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## Short Communication

## Multiple traces of monkeypox detected in non-sewered wastewater with sparse sampling from a densely populated metropolitan area in Asia



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## HIGHLIGHTS

- First dataset on monkeypox viral DNA detection from wastewater in Thailand
- Monkeypox viral DNA tracing is possible with sparse sampling events.
- Non-sewered locations showed positive signals for monkeypox DNA.
- Sanger sequencing confirmed the identification of the monkeypox virus.
- Findings expand the use of wastewater surveillance in resource limited countries.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

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## ABSTRACT

The monkeypox virus is excreted in the feces of infected individuals. Therefore, there is an interest in using viral load detection in wastewater for sentinel early surveillance at a community level and as a complementary approach to syndromic surveillance. We collected wastewater from 63 sewer and non-sewered locations in Bangkok city center between May and August 2022. Monkeypox viral DNA copy numbers were quantified using real-time polymerase chain reaction (PCR) and confirmed positive by Sanger sequencing. Monkeypox viral DNA was first detected in wastewater from the second week of June 2022, with a mean copy number of 16.4 copies/ml ( $n = 3$ ). From the first week of July, the number of viral DNA copies increased to a mean copy number of 45.92 copies/ml. Positive samples were Sanger sequenced and confirmed the presence of the monkeypox virus. Our study is the first to detect monkeypox viral DNA in wastewater from various locations within Thailand. Results suggest that this could be a complementary source for detecting viral DNA and predicting upcoming outbreaks.

Human monkeypox is a zoonotic viral disease that occurs mainly in the rainforests of Central and Western Africa (Peiró-Mestres et al., 2022; Thornhill et al., 2022; Wannigama et al., 2022). Since early May 2022, over 60,000 monkeypox virus infections have been reported across 75 countries, prompting the World Health Organization to declare this outbreak a public health emergency of international concern. The monkeypox virus is a double-stranded DNA virus belonging to a member of the genus *Orthopoxvirus* (family Poxviridae, subfamily Chordopoxvirinae) (Thornhill et al., 2022). It has been found to infect various tissues, including the heart, brain, ovaries, and lymphoid tissue (Thornhill et al., 2022). The primary transmission mode is via close physical contact, including skin-to-skin contact, exchange of body fluids, and respiratory droplets at close range (Miura et al., 2022; Peiró-Mestres et al., 2022; Thornhill et al., 2022; Weinstein et al., 2005).

Monkeypox viral shedding via feces has been previously reported in the stool of infected individuals (Adler et al., 2022; Peiró-Mestres et al., 2022; Sklenovská and Van Ranst, 2018; Tiwari et al., 2023). Therefore it follows that the circulation of the virus within a population may increase the viral load in sewer systems (Wolfe et al., 2022). Wastewater surveillance could be a powerful tool to monitor the virus in communities. It can estimate asymptomatic or pre-symptomatic and symptomatic transmission and predict future waves (Ahmed et al., 2021; Wannigama et al., 2021). However, up to now, little information has been available on monkeypox viral DNA in wastewater. Detection of monkeypox, specifically in Thailand, has not been investigated before, and only seven cases have been reported at the time of writing this manuscript (<https://ddc.moph.go.th/en>).

To assess the presence of monkeypox DNA in wastewater, we retrieved samples from an ongoing SARS-CoV-2 wastewater surveillance program (Wannigama et al., 2021) collected between May and August 2022. Samples were collected twice a month (every two weeks) from 63 locations (shopping centers, condominium complexes, office complexes, food markets, wastewater treatment plants, and entertainment venues) in Bangkok province, covering 51 sub-districts. The monkeypox DNA copy number in samples was

quantified by real-time PCR. E5R/OPG117 and N3R/OPG016 genes were amplified by PCR from the DNA of RT-PCR positive samples collected on the 4th week of August, and Sanger sequencing was conducted to validate the RT-PCR results (Amarasiri et al., 2020; Covarrubias et al., 2022; Kumar et al., 2018; Lum et al., 2022; Shu and McCauley, 2017) (Details are available in the supporting information). All those locations have closed wastewater storage systems.

