Monkeypox Virus in Wastewater Samples from Santiago Metropolitan Region, Chile

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Sewage surveillance provides useful epidemiologic and public health information on viral infections at the population level. We detected monkeypox virus DNA from sewage samples covering 85% of the population in Santiago Metropolitan Region, Chile. We also isolated infective viruses from those samples. Wastewater surveillance could complement clinical surveillance for monkeypox virus.

On May 13, 2022, the World Health Organization raised an alert caused by a large increase in the number of infections by monkeypox virus (MPXV), which causes mpox, a zoonotic disease endemic to some countries of Central and West Africa that has rapidly expanded to nonendemic countries (*1*). A case of MPXV infection in Chile was confirmed on July 17, and since then, >1,400 cases and 2 deaths related to mpox have been reported during the outbreak, according to the Chile Ministry of Health (*2*). The Santiago Metropolitan Region in Chile is the most populated region in the country, accounting for >40% of the total population and most (81%) of the reported MPXV infections (*2*).

Wastewater surveillance has been demonstrated as a key contributor in monitoring viruses, such as poliovirus and SARS-CoV-2, enabling tracking of new variants and, thus, providing an accurate view of infections at the population level (*3*–*5*). In this regard, MPXV detection in sewage samples has also been proposed as a useful complement to clinical surveillance (*6*–*9*). Because stigma and discrimination associated with certain infections limit the willingness of at-risk persons to consult hospital centers, wastewater-based epidemiology (WBE) becomes even more useful because anonymous pooled samples enable visualization of the contributions of a community without revealing individual identities (*10*).

We report wastewater monitoring of MPXV DNA in sewage samples from 3 wastewater treatment plants (WWTPs), accounting for 85% of the overall sewage from Santiago Metropolitan Region, representative of ≥5.5 million persons. We also report the presence of infective MPXV in those samples.

The Study

We collected 21 raw samples of wastewater during April–September 2022 from the WWTPs El Trebal (n = 6), La Farfana (n = 6), and La Higuera (n = 9). We collected samples in 1,000-mL sterile propylene flasks, transported them to the Laboratory of Environmental Virology at Universidad de Chile Faculty of Medicine, and stored them at 4°C until processing.

We concentrated the samples by ultracentrifugation according to the protocol described by Fumian et al. (*11*). We resuspended the pellet obtained in 200 µL of phosphate-buffered saline and stored at −80°C until use.

We used 200 µL of concentrated viral particles to isolate DNA with the QIAamp DNA MiniKit (QIAGEN, https://www.qiagen.com), according to the instructions provided by the supplier. We mixed 5 µL of DNA with the TaqMan Microbe Detection Monkeypox Vi07922155_s1 (ThermoFisher Scientific, https://www.thermofisher.com) and the TaqPath 1-Step Multiplex Master Mix (ThermoFisher Scientific) for the specific detection of MPXV DNA in a QuantStudio 5 real-time PCR machine (ThermoFisher Scientific). A pMG-Amp plasmid carrying the synthetic MPXV

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Table. Monkeypox virus detection in wastewater samples from 3 areas, Santiago Metropolitan Region, Chile, 2022*

Date	La Farfana	El Trebal	La Higuera
Apr 11	ND	ND	–
Apr 19	–	–	ND
Apr 22	ND	ND	–
May 5	ND	ND	–
May 16	–	–	ND
May 19	ND	ND	–
Jun 6	–	–	ND
Jun 16	ND	ND	–
Jul 18	36	–	ND
Jul 22	ND	ND	–
Aug 12	ND	ND	–
Aug 16	35	171	ND
Aug 18	ND	ND	–
Sep 15	ND	ND	895
Sep 20	2,231	960	ND

*Values are genome copies/mL. ND, not done; –, negative.

