Wastewater analysis of Mpox virus in a city with low prevalence of Mpox disease: an environmental surveillance study

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Summary

Background Tracking infectious diseases at the community level is challenging due to asymptomatic infections and the logistical complexities of mass surveillance. Wastewater surveillance has emerged as a valuable tool for monitoring infectious disease agents including SARS-CoV-2 and Mpox virus. However, detecting the Mpox virus in wastewater is particularly challenging due to its relatively low prevalence in the community. In this study, we aim to characterize three molecular assays for detecting and tracking the Mpox virus in wastewater from El Paso, Texas, during February and March 2023.

Methods In this study, a combined approach utilizing three real-time PCR assays targeting the C22L, F3L, and F8L genes and sequencing was employed to detect and track the Mpox virus in wastewater samples. The samples were collected from four sewersheds in the City of El Paso, Texas, during February and March 2023. Wastewater data was compared with reported clinical case data in the city.

Findings Mpox virus DNA was detected in wastewater from all the four sewersheds, whereas only one Mpox case was reported during the sampling period. Positive signals were still observed in multiple sewersheds after the Mpox case was identified. Higher viral concentrations were found in the pellet than in the supernatant of wastewater. Notably, an increasing trend in viral concentration was observed approximately 1–2 weeks before the reporting of the Mpox case. Further sequencing and epidemiological analysis provided supporting evidence for unreported Mpox infections in the city.

Interpretation Our analysis suggests that the Mpox cases in the community is underestimated. The findings emphasize the value of wastewater surveillance as a public health tool for monitoring infectious diseases even in low-prevalence areas, and the need for heightened vigilance to mitigate the spread of Mpox disease for safeguarding global health.

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Research in context

Evidence before this study

The Mpox outbreak in 2022 was a public health emergency of international concern, with over 86,000 confirms cases reported across 110 countries. The reported number of confirmed cases may not fully capture the actual number of infections, indicating a potential underestimation of the outbreak's true extent. Wastewater surveillance has emerged as a complementary public health tool for monitoring infectious disease agents and has the potential to provide an early warning of viral transmission in the community. We conducted a comprehensive search on PubMed, up until June 22, 2023, using the search terms: "wastewater" AND "monkeypox" OR "mpox". Our search yielded a total of 18–20 published papers, including 8 research articles. Upon reviewing these studies, we found reports of Mpox virus detection in wastewater samples from various countries, including the United States, Poland, Spain, Netherlands, Italy, France, and Thailand. Detection of Mpox virus in wastewater is challenging due to its relatively low prevalence in the community, uncertain viral shedding rate from infected individuals into wastewater, and potential inhibitors in the sample, a common issue in pathogen detection in wastewater-based epidemiology. These factors can lead to false-negative detections and underestimation of the infection burden.

Added value of this study

This study utilized a combined approach with multiple molecular assays and demonstrated enhanced detection accuracy of the Mpox virus in wastewater samples from a border population with low disease prevalence. To our knowledge, this is the first study reporting the detection of Mpox virus in wastewater in the City of El Paso, TX. Specifically, Mpox virus DNA was found in wastewater samples from all four communities in the city, despite only one reported case during the sampling period. Even after the case was identified, positive signals were still present in multiple communities. Notably, viral concentration showed a progressive increase 1–2 weeks prior to the reported case. Further sequencing and epidemiological analysis supported the presence of unreported Mpox infections in the city.

Implications of all the available evidence

This study highlights the value of wastewater monitoring as a complementary tool in infectious disease surveillance. Detecting unreported Mpox virus infections through wastewater analysis is crucial for understanding the true disease extent and emphasizes the need for improved surveillance and control measures, particularly in low-prevalence areas. It underscores the importance of maintaining vigilance and proactive public health response to mitigate the spread of Mpox disease in the community.

