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Journal of Applied Microbiology, 2025, 136(3), lxaf066
https://doi.org/10.1093/jambio/lxaf066
Advance access publication date: 17 March 2025
Review Article

JOURNAL OF APPLIED MICROBIOLOGY
OXFORD UNIVERSITY PRESS

A systematic review on the utility of wastewater surveillance for monitoring yellow fever virus and other arboviruses

Caleb Morin[1] **and Zina Alfahl**[1,2,*]

[1]Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, University of Galway, Galway, H91 TK33, Ireland
[2]Centre for One Health, Ryan Institute, University of Galway, Galway, H91 TK33, Ireland

*Corresponding author. Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, University of Galway, Galway, H91 TK33, Ireland. E-mail: zina.alfahl@universityofgalway.ie

Abstract

Aims: This review aims to examine wastewater surveillance for the detection of yellow fever virus (YFV) and related arboviruses, focusing on concentration and extraction methodology, viral decay kinetics, and quantification techniques.

Methods: A literature search was conducted across 5 databases: PubMed, Science Direct, Web of Science, Embase, and Google Scholar following the PRISMA guidelines. Studies included were original scientific articles published between April 2014 and April 2024. Human research studies investigating wastewater surveillance and YFV or other arboviruses/flaviviruses were assessed.

Results: A total of 17 studies were included in this review. YFV was not detected in population-based wastewater samples; however, successful detection of similar viruses suggests potential for YFV monitoring with wastewater surveillance. YFV-spiked wastewater studies reveal similar concentration efficiency and decay rates between arboviruses. Effective concentration methods for YFV likely include centrifugation ultrafiltration and solid pellet extraction. YFV and arboviruses decay faster at higher temperatures, though YFV remains detectable for several days at these temperatures.

Conclusions: Wastewater surveillance presents a promising approach for monitoring YFV and other arboviruses. However, further research is needed to overcome existing limitations and enhance its effectiveness.

Impact Statement

Yellow fever virus (YFV) remains a significant public health threat. Surveillance of YFV is essential for identifying outbreaks. This review explores wastewater surveillance for YFV and other arboviruses.

Keywords: yellow fever; yellow fever virus; arboviral disease; flavivirus; wastewater surveillance

Introduction

Yellow fever is an acute viral haemorrhagic fever caused by the yellow fever virus (YFV), which belongs to the *Flaviviridae* family and is transmitted by mosquitoes (Monath and Vasconcelos 2015). Yellow fever is a significant public health threat which is estimated to cause 51 000–78 000 deaths annually (Garske et al. 2014, Gaythorpe et al. 2021). Yellow fever is largely preventable, given that there has been a safe and effective vaccine available for the past 80 years. However, vaccine supply is limited and can be difficult to deliver to remote areas. Although vaccination and vector control efforts have mitigated its impact, YFV remains a significant public health threat, particularly in Africa and South America. It is evident that enhanced monitoring of YFV is crucial for informing public health policy such as expanded vaccine delivery, mosquito bite prophylaxis, and targeted YFV testing, especially given projections that the YFV vector could find a more favourable environment in the future (Iwamura et al. 2020). Given its relatively low cost, success in COVID-19 monitoring, and emergence in pathogen surveillance (Kilaru et al. 2023), wastewater surveillance is a promising surveillance system, and its feasibility in monitoring YFV warrants investigation.

Like other flaviviruses, YFV is an arbovirus (arthropod-borne virus) (Kilaru et al. 2023). Broadly, arboviruses are a group of viruses that are transmitted to humans via the bite of infected insects, predominantly mosquitoes (Simmonds et al. 2017). Arboviruses survive in sylvatic transmission/maintenance cycles between non-human primates and mosquitoes (Holbrook and Holbrook 2017). These infected mosquitoes can transmit arboviruses to humans in the urban cycle (Kilaru et al. 2023). Some of the most well-known and clinically relevant arboviruses include: Dengue virus (DENV), Japanese encephalitis virus (JEV), West Nile virus (WNV), Zika virus (ZIKV), and YFV. All of these viruses are enveloped, positive-sense single-stranded RNA genome (9–13 kilobase genome), icosahedral capsid, 40–60 nm diameter viruses, which enter cells via membrane fusion, replicate in the cytoplasm, and shed via budding (Simmonds et al. 2017). However, these viruses vary in their incubation period, case fatality rate, geographic distribution, and symptoms (Ishikawa et al. 2014, Rudolph et al. 2014, Holbrook and Holbrook 2017).

Furthermore, these viruses differ in their mosquito vectors (Kain et al. 2022). The principal, but not only, mosquito re-

Received 7 October 2024; **revised** 13 February 2025; **accepted** 13 March 2025

sponsible for YFV transmission is the *Aedes aegypti* mosquito (Simoy et al. 2015). There is concern that, particularly with climate change, strains and serotypes of DENV, and strains of YFV could continue to evolve (Tabachnick 2016), highlighting the need for improved surveillance measures. Additionally, it is believed that climate change and human activity may expand the geographic distribution of the *A. aegypti* mosquito, increasing the prevalence of vector-borne diseases spread by this mosquito, including YFV (Kraemer et al. 2019). Modelling also indicates that the Northern Hemisphere will continue to become more suitable for *A. aegypti* (Iwamura et al. 2020, Laporta et al. 2023). This highlights the potential for YFV vectors to move into northern latitudes and underscores the importance of monitoring this migration.

Estimating the probability of severe disease in YFV infections is challenging, but one estimate suggests it to be ∼12%, with a fatality rate of 47% among those with severe disease (Johansson et al. 2014). Moreover, the probability of asymptomatic YFV infection is estimated to be ∼55% (Johansson et al. 2014). The high proportion of asymptomatic infections for YFV and other arboviruses presents challenges in accurately recognizing outbreaks and characterizing disease burden. Furthermore, the nonspecific symptoms such as fever, headache, nausea, and myalgia are associated with mild and moderate cases of yellow fever (Domingo et al. 2018). Other arbovirus infections can be mistaken for flu-like symptoms or other arbovirus infection symptoms, further impairing outbreak recognition and surveillance, particularly in regions where healthcare resources/surveillance programmes are limited, or in regions where cases of YFV and other arboviruses are uncommon (Colombo et al. 2017).

Due to the large proportion of asymptomatic, subclinical, or nonspecific symptom-associated cases, surveillance of YFV is critical in outbreak management. Currently, passive case detection or syndromic surveillance with laboratory confirmation is the mainstay surveillance method for YFV (Chen and Wilson 2020). Despite overall improvements in YFV monitoring, it is possible that other facets of YFV surveillance will prove useful in reducing/controlling YFV epidemics, aligning with the World Health Organization's (WHO's) goal of eliminating YFV epidemics by 2026 (WHO 2017). Wastewater surveillance may offer a cost-effective YFV monitoring strategy to supplement current surveillance efforts and inform targeted vaccine, vector control, and educational programmes, particularly in resource-limited settings.