DNA fragment 5′-GTGTCTGAATCGTTCGATTA-ACCCAACTCATCCATTTTCAGATGAATAGAGT-TATCGATTCAGACACATGCTTTGAGTTTT-GTTGAATCGATGAGTGAAGTATCATCGGTTG-CACCTTCAGATGC-3′, which contains the target sequence of the primers, was synthesized at Macrogen Inc. (https://www.macrogen.com). We used that plasmid as a positive control and as a template for the calibration curve enabling the quantification of MPXV genome copies per milliliter. We cloned the amplified DNA fragment into pGEM-T Easy Vector (Promega, https://www.promega.com) and transformed it into *Escherichia coli* JM109. Five clones from each sample were sequenced at Macrogen Inc. and compared with MPXV sequences from the 2022 outbreak.

From the 21 sewage samples collected and analyzed from the 3 WWTPs, we detected MPXV DNA in 6 (Table). Consistent with earlier cases of mpox reported in Chile, viral DNA was detected in sewage samples collected in July (La Farfana), August (La Farfana, El Trebal) and September (La Farfana, El Trebal, La Higuera), but not in April or May (Table).

Quantification of MPXV DNA in sewage showed viral loads ranging from 35 to 2,231 genome copies/mL (Table). Higher viral loads in sewage samples correlated with an increase in the number of cases reported by the Chile Ministry of Health in Santiago. Sequencing of the 106-bp amplified DNA fragment from wastewater samples showed 100% homology with MPXV sequences from the 2022 outbreak reported from Germany, the Netherlands, Italy, France, the United Kingdom, the United States, and Chile (Figure 1).

To determine whether the sewage samples contained viable MPXV, we used the samples that had the highest viral load to inoculate VeroE6 monolayers (ATCC CRL-1586). For this procedure, we infected cells with a mixture of MPXV DNA–positive sewage samples and culture medium and collected the supernatant after 7 days for PCR detection. We stored the remaining supernatant and performed a second round of infection by using the supernatant from the first infection. We used positive and negative controls in separate plates to avoid cross-contamination. At 24- and 48-hours postinfection, we collected supernatant for MPXVDNA detection by PCR.

In addition, we inoculated 300 µL of sample AF0922 supplemented with 700 µL of Dulbecco's Modified Eagle Medium plus 2% fetal bovine serum (FBS) into VeroE6 cells in a 6-cm plate. After 2 hours of incubation, we replaced the medium with 5 mL of Dulbecco's Modified Eagle Medium plus 2% FBS. After 7 days, we fixed infected cells with 4% glutaraldehyde and performed negative staining for electron microscopy observation (Appendix Figure, https://wwwnc.cdc.gov/EID/article/29/11/23-0096-App1.pdf) All procedures related to viral isolation were performed in a Biosafety Level 3 laboratory at Unidad de Virología Aplicada, Pontificia Universidad Católica de Chile, Santiago.

We detected a high viral DNA load in the supernatant at day 7 postinoculation, suggesting the presence of infective MPXV in sewage samples (Figure 2, panel A). We were not able to detect MPXV DNA from cells inoculated with samples that tested negative for the virus (Figure 2, panel B). Electron

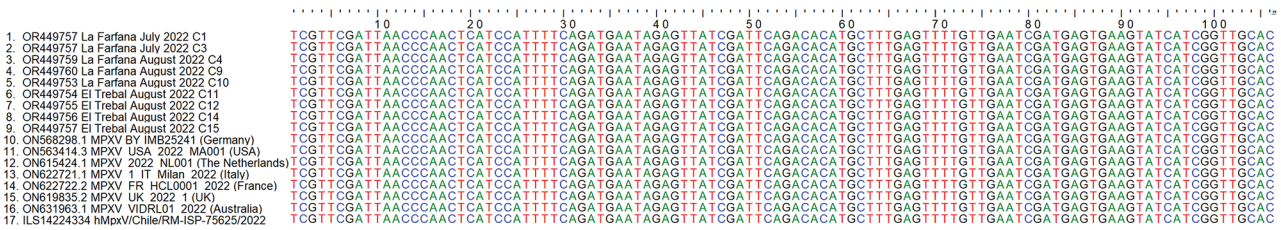

Figure 1. Comparison of nucleotide sequences of the MPXV amplicon obtained for wastewater samples from Santiago Metropolitan Region, Chile (sewage samples 1–9), with reference sequences obtained from other countries during the 2022 mpox outbreak. The 106-bp amplicon generated by quantitative reverse transcription has 100% homology with MPXV sequences obtained in 2022 from cases reported by different countries. GenBank numbers and location and date of isolation are provided for the 9 Chile sample sequences obtained in this study; GenBank or GISAID (https://www.gisaid.org) accession numbers and country are provided for reference sequences. MPXV, monkeypox virus.

