Wastewater surveillance of MPOX virus in domestic sewage

Lopes, B.C. *, Mota, C. R. *

*Department of Sanitary and Environmental Engineering, Federal University of Minas Gerais, 6627 Avenida Antônio Carlos, Pampulha, Belo Horizonte, MG, Brazil.

Highlights:

- First study to assess MPOXV presence in sewage during a Brazil outbreak.
- Low levels of MPOXV DNA were detected in raw sewage.
- Unclear case incidence in sewer sheds.

Keywords: MPOXV, wastewater, WBE.

INTRODUCTION

The monkeypox virus (MPOXV) is a zoonotic disease caused by the Orthopoxvirus, first identified in North Africa in the 1970s. It can spread between humans through direct contact with skin lesions, body fluids, and respiratory particles[1]. In 2022, an MPOXV epidemic emerged outside Africa, leading to the World Health Organization's global public health emergency declaration in 2023[2]. As of now, 86,500 cases have been reported worldwide in 110 countries, with 10,878 cases and 15 deaths in Brazil, according to the U.S. Centres for Disease Control and Prevention (CDC)[3]. Since the first reported cases, there has been a rapid increase in public health infrastructure to detect diseases, including increased testing and educational efforts4. However, equipment availability and clinical awareness limit practical access to testing. An alternative approach to public health surveillance, such as domestic sewage surveillance, provides a means to understand the emerging epidemiology of monkeypox transmission and situational awareness of public health [5]. The sewage surveillance has been used to monitor the presence of poliomyelitis virus [6] and, more recently, to monitor SARS-CoV-2, which causes COVID-19[7]. Rapid identification of outbreak areas and population clusters is crucial for infection control. The main goal of this study is to identify the community circulation of MPOXV in potentially symptomatic, asymptomatic, or pre-symptomatic individuals through sewage systems in the city of Belo Horizonte, Brazil.

METHODOLOGY

During the period from May to December 2022, sewage samples were collected weekly at the Onça Effluent Treatment Plant (ETE) and stored at 4°C until analysis. The viral concentration method based on an electronegative membrane was used. The genetic material present in the membrane was extracted with the Allprep DNA/RNA Virus (Qiagen) kit. To quantify MPOXV DNA, assays were performed

following the protocol recommended by the CDC[8], using the Fast Kit Advanced TaqMan (Thermo Fisher Scientific). Each reaction contained 50 to 100 ng of nucleic acids in ten replicates within a volume of 20 µl per reaction. Positive and negative controls were included in each run. The reactions were conducted in CFX96 (Biorad), with concentrations of 800 nmol of each primer and 250 nmol of the probe, following the standard amplification conditions. To standardise the reactions and evaluate the detection sensitivity of MPOXV, 10 dilutions of MPOXV DNA were prepared in 1X TE (Tris-EDTA – Thermofisher buffer) with pH 8.0 [9].

RESULTS AND CONCLUSIONS

Based on the quantitative polymerase chain reaction (qPCR) results provided in Figure 01, our observations indicate that the viral presence in the sewage samples was predominantly detected in very small quantities, making it difficult to quantify the exact number of viral gene copies per millilitre of sewage. This aligns with the findings of previous studies[10,11,] which have also encountered challenges in accurately quantifying the genetic material of the MPOXV virus in sewage. This difficulty in detection is believed to be attributed to the disease's epidemiological nature, where the population's transmission occurs at a slower rate compared to that of COVID-19 and requires direct contact with an infected individual.

During the study period, the raw sewage samples from the Onça WWTP, which were taken on June 26 and September 11, 2022, were considered positive. The samples are above the proposed detection limit. The results agree with those reported in airport sewage[9] and treatment plants [10–12]. Also, the correlation between the MPOX cases and the percentage of positive reactions in qPCR was weak (Figure 2). It was impossible to translate the concentration of MPOXV DNA in wastewater to a predicted number of cases in the sewer shed.

This groundbreaking study, conducted in Brazil, represents the first instance of identifying MPOXV in sewage samples. Our innovative approach to modifying the standard sewage surveillance infrastructure to detect a non-enteric, non-respiratory virus-like MPOXV demonstrates significant potential for utilising this method as an additional tool for public health monitoring in the future.