Introduction

The Mpox (formerly referred to as monkeypox) outbreak in 2022 was a significant public health event. The World Health Organization declared the rapid spread of Mpox as a public health emergency of international concern on July 23, 2022. As of September 2023, there have been more than 3000 cases reported in Texas, and globally 89,000 confirmed cases in 115 countries/regions, of which 108 had no historically reported Mpox infections.[1] On May 11, 2023, the WHO declared the global emergency over after 10 months but emphasized the importance of long-term disease management. The Mpox virus is a linear, double-stranded DNA virus in the genus *Orthopoxvirus*. The Mpox virus (MPXV) was first isolated from a sick monkey in 1959[2] and the first human infection was confirmed in 1970 in a 9-month-old newborn in the Democratic Republic of the Congo.[3] As a zoonotic disease, Mpox can be transmitted through close contact with skin lesions of an infected person, and exposure to blood, body fluids, cutaneous or mucosal lesions from infected animals.[4] It may also be transmitted at close range by the respiratory route.

Wastewater surveillance is a promising approach for monitoring community outbreaks of infectious diseases including the COVID-19 and Mpox. Wastewater collects viral signals excreted by infected individuals irrespective of clinical symptoms or presentation. This inclusiveness enables us to track the epidemic progression and estimate the magnitude of infections in the community served by a wastewater treatment facility (also called as 'sewershed').[5–8] In addition, wastewater surveillance can capture the shedding of viral particles prior to the onset of symptoms and/or outbreak detection by health authorities, providing an early warning of emerging outbreaks in the sewershed.[9–13] For example, Wolfe et al. reported MPXV DNA detection in multiple wastewater sampling sites in California and some sites detected Mpox before identification of cases.[13]

The Centers for Disease Control and Prevention (CDC) recommended two real-time PCR assays for the detection of MPXV (C22L)[14] and non-variola orthopoxvirus (F8L)[15] in June 2022. We previously confirmed that the sequence of primers and probes in the two assays matches >99.4% and >99.1% of global Mpox genomes, respectively.[16] Two nucleotide mismatches were also found in the primers of C22L assay, and mismatch-corrected primers (C22L_m) showed an improved detection sensitivity with the 100% limit of detection of

2.7 copies of DNA per reaction. We also identified that an additional assay targeting MPXV F3L gene[17] has a high homology to >99.7% of Mpox genomes. In this study, we aim to characterize three molecular assays for detecting and tracking the Mpox virus in wastewater from El Paso, Texas, during February and March 2023.

Methods

Study design

We conducted an environmental surveillance study using wastewater samples collected from the City of El Paso, TX. Our aim was to examine the presence of Mpox virus DNA in the wastewater and compare with clinically reported case data. This study was reported according to the "Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)" guidelines[18] (Table S1).

Wastewater sample collection and processing

Weekly, 24-h composite, raw influent wastewater samples were collected from the City of El Paso, Texas from February 20 to March 27, 2023. There are four wastewater treatment facilities, Fred Hervey (FH), Haskell R. Street (HS), John T. Hickerson (JT), and Roberto Bustamante (RB), collectively serving a total of 751,982 individuals in the city (Table S2). Each facility serves a specific city region, separated by the natural barrier of the Franklin Mountains.[19] Samples were collected by El Paso Water utility and shipped overnight on ice in a secondary container to the laboratory at Houston for analysis. The data of total wastewater flow volume over the last 24 h at each sampling site and sampling date were provided in the Table S3. Samples were processed on the day of receipt. A total of 24 raw wastewater samples were collected. Each sample was separated into pellet and supernatant portions, as viral signals have been found in both fractions.[6,13,20,21] Specifically, 2 mL of raw wastewater was aliquoted and centrifuged to collect the pellet for DNA extraction. For supernatants, 40 mL raw wastewater samples were vacuum filtered through a 0.22 μm polyether sulfone membrane to remove cell debris and solid materials. Supernatants were used to concentrate viral particles as described below.

Viral concentration and DNA extraction

Viral enrichment was performed based on previous methods.[9,22] Briefly, 15 mL of filtrates were concentrated with 30 kDa Amicon Ultra Centrifugal Filter (Sigma, Cat#: UFC9010) by centrifugation (3900 rpm for 20 min) to 150–200 μl subsequently used for DNA extraction using QIAamp DNA Mini Kit (Qiagen, Cat#: 51306). Following the manufacture's protocol, the concentrates were resuspended with 200 μl lysis buffer. For pellet, lysis buffer was directly added into the pellet for DNA extraction. Proteinase K was added into the sample to facilitate the lysis followed by 2 h incubation at 56 °C. DNA extraction was performed based on the standard procedures provided by the kit and 100 μl nuclease-free H_2O was used to elute DNA extracted from pellets and supernatants. Both pellet and supernatant DNA were individually tested for MPXV using real-time quantitative PCR (Bio-Rad, CFX Opus 96, USA).