Wastewater surveillance was first implemented in the 1960s as a tool to detect poliovirus (Kissova et al. 2022). The application of wastewater surveillance has accelerated during the COVID-19 pandemic, where it has been used to infer disease transmission, address testing gaps, and predict variant transmission (Keshaviah et al. 2021, Karthikeyan et al. 2022). Wastewater surveillance has been investigated as a potential tool for monitoring a wide range of infectious agents, including arboviral diseases (Lee et al. 2022, Kilaru et al. 2023). However, the feasibility, challenges, implementation, and future of wastewater surveillance for YFV monitoring remain unclear, particularly in the setting of potential expansion of YFV vectors. Furthermore, the link between wastewater surveillance and population health is not clearly understood for many pathogens, including YFV. Moreover, questions persist regarding how wastewater surveillance should inform public health interventions.

Given the importance of YFV as a significant public health threat, this systematic review aims to understand how wastewater surveillance can be used to monitor YFV. By synthesizing existing research, we seek to understand how this innovative approach can provide valuable insights into the prevalence and transmission dynamics of YFV. This review will evaluate the effectiveness, challenges, and potential of wastewater surveillance as a tool for public health monitoring, ultimately contributing to improved strategies for managing and controlling YFV and other arboviral outbreaks.

Materials and methods

Protocol and registration

The methods for this systematic review are described in a protocol registered with the International Prospective Register of Systematic Reviews (PROSPERO: CRD42024551993). Findings were reported according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Moher et al. 2009) (Table S7).

Study eligibility criteria

Inclusion criteria included original research studies published in English language and human studies or studies in the context of humans. Due to the topic of this systematic review, the population of interest was broad and included human populations, communities, or facilities (hospitals, trains, aeroplanes, ships, and dwellings) with sewage systems, regardless of wastewater treatment process or type. Randomized trials and observational studies related to wastewater surveillance and YFV, flaviviruses, and other arboviruses were included to examine the success, challenges, and feasibility of wastewater surveillance on related viruses. Books, book chapters, reviews, protocols, and abstracts were excluded. Preprints were not considered to minimize the risk of bias and ensure credibility. Reports were screened for inclusion/exclusion criteria by Caleb Morin (CM) and were checked by Zina Alfahl (ZA). Disagreements between individual's judgements were resolved by re-examining reports together, highlighting specific sections of disagreements, and explaining the rationale for inclusion/exclusion to reach a consensus agreement.

Data sources and searches

A systematic search was conducted across 5 electronic databases: PubMed, Science Direct, Web of Science, Embase, and Google Scholar between 01 April 2014 and 01 April 2024. The rationale for this time period was to only include studies using modern wastewater surveillance methods on arboviruses in their current geographic distribution. This time period was thought to best represent current wastewater infrastructure and wastewater surveillance limitations, while balancing comprehensiveness. Boolean operators were used to enhance search terms, including YFV, yellow fever, YFV, arboviral disease, arbovirus, arthropod-borne virus, *A. aegypti*, wastewater surveillance, wastewater-based epidemiology, wastewater-based surveillance, sewage surveillance, flavivirus, *Flaviviridae*, viral haemorrhagic fever, and environmental surveillance. Full details of the search strategy are available in the supplementary material (Table S1).

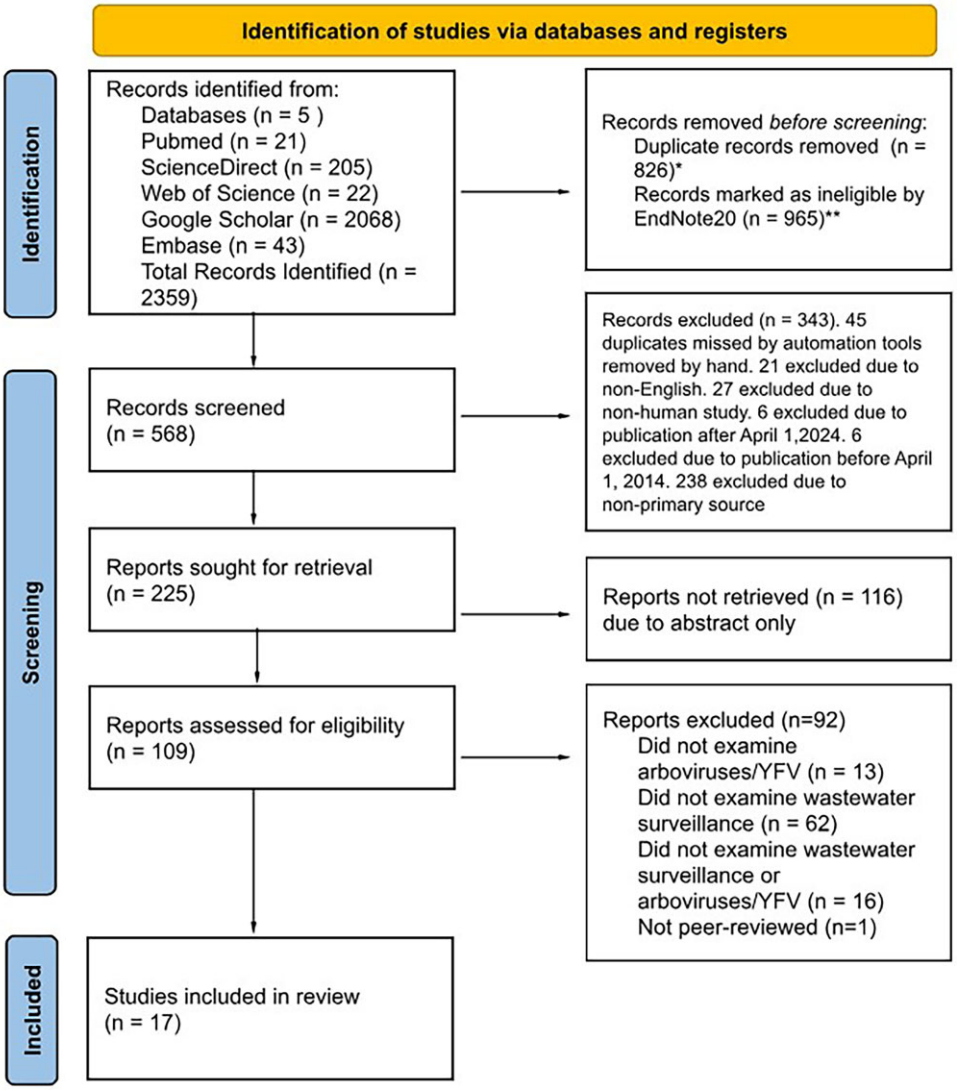

Figure 1. Flowchart of new studies (published between 15 December 2017 and 21 February 2024) included in the review based on the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) protocols. ∗Initial duplicate removal was performed using EndNote software and was checked manually for correctness. ∗∗Of the 965 records marked ineligible by automation tools, 843 reports were excluded due to being marked 'book' or 'book chapter' by EndNote20. 122 reports were automatically excluded due to publication date before 2014. Confirmatory manual review of 200 (20.7%) of these reports revealed no errors in exclusion.