Monkeypox viral DNA was detected in wastewater starting the last week of June 2022. The positivity rate for monkeypox DNA increased from 4.76 % ( $n = 3$ ) to 9.52 % ( $n = 6$ ) between the 4th week of June and the 4th week of August (Table 1). The average copy number of monkeypox DNA detected in the 4th week of June was 16.4 copies/ml ( $n = 3$ ). Average viral DNA concentration increased to 46 copies/ml by the 4th week of July ( $n = 5$ ) and to 80 copies/ml by the 4th week of August ( $n = 6$ ) (Fig. 1 and Table 1). Quantification of monkeypox viral DNA in samples taken a year ago confirmed the absence of the monkeypox virus in those samples. The Sanger sequencing of E5R/OPG117 and N3R/OPG016 gene fragments confirmed the monkeypox virus's identification and validated the PCR findings. Preliminary analysis (Figs. 1c and d) shows that the monkeypox virus DNA from wastewater samples belongs to the West African clade and is closely related to the genomes from the 2022 outbreak, including genomes from Thailand monkeypox cases. All the monkeypox DNA-positive locations were commercial or public venues with closed non-sewered systems. Thailand's first monkeypox case was reported on July 21, 2022, in a 27-year-old male who had been sick for a week, while the second case was reported on July 28, 2022: a 47-year-old male, who had symptoms since July 12, 2022. Subsequently, third (August 3, 2022), fourth (August 5, 2022), fifth (August 15, 2022), sixth (August 21, 2022), and seventh (August 28, 2022) cases were reported. According to the press releases, most patients had symptoms a week preceding positive diagnoses. They had been to entertainment venues or crowded places with tourists and locals, which may correspond to the wastewater findings. Unfortunately, due to the personal data protection act, we cannot access complete contact tracing details of the positive cases.

**Table 1**

Monkeypox viral DNA copies in wastewater samples from six positive locations among the 63 tested locations in Bangkok city from June second week to August fourth week.

Month	Sampling Locations	Monkeypox viral DNA (copies/mL) <sup>a</sup>	Standard deviation	Cumulative cases national (total Monkeypox cases for each month) <sup>b</sup>	Monkeypox viral DNA positivity rate (%)		
June fourth week	B10	11.35	3.15	0	4.76		
	B12	13.22	2.56				
	B23	24.68	2.50				
July Second week	B10	35.48	1.50		2	4.76	
	B12	19.14	3.52				
	B23	21.0	2.31				
July fourth week	B10	31.30	1.01			5	7.93
	B12	35.67	1.00				
	B23	62.89	2.25				
	B34	48.71	4.00				
	B55	51.84	3.74				
August second week	B10	51.8	2.00				5
	B12	60.78	2.24				
	B23	75.74	3.52				
	B34	80.09	4.10				
August fourth week	B55	82.43	2.10	5	9.52		
	B10	75.12	3.78				
	B12	68.44	5.69				
	B23	88.07	5.82				
	B34	90.16	3.67				
	B55	93.43	4.75				
	B37	64.87	4.63				

<sup>a</sup> Average copy number of monkeypox viral DNA at positive locations.

<sup>b</sup> Total confirmed monkeypox cases in Thailand obtained from Department of Disease Control, Ministry of Public Health, Thailand. <https://ddc.moph.go.th/en>.

Detected amounts of monkeypox DNA have increased regarding the quantity detected at positive sampling sites and the number of positive sampling sites. These results suggest the growing transmission of monkeypox and the exact time of monkeypox virus 2022 circulating in Thailand was far earlier than July 2022 (The first positive human case). Unfortunately, we could not match these results to case or contact tracing data. However, phylogenetic analysis of the sequence of E5R/ OPG117 and N3R/OPG016 gene reflect that wastewater samples are clustering with positive clinical cases from Thailand and other geographical origins connected to the monkeypox 2022 outbreak (Xiong et al., 2022). Thailand opened its borders in late May 2022 and is a major tourist destination for travelers from Europe and North America, where many monkeypox cases are currently reported. Thailand is a major risk area for emerging infectious diseases because it is a key destination for the sex tourism industry, where infections can appear and spread easily and quickly across the region (Kueakulpattana et al., 2021). Therefore, we believe that number of individuals carrying the monkeypox virus may be higher in the community than in reported cases. We observed low monkeypox viral DNA concentrations during the sampling period, and studies from US and Europe reported similar findings (de Jonge et al., 2022; Girón-Guzmán et al., 2022; La Rosa et al., 2022; Wolfe et al., 2022). However, all the positive samples in our study are from closed, non-sewered locations collected in sparse sampling events compared to frequent sampling from centralized wastewater treatment plants as in reported studies (de Jonge et al., 2022; Girón-Guzmán et al., 2022; La Rosa et al., 2022; Wolfe et al., 2022). Therefore, our findings strongly emphasize that tracing monkeypox viral DNA in wastewater is feasible even with sparse sampling, including non-sewered wastewater, which allows the application of wastewater-based surveillance to countries/regions with lower resources without established sewer systems. Since Bangkok province does not have an organized wastewater infrastructure like developed countries and it's difficult to track emerging infectious diseases based entirely on centralized wastewater treatment plants (Wannigama et al., 2021). To overcome this challenge, we selected 90 % of sampling locations in commercial or public venues based on population density, accessibility via the transportation system, and frequency of visitation.