Real-time quantitative PCR (RT-qPCR)

The eluted DNA was used to test MPXV by probe-based RT-qPCR. Briefly, PCR Multiplex Supermix (Bio-Rad, Cat #: 12010220) was mixed with the primers, probe, and nuclease-free H_2O (VWR, Cat #: 10220-402) and then added to respective wells of a 96-well PCR plate (Bio-Rad, Cat #: HSL9605). The final concentration for the primers and probe is 750 nM and 375 nM, respectively. The reaction was run with the following program: 95 °C for 3 min for denaturation, followed by 48 cycles of denature (95 °C 3 s) and anneal/extend (60 °C 30 s). All primers, probes, and synthetic gene fragments for MPXV, cowpox, and horsepox viruses were synthesized from Integrated DNA Technologies and their sequence was provided in Table S4. Detailed procedures for the RT-qPCR can be found in our previous work.[16] The standard curves for F3L and F8L using different DNA templates were generated by serial ten-fold dilutions of the synthesized gene fragment from 3.64 to 3.64e6 copies/μl. Each concentration has 6 technical replicates, and the mean value with standard deviation is used for plotting. Two-fold serial dilutions were further used to determine the limit of determination (LOD) for both assays with different DNA templates. We conducted 16~22 technical replicates for concentrations near the limit of detection. The cycle threshold (*Ct*) value was exported by the built-in software with manual confirmation of a sigmoidal amplification curve.

For wastewater samples, we used the F3L, F8L, and C22L_m assays to test MPXV in the supernatant and pellet of each sample. Three technical PCR replicates were performed for each assay. Negative controls (i.e., adding the same volume of nuclease-free H2O with no DNA template) were included for each running with a total of 16~24 replicates. To minimize the potential cross-contamination, we added the DNA for positive controls after all the other samples were prepared and before the sealing of the 96-well plate, followed by centrifugation at 1000 rpm for 1 min before the real-time PCR running. For all the real-time PCR results, *C*t value above 42 was considered negative.[23,24]

RT-qPCR inhibition measurements were conducted on 12 pellet DNA and 8 supernatant DNA extracted from wastewater samples collected on February 20, March 6, and March 27, 2023. To minimize the impact of endogenous Mpox viral DNA, 2 μl ~10^6 copies/μl of a synthetic DNA fragment (MPXV-F3L) were spiked into 18 μl of each DNA sample or H2O control, followed by RT-qPCR using F3L assay. The inhibition levels were calculated using the formula: Inhibition level = $(1-10^{\Delta Ct/m})\times 100\%$, where ΔCt represents the difference

between the Ct values of the spiked sample DNA and spiked H2O control, and *m* is the slope of the standard curve for F3L.[25] Two technical replicates were performed for each sample and the mean value were used for this analysis. Negative inhibition level was considered as no inhibition.

Sanger sequencing was performed to verify the amplified sequence. Specifically, we used the forward and reverse primers in the C22L_m assay (Table S4) to amplify wastewater samples' DNA using Ultra II Q5 Master Mix (New England BioLabs, Cat#: M0544L). The size of C22L_m amplicon is 90 bp. PCR products were then sent to Eton Bioscience Inc. for Sanger sequencing using the C22L_m forward or reverse primer.

Wastewater data normalization and visualization

Viral concentrations (genome copies per µl of RNA) were derived from *Ct* values in real-time PCR using the established standard curves for each assay (Table S5). The concentration was then converted to genome copies per microliter of wastewater by multiplying the dilution factor, i.e., volume of eluted RNA/volume of starting wastewater material. To account for variations in wastewater flow, we used viral load per 100,000 inhabitants as an indicator of viral infection trends.[26,27] Specifically, we multiplied viral concentrations by the total wastewater volume collected over the past 24 h at the sampling site (Table S2) and divided by the served population size (Table S1), which was adjusted to a standard of 100,000.