Study selection, data extraction, and quality assessment

Data from relevant studies were extracted independently by CM and were reviewed independently by ZA. The extracted data included: author, year, country, study objective, relevant virus(es) of investigation, type of wastewater investigation, number of collection locations, detection method(s), quantity detected (if applicable), and rate of viral decay (if applicable). Disagreements between individual's judgements were resolved by revising the study and making a consensus agreement.

The risk of bias was assessed using the Mixed Methods Appraisal Tool (MMAT) (Hong et al. 2018), which was applied to appraise reports and address the risks of bias at an individual study level. Due to the relative novelty of wastewater surveillance, particularly in the setting of arboviral and YFV monitoring, no subject-specific risk of bias assessment was available. The MMAT was used due to its ability to appraise a broad range of empirical observational and experimental studies, which was expected in this literature review.

Data extraction process and data items

Data extraction forms were developed from the template provided by the JBI guidelines (Peters et al. 2020). Data were extracted from the articles into a Microsoft Excel file and organized under the relevant headings by the lead author. Once the process was complete, the data were again reviewed and displayed in tabular form.

Results

Selection of sources of evidence

The database search produced 2359 articles, which were subsequently screened against the inclusion and exclusion criteria as described in section 'Study eligibility criteria'. The titles and abstracts of all studies were screened and a total of 2251 articles were excluded, as they did not meet the inclusion criteria. Following a review and removal of duplicate articles, a total of 17 articles remained for inclusion in this review (Fig. 1).

Reports with detection of flavivirus in wastewater

Table 1 provides a summary of the studies included. Overall, the 17 included studies included in this review were conducted in 8 countries, including the USA ($n = 7$), Singapore ($n = 3$), Taiwan ($n = 2$), China ($n = 1$), North Korea ($n = 1$), Australia ($n = 1$), Uganda ($n = 1$), and Nepal ($n = 1$). Of the studies included in this review, 11/17 (65%) studies directly investigated population-based wastewater to detect and/or quantify viruses in wastewater (O'Brien et al. 2017, McCall et al. 2020, Chandra et al. 2021, Thakali et al. 2022, Boehm et al. 2023, Chandra et al. 2023, Fanok et al. 2023, Kuo et al. 2023, Wolfe et al. 2023, Li et al. 2024, Wong et al. 2024), whereas 5/17 (29%) studies investigated virally spiked wastewater to characterize viral persistence, viral decay, concentration methods, and/or extraction methods (Muirhead et al. 2020, Yang et al. 2021, Chen et al. 2023, Harrison et al. 2023, Shen et al. 2024). One study (6%) investigated both archived population-based wastewater to detect ZIKV, and used spiked wastewater to characterize viral persistence and recovery in wastewater (Zhu et al. 2023).

YFV and arboviral detection and quantification

Among the studies solely examining population-based wastewater ($n = 11$; 65%) or examining both population-based wastewater and spiked wastewater ($n = 1$; 6%), three (18%) studies were unable to detect flaviviruses or arboviruses, each for different reasons. Boehm et al. (2023) were unable to detect WNV nucleic acid in the wastewater, which was consistent with the absence of clinical cases in the population served by the wastewater treatment plant where these samples were collected. Moreover, Thakali et al. (2022) were unable to detect DENV from several wastewater collection sites in Nepal, despite the relatively high DENV cases in the area at the time of sampling. Notable reasons for this inability to detect DENV were explained by the authors, which include storage of wastewater at −25°C leading to viral degradation, inadequate viral concentration methods using the electronegative membrane-vortex (EMV) method, and potential mismatch between confirmed clinical dengue cases and sampling timing (Thakali et al. 2022). Finally, Zhu et al. (2023) analysed water samples stored at −80°C from Pau de Lima, Salvador, Brazil, which were collected from 2015 to 2016 during a ZIKV outbreak. The study did not detect ZIKV in the archived samples, likely because ZIKV RNA was absent. There was no evidence of DNA polymerase inhibition and the spiked bovine respiratory syncytial virus vaccine, which was used as an extraction control for ssRNA viruses, was detected (Zhu et al. 2023).

Apart from (2; 12%) studies where wastewater was spiked with YFV (Chandra et al. 2021, Chandra et al. 2023), none of the included studies detected or quantified YFV from population-based wastewater. To the best of our knowledge, detection of YFV in non-spiked wastewater has not yet been achieved. However, detection of similar viruses, including JEV, DENV, and CHIKV was achieved by (4; 24%) studies (O'Brien et al. 2017, Fanok et al. 2023, Wolfe et al. 2023, Wong et al. 2024), while detection of other *Flaviviridae* members of variable relation to YFV was achieved by an additional (5; 29%) studies (McCall et al. 2020, Yang et al. 2021, Kuo et al. 2023, Li et al. 2024, Shen et al. 2024).

DENV clinical cases were compared in the study by Wolfe et al. (2023), where wastewater solids from three locations serving the Miami-Dade County, Florida, USA. In this study, DENV-3 was detected at 21% of the samples across the three sampling locations and concentrations ranged from below detection limit to 4.1×10^3 copies/g. During the wastewater sampling time frame, there were 165 reported cases of DENV in the county; 132 travel-associated and 33 locally acquired. Based on these confirmed cases and the estimated number of individuals that these three wastewater treatment locations served, it was estimated that DENV-3 could be detected in a population with a weekly DENV-3 incidence of 0.77–4.23 cases/1 million people. Finally, although DENV clinical cases were incorporated to predict potential wastewater detection from the population, the temporal relation between clinical cases and wastewater detection/quantification of DENV, though challenging to investigate, was not explored (Wolfe et al. 2023).

In addition to monitoring clinical cases in conjunction with wastewater surveillance, Wong et al. (2024) investigated mosquito pools in the vicinity of a ZIKV outbreak in Singapore between 15 and 25 May 2023. Wastewater signals were positive for ZIKV RNA in the area of the outbreak and this positivity coincided with the peak in reported ZIKV cases. Positive and equivocal ZIKV RNA in wastewater was also found to be correlated with the number of new weekly ZIKV cases in the area of concern ($r = 0.82$, $P = 0.025$, 95% Confidence Interval = 0.17–0.97). Statistical comparison was not made between mosquito and wastewater positivity, though peak detection of ZIKV-positive *Aedes* mosquitoes coincided with wastewater positivity (Wong et al. 2024).

