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Association between the number of symptomatic mpox cases and the detection of mpox virus DNA in wastewater in Switzerland: an observational surveillance study

Claudia Bagutti[a], Monica Alt Hug[a], Philippe Heim[a], Evelyn Ilg Hampe[a], Philipp Hübner[a], Timothy R. Julian[b], Katrin N. Koch[c], Kerstin Grosheintz[d], Melanie Kraus[d], Carla Schaubhut[d], Rahel Tarnutzer[d], Eva Würfel[d], Simon Fuchs[d], Sarah Tschudin-Sutter[e]

[a] State Laboratory of Basel-Stadt, Basel, Switzerland
[b] Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland
[c] Cantonal Office of Public Health, Department of Economics and Health, Canton of Basel-Landschaft, Liestal, Switzerland
[d] Department of Health, Canton of Basel-Stadt, Basel, Switzerland
[e] Division of Infectious Diseases & Hospital Epidemiology, University Hospital Basel, Switzerland / University of Basel, Basel, Switzerland

Summary

AIM OF THE STUDY: The COVID-19 pandemic has drawn attention to the benefit of wastewater-based epidemiology, particularly when case numbers are underreported. Underreporting may be an issue with mpox, where biological reasons and stigma may prevent patients from getting tested. Therefore, we aimed to assess the validity of wastewater surveillance for monitoring mpox virus DNA in wastewater of a Central European city and its association with official case numbers.

METHODS: Wastewater samples were collected between 1 July and 28 August 2022 in the catchment area of Basel, Switzerland, and the number of mpox virus genome copies they contained was determined by real-time quantitative PCR. Logistic regression analyses were used to determine the odds of detectability of mpox virus DNA in wastewater, categorised as detectable or undetectable. Mann–Whitney U tests were used to determine associations between samples that tested positive for the mpox virus and officially reported cases and patients' recorded symptomatic phases.

RESULTS: Mpox virus DNA was detected in 15 of 39 wastewater samples. The number of positive wastewater samples was associated with the number of symptomatic cases (odds ratio [OR] = 2.18, 95% confidence interval (CI) = 1.38–3.43, p = 0.001). The number of symptomatic cases differed significantly between days with positive versus negative wastewater results (median = 11 and 8, respectively, p = 0.0024).

CONCLUSION: Mpox virus DNA was detectable in wastewater, even when officially reported case numbers were low (0–3 newly reported mpox cases corresponding to 6–12 symptomatic patients). Detectability in wastewater was significantly associated with the number of symptomatic patients within the catchment area. These findings illustrate the value of wastewater-based surveillance systems when assessing the prevalence of emerging and circulating infectious diseases.

Claudia Bagutti
Kantonales Laboratorium
Basel-Stadt
Kannenfeldstrasse 2
CH-4056 Basel
claudia.bagutti[at]bs.ch

Introduction

Mpox has been described as an endemic zoonotic disease in Western and Central Africa since the 1970s [1]. Individual cases and small outbreaks outside the endemic regions were import- and travel-related and did not persist. In May 2022, a novel mpox clade (IIb [2, 3]) emerged in non-endemic countries, mainly in Europe. Since mpox clade IIb is primarily transmitted sexually, it is likely associated with stigma. Therefore, hesitancy to get tested may impair official reporting of mpox infections [4]. Furthermore, case numbers are likely underestimated due to the disease's non-specific symptoms (especially at disease onset), such as fever, myalgia, fatigue, and headache, asymptomatic course [5], and long incubation time of up to 21 days [6]. Therefore, independent and unbiased surveillance systems are needed to estimate the true prevalence of mpox cases.

Studies from the US, France, Italy, Spain, and the Netherlands [7–11] reported successfully detecting mpox virus DNA in wastewater and identifying the clade [12]. Amongst these recently published studies, two compared their wastewater data with mpox case data but did not perform correlation analyses [7, 10]. In their most recent study, Wolfe et al. demonstrated a significant correlation between wastewater and case data at four out of nine sewer sites where more than 10 mpox cases were reported [8].

To further assess the validity of wastewater surveillance for monitoring mpox virus DNA and its association with official case numbers, we used an established and representative wastewater monitoring system for the catchment area of a Central European city [13].

Material and methods

Sample collection and analysis

Wastewater samples were collected from the local wastewater treatment plant (ProRheno AG) that receives wastewater from the catchment area of Basel, Switzerland, which has 273,075 inhabitants (including 201,971 in the political district of Canton Basel-City, Switzerland, 67,388 in the Canton Basel-Country, and 3716 in parts of three municipalities in Germany and France). Twenty-four-hour composite samples were collected daily (except for some days, generally on weekends or around the national holiday on 1 August, when 48- or 72-hour composite samples were collected; table S1) and contained 500 ml of wastewater. Samples were stored at 4°C for up to 72 hours before further processing. Total nucleic acids were concentrated and extracted from 40 ml of wastewater using the Maxwell® RSC Enviro Total Nucleic Acid Kit (Promega) twice weekly. Sampling and RNA extraction were conducted as part of the wastewater-based surveillance system established for COVID-19. Mpox virus DNA was detected in the samples collected from 1 July to 28 August 2022 and in archived samples from 4 August 2021, 11 August 2021, and 20 August 2021 using the VIASURE mpox Virus Real-Time PCR Detection Kit (Ruwag, Switzerland). The number of gene copies per litre of wastewater was calculated to account for the initial wastewater volume (40 ml), the concentration factor during the pre-extraction procedure (500), and the eluate volume (40 µl); one gene copy per PCR equals 500 gene copies per litre. The result in cycle threshold (Ct) values is shown in figure 1. The result was assessed qualitatively as "positive" (Ct <39) or "negative" (Ct ≥39) (table S1).