Sequence alignment and mismatch analysis

Complete genome sequences were downloaded from National Center for Biotechnology Information (NCBI) as fasta files, including 3282 genomes for human Mpox viruses, 98 Cowpox viruses, 4 Horsepox viruses, 58 Smallpox viruses, 112 Vaccinia viruses, and 6 Buffalopox viruses released in the NCBI database as of February 13, 2023. The sequence was imported into R (version 4.1.3) and combined as the genome database for analysis. We aligned each of the oligos (including forward primer, reverse primer, and probe) sequences and their reverse complements to the database and computed the percentage of genomes where the oligo sequences were a 100% match. For non-matching oligos, sequences were extracted and aligned using SnapGene (https://www.snapgene.com/) to identify the specific nucleotides that differ between the oligos and the genomic data. The major types of sequence variation(s) are listed in Fig. 1 and Table S6. These analyses were performed with customized R scripts.

Clinical case data

The reported Mpox case data were obtained from the Department of Public Health in the City of El Paso. Details including the date of reporting, test results, and the geographical locations of cases were provided in Table S7. Individuals displaying signs or symptoms were assessed by healthcare providers. If Mpox was suspected, real-time PCR testing was conducted. Upon confirmation, these individuals were advised to self-isolate for two to four weeks or until all symptoms, including complete rash healing, had resolved.

Role of the funding source

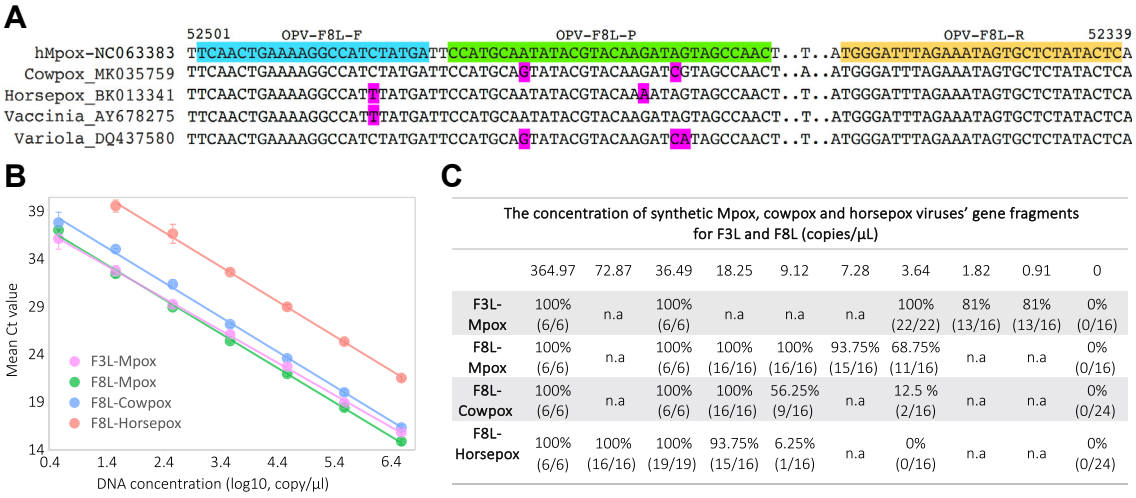
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

We first evaluated the analytical sensitivity and limit of detection of F3L and F8L assays using real-time quantitative PCR (RT-qPCR) with synthetic gene fragments covering corresponding genomic regions of MPXV and other *orthopoxvirus* viruses (Fig. 1). By testing the F3L assay using serial tenfold dilutions of MPXV DNA, we found that F3L detected MPXV DNA across 7-log concentrations from 3.64 to $3.64*10^6$ copies/µl with an amplification efficiency of 97.62% and $R^2 > 0.999$ (Fig. 1B and Table S5). The limit of detection with 22 replicates shows that the F3L assay is 100% sensitive to MPXV detection at 3.64 copies/µl (7.28 copies/reaction, Fig. 1C). Sixteen replicates of controls with no DNA template are negative.

The F8L assay was designed for broad detection of viruses in the genus *orthopoxvirus*; however, the homology between the sequence of primers/probe and species in the genus varies (Fig. 1A). We therefore aligned the sequence of primers and probe of the F8L assay against 6 *orthopoxvirus* species including 3282 genomes for human Mpox viruses, 98 cowpox viruses, 4 horsepox viruses, 58 smallpox viruses, 112 vaccinia viruses, and 6 buffalopox viruses released in the NCBI database as of February 13, 2023. Overall, the F8L primers and probe have the highest full match to MPXV genomes (99.8%) and have 1∼3 nucleotide mismatches to most of the genomes (52%∼100%) in other species (Table S6), suggesting that the F8L assay may have varied detection sensitivity to different *orthopoxvirus* species.