Fanok et al. (2023) retroactively investigated wastewater samples for the monitoring of SARS-CoV-2 to survey JEV, which was detected in pigs and mosquitoes between January 2022 and March 2022. Importantly, effluent from the piggery was not tributary to the two municipal wastewater sampling sites where wastewater samples were taken, indicating that any virus from the piggery was not thought to enter the sampling sites. JEV was detected in samples from mid-February to early March 2022, which coincided with both chicken antibody results, a mosquito grind and human cases of JEV (Fanok et al. 2023).

Metagenomic sequencing was undertaken by (5; 29%) studies to assess viral disease and viral diversity in the wastewater samples of the population they measured (O'Brien et al. 2017, McCall et al. 2020, Yang et al. 2021, Kuo et al. 2023, Li et al. 2024). Similarly, Shen et al. (2024) used metatranscriptomic data mining to assess viral ecology. Together, all reports detected members of the *Flaviviridae* family. More specifically, Li et al. (2024) and O'Brien et al. (2017) identified the flavivirus genus, while McCall et al. (2020), Kuo et al. (2023) and Yang et al. (2021) identified other genera within the *Flaviviridae* family, including hepatitis C virus. While the *Flaviviridae* detected in these reports were not quantified, the number of genes, relative abundance, occurrence frequency and number of hits were reported. Detection and quantification methods are summarized in Table S3.

YFV and arboviral decay in wastewater

In addition to the population-based investigations, (6; 35%) studies investigated spiked wastewater to characterize arboviral kinetics in wastewater (Muirhead et al. 2020, Chandra et al. 2021, Chandra et al. 2023, Chen et al. 2023, Harrison et al. 2023, Zhu et al. 2023), and (4; 24%) of these studies in-

Table 1. General characteristics of selected studies.

Author, Year	Country	Study aims	Relevant virus(es) investigated	Type of wastewater investigation	Number of collection locations	Detection method(s)	Flavivirus detected	Quantity detected	Decay rate
Boehm et al. (2023)	USA	Investigate the occurrence of pathogen nucleic acids in wastewater samples.	WNV	Population-based wastewater	2	Reverse transcriptase PCR	No	NA	NA
Chandra et al. (2023)	Singapore	Examine clarification, concentration, and extraction methods for detection of RNA signals in wastewater.	YFV, ZIKV, DENV2, DENV3,	Spiked wastewater	3	Reverse transcriptase PCR	Yes (spiked)	YFV recovery: 75% from centrifugation, 91% from filtration (Table S5 *)	NA
Chandra et al. (2021)	Singapore	Investigate the decay kinetics of a panel of arboviruses. Analyse heat inactivation and cryopreservation on decay kinetics.	YFV, ZIKV, DENV2, DENV3	Spiked wastewater	4	Reverse transcription PCR	Yes (spiked)	NA	YFV T90 of 2.612 days at 37°C, 4.403 days at 25°C, and 72.363 days at 6°C (Table S4*)
Chen et al. (2023)	Taiwan	Evaluate recovery methods and qPCR for DENV and a noncognate spiked control in artificially loaded water samples.	DENV	Spiked wastewater	1	Reverse transcriptase PCR	Yes (spiked)	% recovery: 32.3 ± 16.3 ultrafiltration of DENV2. 34.4 ± 8.6 ultrafiltration of DENV1. 21.2 ± 34.4 ultrafiltration of DENV3. 91.8 ± 70.6 ultrafiltration of DENV4. (Table S5*)	NA
Fanok et al. (2023)	Australia	Demonstrate the capability of wastewater-based surveillance for detecting cases of JEV infection in the community.	JEV	Population-based wastewater	2	Reverse transcriptase PCR and NGS	Yes	Mean Cq of 37.97 from 8 positive samples at 1:10 dilution. (Table S3*)	NA
Harrison et al. (2023)	USA	Investigate the persistence of model virus genomes in wastewater, the protective effects of virus capsids, and the relative decay rates of the genome and infectious viruses.	(+)ssRNA viruses	Spiked wastewater	1	qPCR and reverse transcriptase PCR	Yes (spiked)	NA	Mean T90 of 0.29 hours for extraviral (+)ssRNA virus. Mean T90 of 6.0 days for encapsulated (+)ssRNA (Table S4*)
Kuo et al. (2023)	Taiwan	Investigate viral diversities in campus wastewater and reclaimed water of a university dormitory using metagenomic sequencing.	HCV (*Flaviviridae*)	Population-based wastewater	2	Shotgun metagenomic analysis	Yes	50 genes detected in wastewater. 84 genes detected in reclaimed water	NA
Li et al. (2024)	USA	Evaluate sampling and virus concentration methods using virus communities concentrated with Virus Adsorption-Elution (VIRADEL) and Polyethylene Glycol (PEG) precipitation.	Unspecified flavivirus	Population-based wastewater	3	Metagenomic and NGS	Yes	Flavivirus detected at 2 sites on Dec 27, 2021 with VIRADEL. Detected at 2 sites on Dec 27, 2021 with PEG, and at 1 site on Jan 17, 2022 with PEG *(Table S3 *)*	NA
McCall et al. (2020)	USA	Assess the diversity of human viral pathogens in untreated wastewater.	HCV (*Flaviviridae*) and CHIKV (arbovirus)	Population-based wastewater	3	Metagenomic, NGS, and reverse transcription PCR	Yes	2 of 18 samples detected HCV	NA