ACKNOWLEDGMENTS

The authors wish to express their gratitude for the financial support extended by the National Council for Scientific and Technological Development (CNPq), the Minas Gerais Research Foundation (FAPEMIG), and the INCT Sustainable Sewage Treatment Plants. This support has been pivotal in advancing our research and contributing to the development of sustainable sewage treatment solutions.

REFERENCE

1. WHO. WHO Director-General declares the ongoing monkeypox outbreak a Public Health Emergency of International Concern. https://www.who.int/europe/news/item/23-07-2022-who-director-general-declares-the-ongoing-monkeypox-outbreak-a-public-health-event-of-international-concern (2022).
2. WHO. Mpox (monkeypox) outbreak 2022. https://www.who.int/emergencies/situations/monkeypox-oubreak-2022 (2022).
3. CDC. 2022 Global Map & Case Count. 2022 Global Map & Case Count (2022).
4. Aden, T. A. *et al.* Rapid Diagnostic Testing for Response to the Monkeypox Outbreak — Laboratory Response Network, United States, May 17–June 30, 2022. *MMWR. Morb. Mortal. Wkly. Rep.* **71**, 904–907 (2022).
5. Girón-Guzmán, I. *et al.* Spanish wastewater reveals the current spread of Monkeypox virus. *Water Res.* **231**, 119621 (2023).
6. Brouwer, A. F., Masters, N. B. & Eisenberg, J. N. S. Quantitative Microbial Risk Assessment and Infectious Disease Transmission Modeling of Waterborne Enteric Pathogens. *Curr. Environ. Heal. Reports* **5**, 293–304 (2018).
7. Mota, C. R. *et al.* Assessing spatial distribution of COVID-19 prevalence in Brazil using decentralised sewage monitoring. *Water Res.* **202**, 117388 (2021).
8. Li, Y., Zhao, H., Wilkins, K., Hughes, C. & Damon, I. K. Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA. *J. Virol. Methods* **169**, 223–227 (2010).
9. La Rosa, G. *et al.* Detection of Monkeypox Virus DNA in Airport Wastewater, Rome, Italy. *Emerg. Infect. Dis.* **29**, 193–196 (2023).
10. Wannigama, D. L. *et al.* Multiple traces of monkeypox detected in non-sewered wastewater with sparse sampling from a densely populated metropolitan area in Asia. *Sci. Total Environ.* **858**, 159816 (2023).
11. Wolfe, M. K. *et al.* Use of Wastewater for Mpox Outbreak Surveillance in California. *N. Engl. J. Med.* **388**, 570–572 (2023).
12. Wurtzer, S. *et al.* First Detection of Monkeypox Virus Genome in Sewersheds in France: The Potential of Wastewater-Based Epidemiology for Monitoring Emerging Disease. *Environ. Sci. Technol. Lett.* **9**, 991–996 (2022).

FIGURES

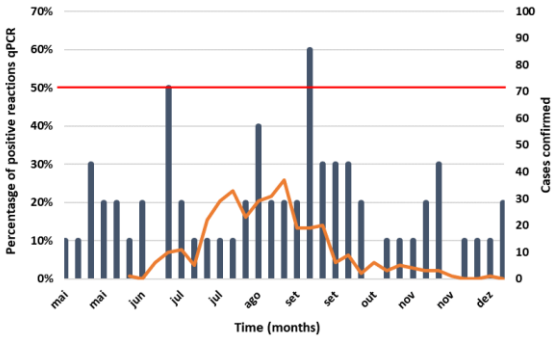

Figure 1 : Positive reactions to the MPOXV target gene and number of reported cases

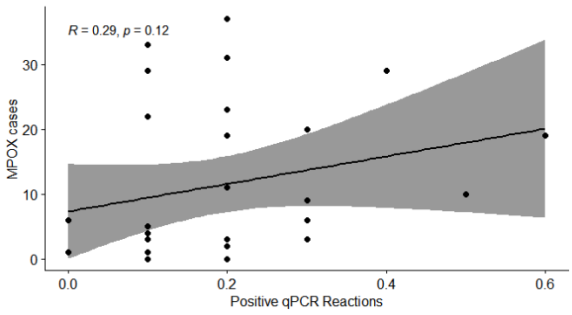

Figure 2: Spearman correlation plot reported MPOX cases and positive reactions in qPCR