To determine how these sequence variations impact detection of orthopoxvirus, we synthesized MPXV, cowpox, and horsepox virus gene fragments covering the F8L region. Standard curves from serial 7-log dilutions show similar amplification efficiencies using MPXV (89.49%), cowpox (88.40%), and horsepox DNA (88.39%) with $R^2 > 0.998$ (Fig. 1B and Table S5). However, the analytical sensitivity ranges between 1.8 and 7.1 *Ct* among the three viral genome fragments. The 100% of detection of the three viral DNA sequences varies from 9.1 to 36.44 copies/µl (Fig. 1C). In total, 24 no-template controls for the F8L assay are negative. These results showed that the F8L assay is most



C

The concentration of synthetic Mpox, cowpox and horsepox viruses' gene fragments for F3L and F8L (copies/µL)

	364.97	72.87	36.49	18.25	9.12	7.28	3.64	1.82	0.91	0
F3L-Mpox	100% (6/6)	n.a	100% (6/6)	n.a	n.a	n.a	100% (22/22)	81% (13/16)	81% (13/16)	0% (0/16)
F8L-Mpox	100% (6/6)	n.a	100% (6/6)	100% (16/16)	100% (16/16)	93.75% (15/16)	68.75% (11/16)	n.a	n.a	0% (0/16)
F8L-Cowpox	100% (6/6)	n.a	100% (6/6)	100% (16/16)	56.25% (9/16)	n.a	12.5 % (2/16)	n.a	n.a	0% (0/24)
F8L-Horsepox	100% (6/6)	100% (16/16)	100% (19/19)	93.75% (15/16)	6.25% (1/16)	n.a	0% (0/16)	n.a	n.a	0% (0/24)

Fig. 1: **Specificity, sensitivity, and limit of detection for the F3L and F8L assays.** (**A**) Alignment of F8L forward primer (highlighted cyan), probe (green), and reverse primer (yellow) against MPXV, cowpox viruses, horsepox, vaccinia virus, and smallpox (variola) virus genomes. Mismatched nucleotides were highlighted in red. (**B**) Standard curves for the F3L and F8L assays for Mpox, cowpox, and horsepox virus DNA detection. The data shown represent the mean of 6 replicates with standard deviations. Trendline equations and coefficients of determination are provided in Table S5. (**C**) Limits of detection of the F3L and F8L assays for Mpox, cowpox, and horsepox DNA fragments. n.a represents 'not tested'. The number of replicates is shown in parentheses.

sensitive in detecting this MPXV genome fragment compared to cowpox and horsepox viruses.

Next, we tested for MPXV in both the supernatant (filtrate) and pellet of wastewater samples collected from February 20 to March 27, 2023, using the F3L, F8L, and C22L_m assays. Positive MPXV signals were observed in the supernatant or pellet or both, with higher viral concentrations typically in the pellet than the supernatant (Fig. S1). Interestingly, higher levels of PCR inhibition were observed in the pellet samples compared to the supernatant (Fig. S3). Overall, the F3L assay detected MPXV in 17/24 (70.8%) samples, C22L_m detected the virus in 14/24 (58.3%) samples, and F8L in 3/24 (12.5%) samples (Fig. 2A). Eleven of 24 (52.4%) samples were detected by more than one assay, and three samples were not detected by any of the three assays. The HS sewershed had the highest F3L assay positivity frequency. Aggregating the viral concentrations by sampling date, viral concentrations in the pellet were higher than in the supernatant (Fig. 2B and Fig. S1). Increased viral concentrations in wastewater were observed from February 27, with a peak in the week of March 6. Accounting for wastewater flow fluctuations, we computed the viral load per 100,000 inhabitants (Fig. S4), which showed a similar trend to the viral concentration data. Additionally, Sanger sequencing of PCR products using a FH pellet sample on March 13 further confirmed a 94% identity match to the MPXV reference genome (Fig. S2).