Table 1. Continued

Author, Year	Country	Study aims	Relevant virus(es) investigated	Type of wastewater investigation	Number of collection locations	Detection method(s)	Flavivirus detected	Quantity detected	Decay rate
Muirhead et al. (2020)	USA	Develop a system for analysis of the persistence of ZIKV RNA in environmental waters using commercially available RNA extraction and detection kits over three temperatures.	ZIKV	Spiked wastewater	1	Reverse transcription PCR	Yes (spiked)	NA (Spiked)	T90 of 230 days at 4°C, 21 days at 25°C, 8.5 days at 37°C (Table S4∗)
O'Brien et al. (2017)	Uganda	Quantify the abundance of human viruses in surface water and wastewater, characterized the viral diversity of water samples, and to established preliminary data that could indicate the possibility of using these methods in future wastewater-based epidemiology studies.	cacipacore virus (flavivirus) *Table 6*	Population-based wastewater	5	NGS, qPCR, and metagenomic sequencing	Yes	Not quantified	NA
Shen et al. (2024)	North Korea	Address the feasibility of metatranscriptomics for understanding the fate of human-derived pathogens in drinking water sources.	Unspecified flavivirus	Population-based wastewater	1	Metatranscriptomic sequencing	Yes	Not quantified	NA
Thakali et al. (2022)	Nepal	Investigate the presence of DENV RNA in the wastewater	DENV	Population-based wastewater	6	Reverse transcriptase PCR	No	NA	NA
Wolfe et al. (2023)	USA	Apply RT-PCR assays for DENV serotypes 1, 2, 3, and 4 to test wastewater solids.	DENV (1, 2, 3, 4)	Population-based wastewater	3	Reverse transcriptase PCR	Yes	Maximum of 4.1×10^3 cp/g (50th percentile, median = non-detect) DENV3 detected in 24 of 112 (21%) samples from all three WWTP sites	NA
Wong et al. (2024)	Singapore	Monitor ZIKV outbreak and mitigate further ZIKV transmission comparing case, entomological and wastewater-based surveillance.	ZIKV	Population-based wastewater	3	Reverse transcriptase PCR	Yes	Not quantified. Area A had a higher frequency and proportion of equivocal samples (20.2%, 34/168, 10 days) and of positive samples (6.0%, 10/168, 4 days).	NA
Yang et al. (2021)	China	Examine human RNA viruses using NGS in sewage water samples.	Hepatitis A Virus (*Flaviviridae* family)	Population-based wastewater	3	NGS	Yes	HAV detected at 4 sites with 22 total hits. total size of 1820	NA
Zhu et al. (2023)	USA	Characterize the persistence and recovery of spiked ZIKV RNA into surface water, wastewater, and a combination of both to examine the potential for detection in open sewers.	ZIKV	Both spiked wastewater and Population-based wastewater	1	Reverse transcriptase PCR	Yes (in spiked) No (in environment)	NA	T90 of 4.17 days to 65 days depending on temperature, spiked medium, and initial concentration (Table S4∗)

Data extraction from included studies. If the number of viruses investigated exceeded 10, a reference to the relevant figure/table outlining the viruses investigated is provided. Quantity detected is reported as % recovery, Cq values, number of genes detected, number of positive sites, or copies per gram depending on study objective and measurement. Decay rate, if applicable, is reported as T90, which refers to the time required for 1-log10 reduction.
Abbreviations: YFV = Yellow fever virus, WNV = West Nile virus, DENV = Dengue virus, MHV = Murine hepatitis virus, WWTP = Wastewater treatment plant, NGS = Next generation sequencing, PCR = Polymerase chain reaction, NA = Not applicable.
∗ Supplemental data sheet

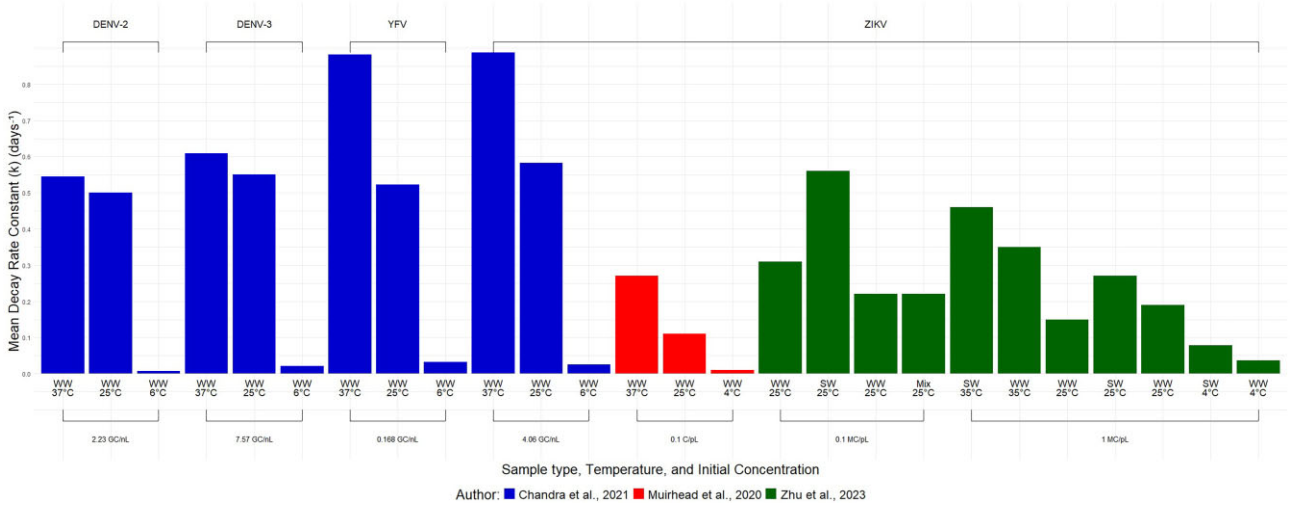

Figure 2. Mean viral decay rate of spiked arboviruses based on sample type, temperature, and initial concentration of the studies examining DENV-2, DENV-3, YFV, and ZIKV. Mean first order decay rate constant is reported as k (days^{-1}), where higher k values suggest faster decay rate. Studies are grouped based first author surname representing investigations carried out by Chandra et al. (2021), Muirhead et al. (2020) and Zhu et al. (2023). Decay rate experimental conditions are indicated below the bars with WW = virally spiked wastewater, SW = virally spiked surface water, and Mix = virally spiked 50:50 mix of wastewater and surface water. Virus type is denoted by horizontal brackets above the bars.

vestigated decay of various viruses at several temperatures, in various wastewater environments. In the studies investigating arboviral decay, mean decay rate constants were reported for each virus investigated and at each temperature (Fig. 2). To investigate the role of encapsulation on viral decay, Harrison et al. (2023) compared extracapsular and encapsulated RNA and DNA, finding that encapsulation reduced the decay rate constant by approximately threefold at 25°C. These findings suggest that encapsulation fosters a protective effect against nucleic acid decay in wastewater, ultimately allowing wastewater surveillance for RNA viruses with envelopes.

Viral decay investigations at various temperatures are also important in understanding wastewater surveillance, particularly for viruses endemic to tropical regions, like YFV. Investigations on spiked, non-attenuated YFV performed by Chandra et al. (2021) revealed that YFV has faster decay in 37°C than 25°C or 6°C with decay being slowest in 6°C. There is a significant increase in the decay rate from 6 to 25°C. However, the T90 (time required for 1-log10 reduction) at 37°C remains on the order of days and is more comparable to the decay rate at 25°C than to the rate at 6°C. Furthermore, decay rates are largely similar between YFV, DENV-2, DENV-3, and ZIKV, with some notable differences. First, YFV appears to decay slowest in 6°C temperatures among the arboviruses investigated, but decays faster than DENV-3 both at 25 and 37°C. Second, YFV decay was most similar to ZIKV decay. On that note, Muirhead et al. (2020) reported a similar trend in ZIKV decay as explored by Chandra et al. (2021), where decay at 4°C was slower than either 25 or 37°C, with the higher two temperatures having more comparable decay rates. A similar trend was observed by Zhu et al. (2023). In addition to spiked wastewater, Zhu et al. (2023) examined both spiked surface water and a 50:50 mix of surface water and wastewater. Viral decay was faster at each given temperature for surface water experiments compared to spiked wastewater. Though only performed at 25°C, the mixed spiked surface water and wastewater had comparable decay rates to ZIKV spiked wastewater. Furthermore, direct comparison between ZIKV decay at different concentrations revealed faster rates of decay for lower initial concentrations of ZIKV (1×10^8 copies/mL vs 1×10^9 copies/mL), suggesting that rate of viral decay is not strictly a first order process, and that initial concentration is a consideration with respect to decay time. This temperature and initial concentration-dependent trend was largely consistent between these studies, as shown in Fig. 2. To compare decay rates between these studies, the first order mean decay rate constant (k) was used and was derived from the following formula: $k = \frac{-\ln(0.1)}{T90}$ where T90 refers to the time for a log10 reduction in viral concentration in days. Units for k are days^{-1}. Large k values reflect faster decay rates, and small k values reflect slower decay rates.