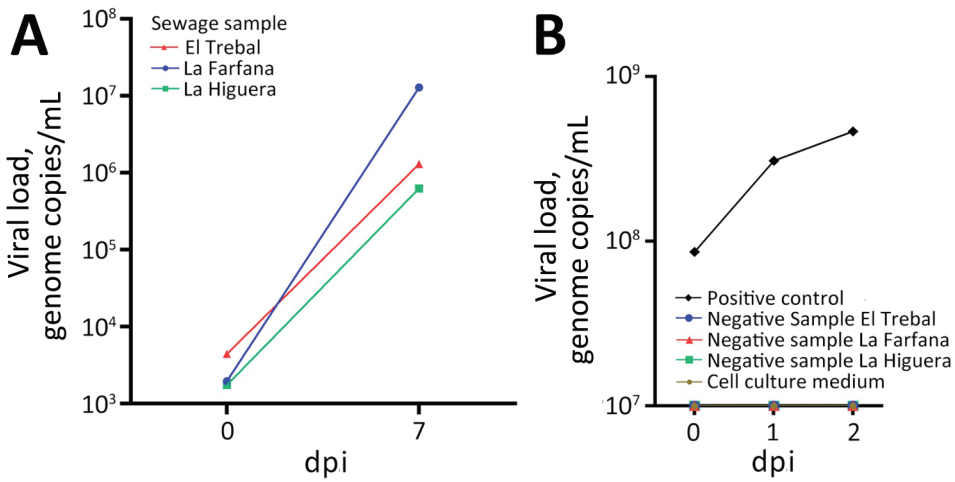

Figure 2. Detection of viral genome of monkeypox virus in wastewater samples from wastewater treatment plants in Santiago Metropolitan Region, Chile. A) PCR results for Vero E6 cell supernatant at 7 dpi. B) PCR results for supernatant samples of Vero E6 cells infected with positive control (cell culture supernatant infected with monkeypox virus) and negative controls (Dulbecco's Modified Eagle Medium plus 2% fetal bovine serum and negative wastewater samples). dpi, days postinfection.

microscopy analyses of VeroE6 cells inoculated with MPXV recovered from sample AF0922 showed intracellular viral particles with an average size of ≈300 nm (Appendix Figure).

Conclusions

WBE has acquired an increasingly useful role in surveillance systems that efficiently detect pathogenic microorganisms. It will also be useful as a tool for control and timely prevention of endemic and emerging infectious diseases.

Using WBE as a complement to universal clinical surveillance enables determination of actual pathogen circulation and its load in a population. For example, WBE has become a useful tool worldwide for visualizing the circulation of SARS-CoV-2 and its variants (*3*–*5*). Therefore, WBE could also complement clinical surveillance of MPXV, enabling estimation of actual circulation and load of the virus in a community (*6*,*9*). However, it will be useful to generate more information regarding virus elimination in an infected person; viral DNA load in stool, urine, semen, saliva, and other secretions; and persistence and infectivity of the virus in the environment and, in particular, in a matrix as complex as wastewater.

In conclusion, we detected MPXV DNA and determined its concentration in wastewater in Santiago, Chile. We were also able to isolate the virus from samples with the highest viral loads. Although detection of viable virus in sewage samples observed in this study generates an alert, there is no information on the risk that this could have for the personnel working in treatment plants. The potential risk for environmental transmission of MPXV is still unknown and thus remains a serious public health issue.

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A.G. participated in the study design; M.A. and C.M.-V. performed the experiments; A.G., R.S.-R., and M.F. analyzed the data; and A.G., R.S.-R., and C.M.-V. wrote the manuscript. All authors approved the final version of the manuscript.

About the Author

Mr. Ampuero is a research scientist in the Virology Environmental Laboratory, Universidad de Chile, Santiago, Chile. His primary research interests are identification and characterization of viruses in wastewater.