Clinically, five Mpox cases have been reported in the City of El Paso since August 2022, with the latest case identified on March 11 during the study period, marking a five-month gap since the prior case (Fig. 2C). In contrast, wastewater sampling revealed Mpox virus DNA in all four sewersheds throughout the city (Fig. S1), both before and after this most recent clinical case. This discrepancy between clinical and wastewater data indicates the possibility of unreported Mpox cases in the city. Furthermore, a surge in wastewater viral concentrations was noted 1–2 weeks before the latest clinical report, reinforming wastewater surveillance as a viable early-warning system for new infections, in line with recent study in California.[13]

Discussion

Wastewater surveillance is being widely implemented as a complementary public health tool to detect infectious pathogens including SARS-CoV-2 and Mpox virus.[13,28–30] Detecting Mpox in wastewater presents greater challenges than SARS-CoV-2 due to its lower community prevalence, resulting in lower viral concentrations. This demands both highly efficient virus concentration techniques and enhanced sensitivity in molecular assays for accurate detection. The magnitude of viral shedding into wastewater per infected individual is also unclear. In addition to skin lesions, Mpox virus DNA has been found in various bodily fluids, including feces, urine, saliva, anorectal swabs, semen, and blood of infected patients.[4,31,32] However, the contribution of Mpox virus DNA from these different sources to wastewater is unclear. Further investigations are necessary to quantitatively assess the role of these sources in shaping the wastewater data.

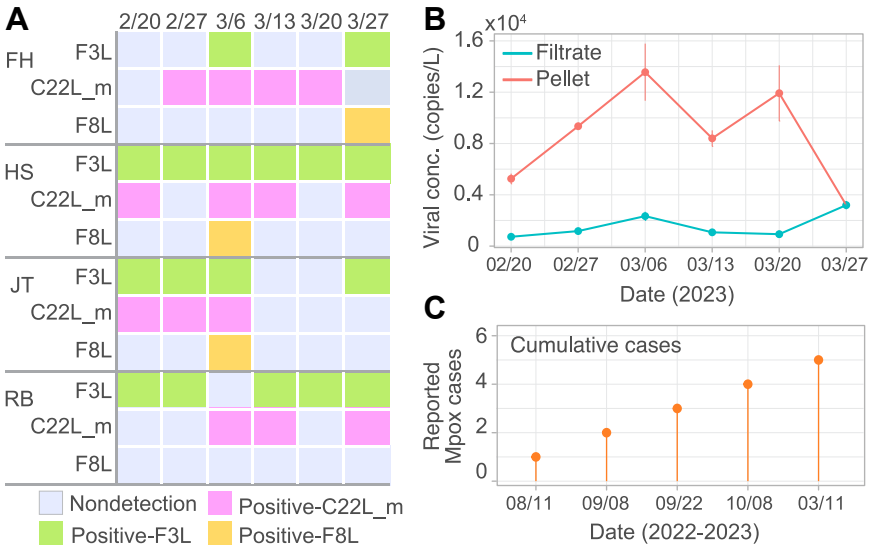


***Fig. 2:* MPXV detection in wastewater using the F3L, F8L, and C22L_m assays.** (**A**) Detection results using the F3L, F8L, and mismatch-corrected CDC (C22L_m) assays for weekly wastewater samples collected between February 20 and March 27, 2023. (**B**) Mean viral concentrations based on the three targets (F3L, F8L, C22L) in wastewater supernatants and pellets. Data are expressed as the mean of four sewersheds and the three assays. Error bars represent the standard error of the mean. (**C**) The total Mpox cases reported in the El Paso City. The most recent case was reported on March 11, 2023.

Moreover, the presence of inhibitors in wastewater samples poses an additional challenge for molecular detection by real-time PCR. Our data indicate that the pellet samples exhibit higher inhibition than supernatant, ranging from 0 to 18% (Fig. S3). Although this study is not focused on quantifying specific inhibitors, it's important to note that such inhibitors can interfere with the accuracy and sensitivity of the assays, potentially leading to false-negative results. To address this challenge, alternative approach such as droplet digital PCR, which is less susceptible to inhibitors, has shown improved detection sensitivity.[13,33,34] However, its high cost restricts its widespread application in wastewater surveillance. Using multiple MPXV detection assays is another approach to address this challenge. Taking the C22L_m assay in HS sewershed as an example, we did not detect MPXV DNA in either the supernatant or pellet for samples collected on February 27 and March 20; however, other samples collected during this time frame were positive. There are many potential reasons for the negative tests including low viral DNA concentrations and PCR inhibitors present in wastewater. As such, results using the F3L and F8L assays were crucial for cross-validation. On the other hand, characterization of these assays helps understand their specificity and detection limit given the thermodynamic variations among primers/probe[16] and evolution of the Mpox virus.[35] F8L, recommended by CDC, was designed for detecting viruses in *orthopoxvirus* with higher sensitivity for MPXV (Fig. 1). Results from F3L and C22L_m assays and sequencing (Fig. S2) further validated the detection of MPXV in wastewater. Hence, the utilization of multiple assays targeting different genomic sites of the virus, coupled with meticulous assay characterization, can enhance detection accuracy, especially for low-abundant targets.