YFV and arboviral extraction and concentration methods

Methods of extraction and concentration are crucial when quantifying and detecting YFV and other arboviruses in wastewater. Accordingly, studies (3; 18%) reported mean recovery efficiency of virally spiked wastewater or surface water, using differing concentration methods (Chandra et al. 2023, Chen et al. 2023, Zhu et al. 2023). Among the several concentration methods investigated by Chandra et al. (2023), centrifugation ultrafiltration generally outperformed other concentration methods for recovery of DENV-2, DENV-3, YFV, and ZIKV. Mean recovery efficiency ranged from 49.62% ± 13.75% to 84.76% ± 17.45% depending on the single-use column used in filtration with the Macrosep Advance 30 kDa cutoff having the highest mean recovery efficiency at 84.76% ± 17.45%. Centrifugation ultrafiltration was performed using 50 mL of spiked wastewater in triplicate. Though not as efficient as centrifugation ultrafiltration, polyethylene glycol (PEG) precipitation was also observed to be successful in recovery of the above-mentioned viruses, an important finding given the relatively low cost of PEG precipitation (LaTurner et al. 2021). PEG precipita-

tion mean recovery efficiency ranged from 20.86% ± 2.90% to 19.82% ± 4.29%, depending on centrifugation protocols. PEG precipitation was performed using 200 mL of spiked wastewater in triplicate. Electronegative and electropositive membrane captures were generally less efficient than PEG precipitation for recovery of these arboviruses, with the exception being electronegative membrane capture with bead-beating, which had a mean recovery efficiency of 28.87% ± 4.87%. These charged membrane capture and elution techniques were performed using 200 mL of spiked wastewater in triplicate. Importantly, recovery efficiency was principally dependent on concentration method type and not by arbovirus type, suggesting that these concentration methods were consistent across DENV-2, DENV-3, YFV, and ZIKV. Therefore, these findings may suggest that the recovery efficiencies among these arboviruses are comparable among concentration using the same methodology (Chandra et al. 2023).

The concept that arboviruses can be successfully recovered from wastewater by ultrafiltration is corroborated by the findings of Chen et al. (2023), where all four serotypes of DENV were efficiently recovered from spiked wastewater using ultrafiltration centrifugation of 50 mL spiked wastewater performed in triplicate. While the mean ± SD recovery efficiencies for DENV-2 and DENV-3 were similar (32.3% ± 16.3% and 21.2% ± 34.4%, respectively) and consistent with the trend observed by Chandra et al. (2023), DENV-4 recovery efficiency was higher (91.8% ± 70.6%). This somewhat contradicts the idea that arboviruses may have comparable recovery efficiencies. However, due to the high variability in the recovery efficiency of DENV-4 and the absence of statistical analysis comparing the recovery efficiency of these serotypes, the significance of this difference remains unclear. Possible reasons for this high variability include serotype differences in primer-template interactions in DENV leading to variable amplification and DENV-4-specific serotype sensitivity to inconsistencies in experimental conditions. This serum-specific variability in PCR recovery rates may limit the accuracy of wastewater surveillance and comparison between serotypes and viruses. However, the DENV-4 serotype was not explicitly investigated by Chandra et al. (2023) and was not implied to be comparable to other arboviruses like YFV. In contrast to ultrafiltration concentration, Chen et al. (2023) observed that DENV could not be successfully recovered from wastewater using skimmed milk flocculation (SMF) enrichment methodology.

Moreover, Zhu et al. (2023) found that % recoveries from ZIKV RNA signals from extraction of 100 mL wastewater supernatant with SMF were consistently low. However, in-triplicate concentration from solid pellet using SMF (in contrast to liquid supernatant) yielded recovery percentages ranging from 5% to 50% depending on experimental conditions, suggesting that ZIKV was preferentially separated through association with solids. Furthermore, extraction of ZIKV was more successful in wastewater or mixed wastewater-surface water than from surface water alone. This method of concentrating arboviruses from solids was similar to the methodology undertaken by the previously mentioned study Wolfe et al. (2023), where it was estimated that wastewater detection of DENV could be possible with 0.77–4.23 weekly dengue cases per 1 million population. Supplementary material (Table S5) provides a summary of YFV and arboviral extraction.

Discussion

Study quality

Given the novelty of wastewater surveillance, no robust or relevant risk of bias assessments exist. In relation to the studies using qPCR to investigate arboviruses, minimum information for publication guidelines was reported in all but 4 studies (Table S6). This lack of MIQE reporting limits the reproducibility and internal validity of the associated studies. Thus, studies without MIQE compliance should be interpreted with caution, as their findings may be less reliable and could introduce bias. A complete table of studies and the use of MIQE guidelines is presented in Table S6. For the external validity of these studies, investigation of specific populations may have limited generalizability to other or global populations. For example, wastewater collected from the USA may not be generalizable to Brazilian wastewater parameters, or wastewater parameters in YFV-endemic locations. While informative regarding concentration methodology and viral decay, spiking of wastewater samples is generally not representative of population-based wastewater investigation. Laboratory-spiked viruses may exist at higher quantities than realistic of population-based wastewater viruses. Thus, virally spiked wastewater was not deemed representative of the target populations in these studies, which is reflected in the risk of bias assessment (Table S2).

Furthermore, given the complexity, novelty, and lack of sensitivity associated with metagenomics, the results from studies using metagenomics should be interpreted with caution. While metagenomics may suggest the presence of a particular pathogen and may be used to guide other surveillance methods, metagenomics alone cannot be used to determine the absolute abundance of viruses. Additionally, contigs related to human viruses may not directly indicate the presence of infectious virus in the community (Li et al. 2024). Another concern with metagenomics is low hits. Low hits might can result from sequencing errors or contamination, which limit the reliability of the findings of these studies (Jurasz et al. 2021). Positive viral presence in metagenomic investigations should be interpreted as less convincing than positive viral presence in population-based PCR investigation of arboviruses. Therefore, the metagenomic investigations included in this review are of lower internal validity compared to population-based PCR investigations directly investigating arboviruses, given the concerns with accurate pathogen prevalence estimates.

