeting JEV and NGS were applied to assess whether WS could detect evidence of community-level infection and complement One Health surveillance systems.

Findings

- JEV RNA was detected in WS samples collected during the same period as a cluster of 9 human cases in SA riverine areas; 2 were confirmed by NGS and a 2nd RT-qPCR.
- Additional wastewater JEV detections were consistent with concurrent mosquito surveillance and sentinel chicken seroconversion, indicating local viral circulation.
- The study provided the first documented detections of JEV in municipal wastewater during an acute outbreak.

Public Health Significance

The findings highlight the potential for WS to strengthen JEV surveillance by providing a geographically defined population-level signal that complements vector, animal, and clinical surveillance. This is particularly important for JEV and other arboviruses which may cause severe disease but for which most infections are asymptomatic and where traditional systems may miss early or low-level circulation.

Lessons Learned

- **Archiving:** Stored WS samples enable retrospective testing and insights.
- **Feasibility:** WS is technically feasible for JEV (1st global proof of concept).
- **Integration:** WS may add value to One Health multimodal surveillance.
- **Timeliness:** Real-time WS with rapid confirmation could enhance early warning.
- **Scalability:** Existing WS can be rapidly adapted for JEV/emerging pathogens.

Conclusion

This study demonstrates that WS can detect JEV during an outbreak in a non-endemic setting. Incorporating agile WS alongside vector, animal, and clinical surveillance during periods of heightened risk may strengthen early detection and outbreak response. Further research is needed to refine methods and explore real-time operational use.

⁵ With acknowledgement of financial support from SA Health and SA Water. Contact Dr Brendon King brendon.king@sawater.com.au

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