A key finding in this work is the discrepancy between clinical and wastewater data–Mpox virus DNA was detected across multiple city regions for six weeks, yet only one clinical case was reported in this period. Based on the policy implemented in El Paso, confirmed cases with Mpox needs to be quarantined for 2~3 weeks or until symptoms resolve.[36] While the recorded case's mobility data is unknown, the likelihood of this individual moving citywide and shedding viral materials into wastewater for six weeks is low. Mpox virus can remain viable for over two weeks in the environment,[37,38] however, this duration doesn't sufficiently explain the repeated positive detections across multiple geographic locations in the city. Additional evidence supporting the unreported cases include: a) positive signals were found on February 20, approximately three weeks before the latest case was reported which exceeds the mean viral incubation period of 5.6–9.1 days[39,40] and b) the penultimate case was reported on October 8, 2022. Persistent viral shedding in patients has been reported for up to 39 days in the literature,[41,42] but has never been reported for over 4 months, suggesting that the signals observed in wastewater on February 20, 2023 are likely not due to shedding from the case on October 8. In sum, the data and analyses suggest the presence of unreported Mpox cases in the city.

As of September 2023, there are over 89,000 Mpox cases reported globally, but the percentage of asymptomatic and unreported MPXV infections remains unknown. Two recent studies found 75%[43] and 5%[44] of asymptomatic infections among 4 and 284 Mpox cases by screening 224 and 583 individuals, respectively. Although the range is large, both studies showed that the current number of Mpox cases is underestimated, which is further supported by our wastewater results reported here. The next important question that arises is how to effectively identify the specific locations of these non-reported infections within the city. To address this, one potential approach is to strategically sample wastewater from upstream communities, such as neighborhoods, housing estates, and even individual buildings.[45–47] Analyzing these upstream wastewater samples can aid in pinpointing the locations with non-reported infections within the city, and facilitate targeted public health interventions.

In summary, we detected MPXV using three distinct molecular assays in wastewater samples collected from El Paso, Texas. Notably, we observed a progressive rise in viral concentrations in the wastewater approximately 1–2 weeks prior to the reporting of a new clinical case. The presence of viral signals in multiple sewersheds both before and after the identification of the sole clinical case strongly suggests the existence of unreported Mpox virus infections within the city during the sampling period. By emphasizing the necessity of utilizing a combined approach with multiple molecular assays, this study contributes to enhancing the detection of Mpox virus DNA in wastewater in a region with low disease prevalence, and helps guide effective prevention and control measures in similar low-prevalence areas worldwide.

Contributors

Conceptualization: FW, KDM; Data curation, analysis, and validation: JO, FW; Methodology: FW; Investigation and visualization: FW, JO; Supervision: FW, KDM; Funding acquisition: JR, EB, KDM, FW; Discussion: JO, CC, SRV, AG, AAO, CM, CXB, ELB, SJC, JD, BMH, MT, HIO, JB, AWM, JR, EB, KDM, FW; Writing—original draft: FW, JO; Writing—review & editing: JO, CC, SRV, AG, AAO, CM, CXB, ELB, SJC, JD, BMH, MT, HIO, JB, AWM, JR, EB, KDM, FW.

Data sharing statement

Data and code scripts for this study are available upon reasonable request to the corresponding author (F.W.).

Ethical approval statement

This article does not contain any studies involving animals or humans performed by any authors. The de-identified Mpox case data were provided by the Department of Public Health in the City of El Paso.

Declaration of interests

The authors declare no competing interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lana.2023.100639.

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