YFV and arboviral detection

Although YFV has not yet been detected or quantified in population-based wastewater surveillance, population-based investigation on related arboviruses/*Flaviviridae* and YFV-spiked experiments highlight wastewater surveillance as a potentially promising tool for detection and monitoring of YFV outbreaks. However, several considerations exist as potential impediments in implementing wastewater surveillance for the monitoring of YFV.

Regarding YFV detection and quantification, previous successes in population-based wastewater surveillance for DENV, ZIKV, and JEV highlight the potential ability to monitor YFV in wastewater (Fanok et al. 2023, Wolfe et al. 2023, Wong et al. 2024). Based on laboratory experiments from Chandra et al. (2021), viral recovery is likely similar among some arboviruses in spiked settings. Moreover, these arboviruses are all enveloped, positive-sense single-stranded RNA viruses

with similar capsids, diameter, entry, replication, and shedding mechanisms (Simmonds et al. 2017). However, given the lack of comparative studies in environmental wastewater, it is unclear if these viruses exhibit similar kinetics from population-based media outside of laboratory settings.

Additionally, variations in shedding rates among these arboviruses may affect the initial virus concentration in wastewater, which could, in turn, impact recovery efficiency and detection. For instance, though it has been established that DENV, ZIKV, WNV, and YFV are shed in the urine, urine positivity varies between arboviruses varies (Lee et al. 2022). Differences in faecal shedding are more poorly characterized and may further contribute to differences in virus concentration in wastewater. While YFV shedding is somewhat well-established from symptomatic individuals in the urine (Domingo et al. 2011, Phan et al. 2020), a better understanding of inter- and intra-individual variability in the quantity of viral excretion would prove fundamental in understanding wastewater quantification of YFV. This is true not only of YFV, but for arbovirus monitoring generally. On that note, among the studies included in this review, clinical case correlates were rarely incorporated into wastewater investigation, meaning drawing conclusions regarding case counts from wastewater surveillance is challenging for arboviruses at this time. Lack of quantification of viral RNA in population-based wastewater also makes these conclusions challenging. However, the mere presence of an arbovirus like YFV in wastewater may be used to imply disease burden or potential outbreak sites, particularly in vaccine and/or wild YFV-naive populations. Nevertheless, the insights gained from studying other arboviruses in population-based settings, as well as from virally spiked samples, are valuable for guiding future wastewater surveillance for YFV.

Another important consideration with YFV is the availability of a vaccine. Unlike many other arboviruses, the presence of a live attenuated vaccine for YFV may introduce important confounders for wastewater surveillance. Since the YFV vaccine has been shown to be shed via urine (Domingo et al. 2011), it is unknown if vaccine-derived nucleic acid will impact population-based wastewater sampling. However, since the vaccine and wild YFV strains are not genetically identical (Davis et al. 2021), it is possible that reverse transcriptase PCR would be able to differentiate between wild and vaccine strains with optimized primer development. Furthermore, though unlikely to be feasible in resource-limited settings, metagenomics sequencing may be even more successful at differentiating between wild and vaccine YFV while characterizing viral ecology in wastewater (O'Brien et al. 2017, McCall et al. 2020, Li et al. 2024).

Given the relatively low expected YFV load in wastewater samples, the concentration of samples emerges as an important consideration when applying wastewater surveillance to YFV. Regarding concentration and extraction methods for YFV, the studies included in this review on other arboviruses have shown that a variety of methods can be used to yield good recovery. It seems that concentration using centrifugation-ultrafiltration, especially with 30 kDa cutoffs, may provide the best recovery (Chandra et al. 2023, Chen et al. 2023). However, other methods like electronegative membrane capture and PEG precipitation have also been shown to be able to maintain relatively high recovery efficiencies (Chandra et al. 2023). SMF concentration directly from wastewater does not appear to be an effective concentration method (Chen et al. 2023). However, SMF concentration of solid pellet does appear to be an effective strategy to concentrate arboviruses from wastewater (Zhu et al. 2023). Similarly, solid pellet extraction and concentration have been shown to be effective in concentrating DENV from non-spiked, population-based wastewater (Wolfe et al. 2023). Thus, solid pellet concentration and extraction of arboviruses seem to be an effective recovery method that bears further investigation, particularly in the setting of arboviruses. Indeed, many of the concentration and extraction methods employed by the included studies were able to generate modest-to-high recovery efficiency, suggesting that the 'best' concentration method may depend more on case-by-case surveillance requirements versus detection method. Interestingly, the general success of several concentrations is also seen with COVID-19 wastewater surveillance. For example, SMF concentration, modified ultrafiltration concentration, PEG precipitation, and aluminium polychloride flocculation have all been used with success to detect SARS-CoV-2 in wastewater samples (Jafferali et al. 2021, LaTurner et al. 2021, Philo et al. 2021). Indeed, until more research is conducted regarding the comparative effectiveness of arbovirus extraction from population-based wastewater samples, it may be that the best concentration method balances extraction efficiency and cost, depending on resource constraints.

Viral decay in wastewater seems to accelerate as a function of temperature, with increased stability observed at lower temperatures and decreased stability at higher temperatures. It is believed that the stability of these arboviruses is attributable to their envelope, which is protective against decay in wastewater (Harrison et al. 2023). While colder temperatures may foster stability, at 37°C the T90 in the range of days suggests that sampling in these temperatures is likely still possible within reasonable timeframes. This was confirmed by Wolfe et al. (2023), who performed DENV wastewater sampling in the tropical climate of Miami-Dade county.

Experimentally, it has been shown that the decay rate of DENV is comparable to that of other related arboviruses, including YFV (T90 within 3.95–6.21 days at 25°C) (Chandra et al. 2021). However, it is unclear if these arboviruses differ in their decay rate outside of laboratory conditions. Furthermore, it is unknown how other variables like pH, sunlight or other organic matter may impact decay rate. Importantly, in many areas where YFV is endemic, open sewage systems may be more common, which opens the possibility of other variables impacting decay rate prior to sampling. In summary, experiments on arboviral decay suggest that, despite accelerated decay at higher temperatures, arboviral extraction remains possible across a broad range of temperatures. However, further research is needed to explore other influencing variables.

There are several limitations associated with this review. Firstly, there is a considerable lack of data regarding wastewater surveillance for arboviruses and YFV specifically. The lack of non-spiked YFV detection is a significant limitation. Lack of population-based YFV wastewater surveillance makes YFV comparison to other arboviruses challenging. Spiked studies do provide valuable insight into virus detection methods and decay, though population-based studies may help to better understand the prevalence and distribution of YFV and other arboviruses in populations. Secondly, although more research has been conducted on YFV-related arboviruses, the applicability of these findings to YFV remains

questionable. Generally, these viruses are phylogenetically closely related and share similar characteristics. However, potential differences in incubation period, case fatality rate, geographic distribution, shedding, and persistence in wastewater may contribute to unforeseen differences in wastewater sampling, concentration, extraction, and quantification methodology. Finally, considerable heterogeneity in the study design of the reports included, including sampling, detection kits/methods, location, and wastewater system infrastructure complicated comparison and synthesis of the extracted data.