This comes with its limitation: we cannot attribute most of these locations to actual catchment numbers or flow due to the nature of non-sewered (Wannigama et al., 2021). However, this approach helped us to successfully identify the potential clusters and transmission in urban and rural communities during the SARS-CoV-2 pandemic (Wannigama et al., 2021). Moreover, our detection methodology is entirely based on standard qPCR techniques, which may be easily adaptable to resource-limited settings compared to droplet digital PCR or whole genome sequencing (de Jonge et al., 2022; Girón-Guzmán et al., 2022). Therefore, we believe that our findings might also advance health equity and provide strong real-world evidence for wastewater-based epidemiology in densely packed urban populations with poor sewer infrastructure.

The fewer number of reported positive cases in Thailand may be attributed to treatment-seeking behavior, LGBTQ people facing stigma and bias over mislabeling monkeypox as a sexually transmitted disease (STD), misdiagnosis with secondary syphilis, and unable to locate blisters in internal organs (rectum or throat cavity) (Girometti et al., 2022; Miura et al., 2022; Tarín-Vicente et al., 2022; Thornhill et al., 2022). Also, the lack of access to testing and the unavailability of diagnostics could steer people away from getting tested and seeking treatments (Girometti et al., 2022; Miura et al., 2022; Tarín-Vicente et al., 2022; Thornhill et al., 2022). According to a recent report, many of those infected individuals experience mild symptoms, including singular lesions or mild rectal pain; for example, a single lesion or sore in the oral cavity or on the genitals (Miura et al., 2022; Peiró-Mestres et al., 2022; Thornhill et al., 2022). Also, some people with monkeypox may not develop any symptoms (Ferré et al., 2022; Miura et al., 2022). Despite the mildness or absence of symptoms, infected people are likely to shed the virus into wastewater via routes connected to the location of the lesion; those shed through urine and feces are most likely to end up in wastewater (Peiró-Mestres et al., 2022). We also determined the absence of monkeypox viral DNA in historical samples (such as those taken a year ago as part of a study into SARS-CoV-2 in wastewater), which may suggest the monkeypox DNA detected in this study is excreted to the wastewater by the infected individuals from the current outbreak.

However, 15.3 % of the country's total population resides in Bangkok. Our results based on biweekly wastewater sampling frequency may not provide a clear view of the overall picture of monkeypox transmission in Thailand. The samples in this study were characterized according to the United States Centers for Disease Control and Prevention (CDC) Monkeypox virus-specific real-time PCR assay (CDC, 2022) like other studies (de Jonge et al., 2022; La Rosa et al., 2022; Wolfe et al., 2022). However, further characterization with Sanger sequencing assured the strength of our findings and the credibility of the CDC PCR assay. In addition, the inability to easily correlate virions in wastewater to the number of infected people makes it challenging to interpret the results. Furthermore, there is limited information regarding the intensity and duration of monkeypox viral DNA shedding in feces throughout infection (Miura et al., 2022). However, our findings provide an opportunity for ensuring an equitable approach to wastewater monitoring even with less frequent sampling, including non-sewered wastewater in countries/regions with lower resources.