References

1. World Health Organization. Mpox (monkeypox) outbreak: global trends, 2022 [cited 2022 Dec 26]. https://worldhealthorg.shinyapps.io/mpx_global
2. Ministry of Health, Department of Epidemiology, Undersecretary of Public Health. Executive report monkeypox, Chile. December 23, 2022 [in Spanish] [cited 2023 Sep 8]. http://epi.minsal.cl/wp-content/uploads/2022/08/Protocolo_vigilancia_viruela_del_mono_22082022.pdf
3. Mousazadeh M, Ashoori R, Paital B, Kabdaşlı I, Frontistis Z, Hashemi M, et al. Wastewater based epidemiology perspective as a faster protocol for detecting coronavirus RNA in human populations: a review with specific reference

to SARS-CoV-2 virus. Pathogens. 2021;10:1008. https://doi.org/10.3390/pathogens10081008
4. Wu F, Lee WL, Chen H, Gu X, Chandra F, Armas F, et al. Making waves: wastewater surveillance of SARS-CoV-2 in an endemic future. Water Res. 2022;219:118535. https://doi.org/10.1016/j.watres.2022.118535
5. Karthikeyan S, Levy JI, De Hoff P, Humphrey G, Birmingham A, Jepsen K, et al. Wastewater sequencing reveals early cryptic SARS-CoV-2 variant transmission. Nature. 2022;609:101–8. https://doi.org/10.1038/s41586-022-05049-6
6. Chen W, Bibby K. Model-based theoretical evaluation of the feasibility of using wastewater-based epidemiology to monitor monkeypox. Environ Sci Technol Lett. 2022;9:772–8. https://doi.org/10.1021/acs.estlett.2c00496
7. Gul I, Liu C, Yuan X, Du Z, Zhai S, Lei Z, et al. Current and perspective sensing methods for monkeypox virus. Bioengineering (Basel). 2022;9:571. https://doi.org/10.3390/bioengineering9100571
8. de Jonge EF, Peterse CM, Koelewijn JM, van der Drift AR, van der Beek RF, Nagelkerke E, et al. The detection of monkeypox virus DNA in wastewater samples in the Netherlands. Sci Total Environ. 2022;852:158265. https://doi.org/10.1016/j.scitotenv.2022.158265
9. Tiwari A, Adhikari S, Kaya D, Islam MA, Malla B, Sherchan SP, et al. Monkeypox outbreak: Wastewater and environmental surveillance perspective. Sci Total Environ. 2023;856:159166. https://doi.org/10.1016/j.scitotenv.2022.159166
10. Nelson B. What poo tells us: wastewater surveillance comes of age amid covid, monkeypox, and polio. BMJ. 2022;378:o1869. https://doi.org/10.1136/bmj.o1869
11. Fumian TM, Leite JP, Castello AA, Gaggero A, Caillou MS, Miagostovich MP. Detection of rotavirus A in sewage samples using multiplex qPCR and an evaluation of the ultracentrifugation and adsorption-elution methods for virus concentration. J Virol Methods. 2010;170:42–6. https://doi.org/10.1016/j.jviromet.2010.08.017

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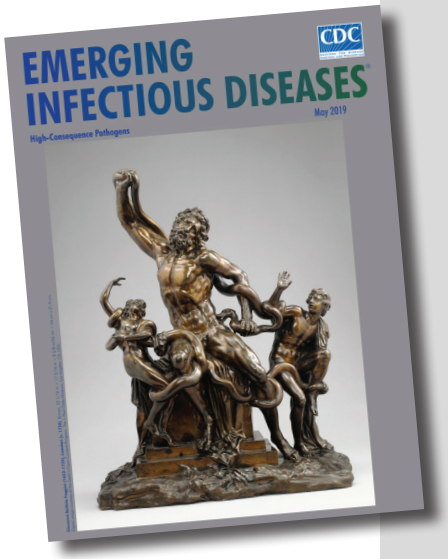
Nipah Virus

[ne´-pə vī´-rəs]



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In 1994, a newly described virus, initially called equine morbillivirus, killed 13 horses and a trainer in Hendra, a suburb of Brisbane, Australia. The reservoir was subsequently identified as flying foxes, bats of the genus *Pteropus* (Greek pteron [“wing”] + *pous* [“foot”]). In 1999, scientists investigated reports of febrile encephalitis and respiratory illness among workers exposed to pigs in Malaysia and Singapore. (The pigs were believed to have consumed partially eaten fruit discarded by bats.)