Recommendations for future studies

Several directions for future research could further advance the study of wastewater surveillance for YFV. Population-based investigations in areas where YFV is endemic or in outbreak areas would provide valuable insight into YFV monitoring outside of laboratory settings. An emphasis should be placed on comparison and correlation of wastewater data with clinical case counts or even other surveillance methods such as mosquito pools to determine the ability of wastewater surveillance to predict outbreaks and characterize disease burden. Longitudinal studies conducted over an extended period of time may also provide insight on temporal and/or seasonal dynamics of YFV transmission, while case studies may provide information on the feasibility and challenges of implementing wastewater surveillance for YFV.

Secondly, it is unclear how wastewater surveillance of arboviruses should inform public health programmes. Currently, YFV is mainly monitored with syndromic surveillance and laboratory investigation, most commonly with enzyme-linked immunosorbent assays (ELISA) and confirmatory PCR (Leong 2018). However, these tests are not available globally. The main strategy for controlling YFV outbreaks is vaccination (Chen and Wilson 2020). The goal of wastewater surveillance is to complement current testing by providing additional detail on potential locations, severities, and timeframes of arboviral outbreaks. Ideally, this information is then used to drive location- and time-specific testing, vaccination, insecticide use, and education campaigns. While there is minimal data on wastewater surveillance of arboviruses informing public health strategy, wastewater data has been used in the past to educate changes in viral public health interventions. For instance, wastewater surveillance was used in Israel in 2013 to monitor a polio outbreak and caused the launch of a successful polio vaccination campaign (Tulchinsky et al. 2013, Manor et al. 2014). More recently, wastewater surveillance has been used to serve as an early warning system (Bibby et al. 2021). Given the presence of efficacious public health interventions for YFV and the previous successes of wastewater surveillance for other viruses in the past, future studies should examine the extent to which wastewater surveillance should inform YFV and arboviral public health interventions.

Moreover, while sample clarification, concentration, and extraction methodology have been explored for other arboviruses, these methodologies have scarcely been examined for YFV. Although these methods are likely to be similar and applicable to YFV monitoring, further method optimization may be needed for YFV.

Similarly, cost-benefit or feasibility analyses may be insightful for YFV monitoring in resource-limited settings given that several concentration methods seem effective. Certain concentration methods may be more feasible for different arboviruses based on differences in arbovirus distribution and the inherently available infrastructure and resources. For example, resource-limited settings may be able to undertake less costly methods like electronegative filtration, which has a moderate start-up cost, but a relatively low per-sample cost for COVID-19 monitoring (LaTurner et al. 2021). This start-up cost is partially driven by the need for high-powered centrifuges required for filtration of larger input volumes. Input volume is another consideration when comparing concentration methods. While higher input volume may increase virus concentration, the processing time may also increase, and there is a natural limit of input volume based on blocking of the filter membrane (Chandra et al. 2023). PEG precipitation is generally slower than ultrafiltration, requiring overnight mixing, and requires more input volume compared to ultrafiltration. This is reflected in the volumes of wastewater used by Chandra et al. (2023) with 50 mL used for ultrafiltration and 200 mL used for PEG precipitation (Chandra et al. 2023). These differences in timing and input volume may be important technical and financial considerations when implementing wastewater surveillance for YFV and arboviruses.

Once cost-benefit analyses have been adequately explored, next steps may include standardization of protocols to foster comparability of data across different studies in different regions. Initially, establishing standardized protocols may improve comparability between arbovirus collection, concentration, storage, and quantification, which could shed light on potential differences in optimal virus-specific wastewater surveillance. From these unified protocols, virus-specific changes could be made to develop arbovirus-specific standardized protocols.

Another limitation is the limited knowledge and investigation of appropriate PCR probes specifically for YFV. Only 2 studies, from the same institute, included in this review used YFV PCR primers and probes. The primers and probes used were the same in these studies and targeted the strain-conserved sequences of the 5′-noncoding region of YFV (Domingo et al. 2012). Notably, the authors mention that differences in PCR inhibition between the arboviruses tested could have been due to primer design and that modifying primers to extend the sequences to include more G/C bindings could help improve amplification in complex matrices (Chandra et al. 2023). YFV and other arbovirus primer and probe optimization are thus important topics of investigation for future studies. Additionally, it is possible that other primers may be more advantageous in the context of population-based wastewater investigation compared to spiked vaccine-strain studies.

Furthermore, more information on YFV shedding in the urine, particularly for subclinical infections, would be helpful in identifying the amount of YFV present or expected during outbreaks. This shedding rate may then be compared to wastewater quantification to better elucidate the success of various concentration, extraction, detection, and quantification methodology outside of laboratory settings. Future investigations may also explore advancements in detection technologies like Next Generation Sequencing (NGS) and PCR to improve the sensitivity and specificity of YFV detection. Improvement of these technologies may also prove beneficial in differentiating between locally ac-

quired, live attenuated vaccine strain, and travel-acquired cases.

Conclusion

In conclusion, while YFV has not yet been detected or quantified in population-based wastewater surveillance, the investigation into related arboviruses and YFV-spiked experiments provides direction for YFV wastewater surveillance. The successful detection and quantification of DENV, ZIKV, and JEV in wastewater provide a promising basis for adapting similar methods for YFV. Nonetheless, challenges such as potentially variable shedding rates, the impact of the YFV vaccine, and the need for optimized concentration and extraction methods must be addressed. Understanding viral decay, especially at temperatures typical of tropical climates, is crucial for effective monitoring of YFV and related arboviruses. Despite the potential of wastewater surveillance for YFV, further research is needed to refine concentration and extraction methodologies, explore viral kinetics, and establish robust population-based studies to enhance YFV detection, outbreak prediction, and inform public health interventions.

Acknowledgements

The authors would like to thank Applied Microbiology International (AMI) for funding and supporting the summer studentship programme.

Author contributions

Caleb Morin (Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing), and Zina Alfahl (Conceptualization, Funding acquisition, Investigation, Writing – review & editing)

Supplementary data

Supplementary data is available at *JAMBIO Journal* online.

Conflict of interest: None declared by all authors. .

Funding

This review was supported by Applied Microbiology International Summer Studentship Grant 2024 (Grant reference no.: 02-303952).

Data availability

Data can be made available upon reasonable request.

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Received 7 October 2024; **revised** 13 February 2025; **accepted** 13 March 2025