#### CRediT authorship contribution statement

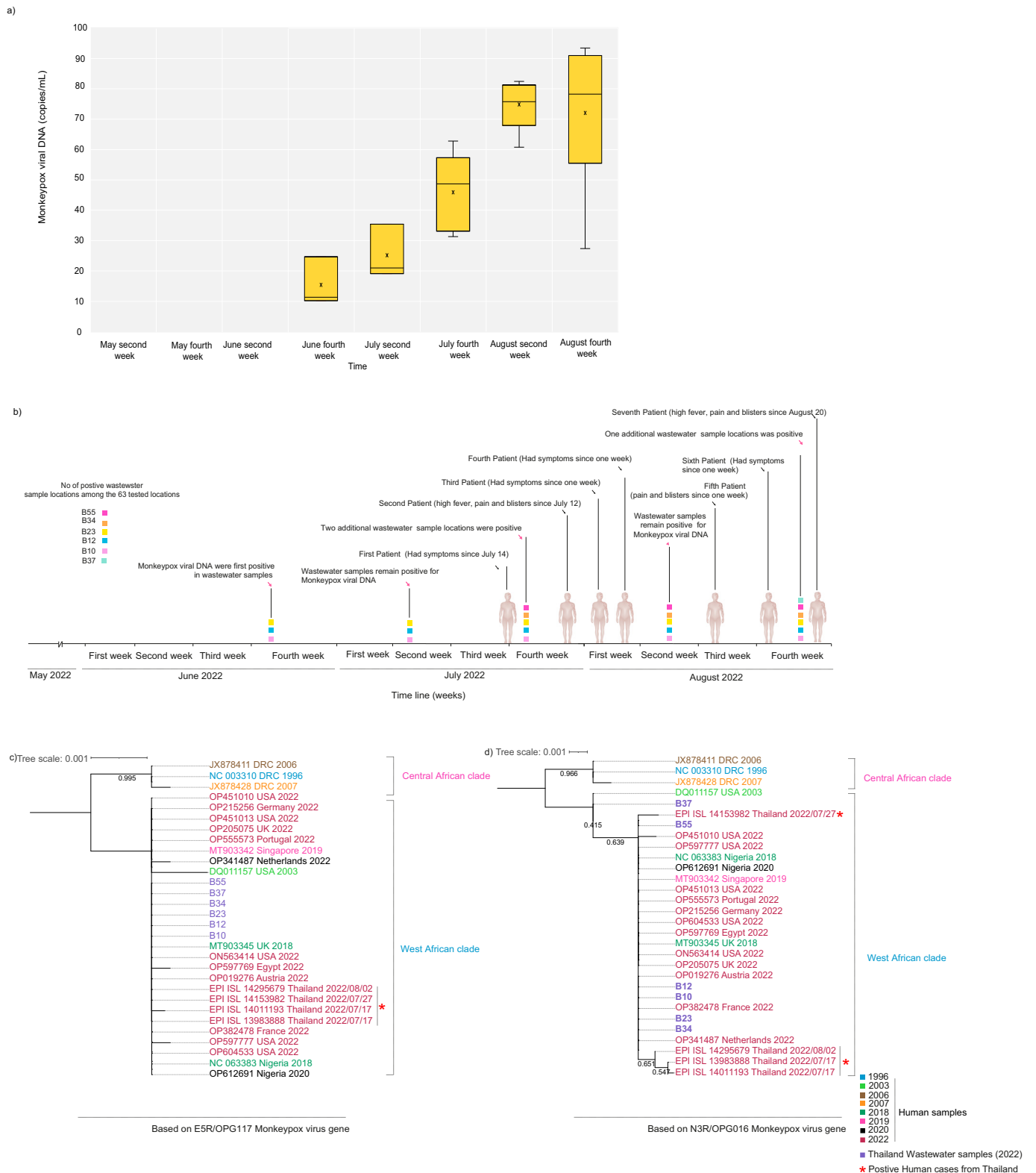
Dharmika Leshan Wannigama conception, funding acquisition, investigation, data curation, formal analysis, supervision, writing the original draft of the manuscript.

Mohan Amarasiri conception, investigation, data curation, formal analysis, supervision, writing the original draft of the manuscript.

Parichart Hongsing conception, funding acquisition, investigation, data curation, formal analysis, supervision, writing the original draft of the manuscript.

Cameron Hurst conception, funding acquisition, investigation, data curation, formal analysis, supervision, writing the original draft of the manuscript.

Charin Modchang data curation, formal analysis, supervision, writing the original draft of the manuscript.



**Fig. 1.** a) Viral loads of monkeypox DNA copies in wastewater at six positive locations among the 63 tested locations in Bangkok from June to August 2022. The lower and upper boundaries of the box (interquartile) represent the 25th and the 75th percentile, respectively. The line within the box corresponds to the median and the cross to the mean of the distribution, while the whiskers indicate the highest and the lowest monkeypox DNA copy values. b) Timeline of the monkeypox wastewater viral DNA detection and monkeypox-positive patients reported. Evolutionary history was inferred using Maximum Likelihood method and Hasegawa-Kishino-Yano model for E5R/OPG117 (c) and Tamura 3-parameter model for N3R/OPG016 (d). The trees with highest log likelihood (−6078.89 and −1778.21) are shown.

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## Ethical approval

Ethics approval was not required for this type of environmental wastewater surveillance study.

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## Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its additional information

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.159816>.

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