The causative agent was determined to be closely related to Hendra virus and was later named for the Malaysian village of Kampung Sungai Nipah. The 2 viruses were combined into the genus *Henipavirus*, in the family *Paramyxoviridae*. Three additional species of *Henipavirus*—Cedar virus, Ghanaian bat virus, and Mojiang virus—have since been described, but none is known to cause human disease. Outbreaks of Nipah virus occur almost annually in India and Bangladesh, but *Pteropus* bats can be found throughout the tropics and subtropics, and henipaviruses have been isolated from them in Central and South America, Asia, Oceania, and East Africa.

References:

1. Centers for Disease Control and Prevention. Outbreak of Hendra-like virus—Malaysia and Singapore, 1998–1999. MMWR Morb Mortal Wkly Rep. 1999;48:265–9.
2. Selvey LA, Wells RM, McCormack JG, Ansford AJ, Murray K, Rogers RJ, et al. Infection of humans and horses by a newly described morbillivirus. Med J Aust. 1995;162:642–5.

https://wwwnc.cdc.gov/eid/article/25/5/et-2505_article

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Appendix

Methods

Wastewater Treatment Procedure

Samples were concentrated by ultracentrifugation according to the protocol described by Fumian et al. (*1*). Briefly, 42 mL of each sample previously homogenized were ultracentrifuged at 100,000 × *g* for 1 hour at 4°C. The supernatant was discarded, and the pellet was resuspended in 3.5 mL of 0.25N glycine buffer, pH 9.5, on ice for 30 min. The solution was neutralized with 3.5 mL of 2× PBS, pH 7.2, and clarified by centrifugation at 12,000 × *g* for 15 min at 4°C. The supernatant enriched in viral particles was further concentrated by ultracentrifugation at 100,000 × *g*, and the pellet was resuspended in 200 µL of 1× PBS and stored at −80°C until use.

Infectivity Assay

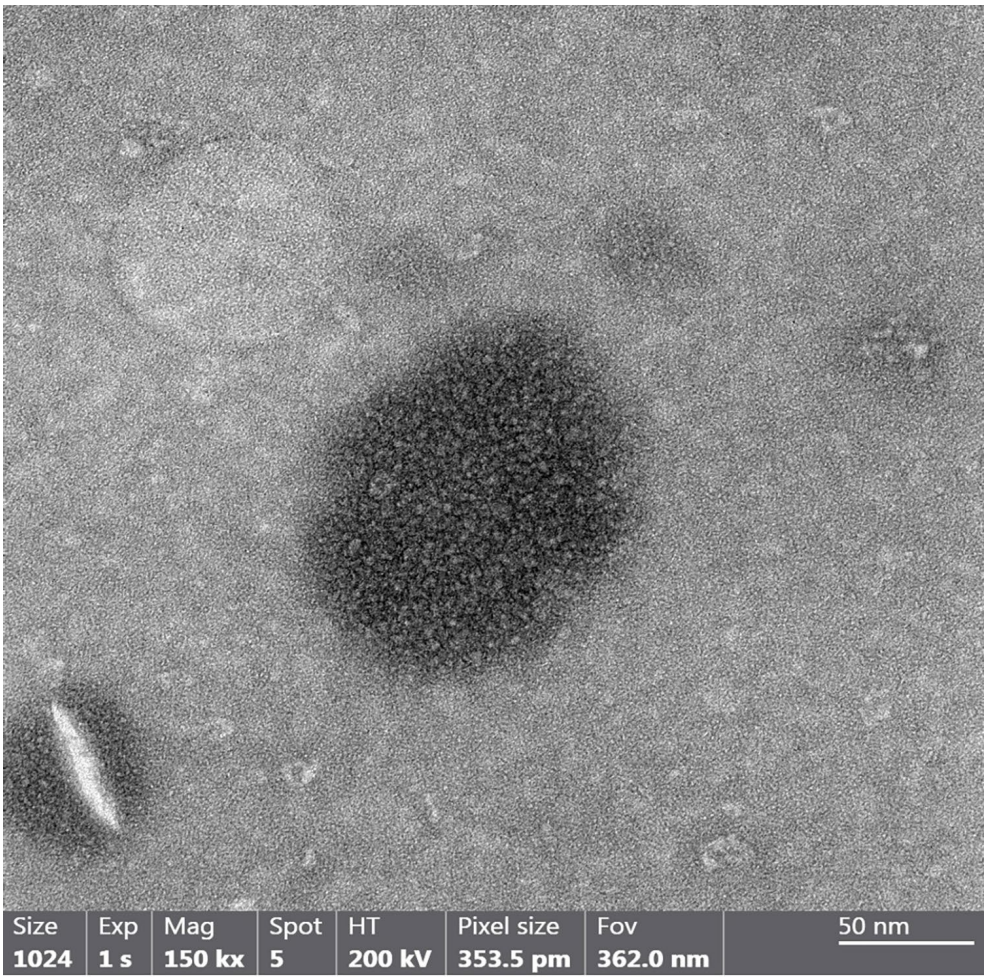
To evaluate whether sewage samples contained viable monkeypox virus, we selected the samples containing the higher viral load from each WWTP and used them to inoculate VeroE6 (ATCC CRL-1586) monolayers. These cells were seeded at 90% confluence in a 6-well plate and inoculated the following day with a mix of 200 µL of monkeypox DNA-positive sewage samples and 200 µL of culture medium (DMEM, 2% FBS, 0.1 mg of penicillin/streptomycin, and 0.2 mg of neomycin). The mixture and VeroE6 cells were incubated for 1.5 hours, and then the mixture was replaced with 500 µL of fresh medium. Seven days later, we scraped and centrifuged the cells, discarded the pellet, and used 250 µL of the supernatant for PCR detection as described above. We stored the remaining supernatant. To avoid cross-contamination, we included positive and negative controls (DMEM, 2% FBS) in separate plates.

For a second round of infection, we seeded VeroE6 cells in a 12-well plate and added 100 µL of supernatants from the previous infection, along with 100 µL of DMEM, 2% FBS in triplicate. We included positive and negative controls (DMEM, 2% FBS and negative sewage samples) in a separate plate. At 24h and 48h postinfection, we collected 250 µL of supernatant for monkeypox virus DNA detection by PCR.

References

1. Fumian TM, Leite JP, Castello AA, Gaggero A, Caillou MS, Miagostovich MP. Detection of rotavirus A in sewage samples using multiplex qPCR and an evaluation of the ultracentrifugation and adsorption-elution methods for virus concentration. J Virol Methods. 2010;170:42–6. PubMed https://doi.org/10.1016/j.jviromet.2010.08.017

2. Reynolds ES. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol. 1963;17:208–12. PubMed https://doi.org/10.1083/jcb.17.1.208



Appendix Figure. Transmission electron microscopy of cultured monkeypox virus from MPXV DNA-positive sewage samples. VeroE6 cells were inoculated with MPXV DNA-positive sample for 7 days, after cytopathic effect was visible. The monolayer was fixed for 18 h in 2.5% glutaraldehyde, 0.1M sodium cacodylate buffer, pH 7.2, postfixed in 1% osmium tetroxide in cacodylate buffer, and washed with distilled water. Samples were stained in block with 2% aqueous uranyl acetate, dehydrated with a series of acetone, and pre-filtered in 1:1 resin:acetone. Samples were placed in pure resin for 4 h (at room temperature) and then embedded in molds with pure resin and polymerized (60°C for 48 h). Thin sections were obtained on a Leica Ultracut R ultramicrotome, placed on copper grids, and stained with 2% aqueous uranyl acetate for 8 minutes and with lead citrate according to Reynolds (*2*) for 4 minutes. The sections were observed in a Thermo-Fisher Talos F200C transmission electron microscope at 200kV (Advanced Microscopy Unit, FCB, Catholic University of Chile). Scale bar indicates 50 nm.