Mpox case data

Data on the number of newly diagnosed mpox cases (new cases counted on the day of diagnosis) and the number of symptomatic cases (accounting for the symptomatic period of each patient) was captured daily and provided by the Health Departments of Basel-Stadt and Basel-Landschaft. Symptomatic case data is based on the mandatory reporting of all PCR- or serological test-confirmed results as specified by federal law [14] and on local health authorities' follow-up calls to all patients. The number of patients still reporting symptoms was determined each day. A formal study protocol was not prepared and published in a respective registry.

Statistical analysis

Logistic regression analyses with robust standard errors were performed to assess associations between the detectability of mpox virus DNA in wastewater and daily mpox cases (absolute numbers and seven-day medians) and the daily number of symptomatic patients (absolute numbers and seven-day medians). We defined the outcome as the detectability of mpox virus DNA in wastewater (categorised as detectable or undetectable). Therefore, odds ratios (ORs) represent the odds of detecting mpox virus DNA in wastewater per symptomatic case (or number of daily cases) or per unit of the seven-day median of symptomatic cases (or seven-day median of the number of daily cases). Furthermore, the number of daily mpox cases and symptomatic mpox cases on days with positive wastewater samples was compared to the number of daily cases on days with negative wastewater samples using the Mann-Whitney U test. All statistical analyses were performed using STATA 16.0 (Stata Corp., College Station, Texas, USA).

Ethical statement

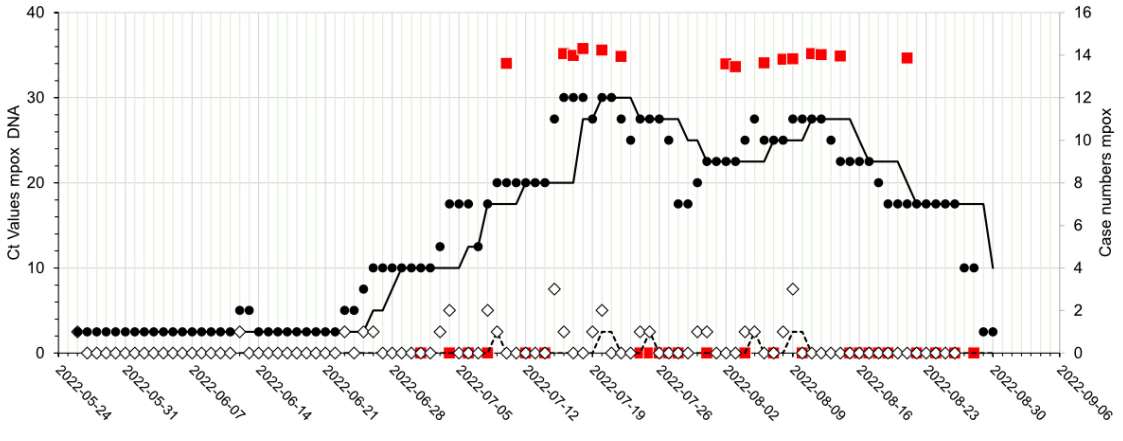
Because this study did not involve human research, no ethical consent was required.

Results

Detection of mpox virus DNA in wastewater

Thirty-nine wastewater samples were analysed during the 59-day study period (table S1). The detected Ct values were between 34 and 36 (1–3 gene copies per 20 µl re-

Figure 1: Detection of mpox virus DNA in wastewater in relation to mpox (mpox) case numbers in the catchment area of the city of Basel during the indicated time period. The mpox virus DNA status of the wastewater samples (n = 39) is indicated (red-filled squares; negative samples on the x-axis, positive samples shown by their Ct values). Open diamonds represent the number of newly reported mpox cases, and closed circles represent the number of symptomatic patients. The corresponding seven-day median curves are shown by black lines (dashed line: newly reported cases, solid line: symptomatic cases). The results of 48- and 72-hour pooled samples were attributed to the corresponding later date.



action, equalling 500–1500 gene copies per litre of wastewater). The number of gene copies detected per litre of wastewater was below the limit of quantification (10 gene copies/reaction). Therefore, the result was only assessed qualitatively as positive or negative. Mpox virus DNA was detected in 15 of the 39 samples analysed (table S1) from 10 July until 21 August 2022 (figure 1). The three samples from the 2021 control period were negative for mpox virus DNA.

Association between mpox virus DNA detected in wastewater and official case numbers

In the logistic regression analyses, the positivity of the wastewater samples was significantly associated with the number of symptomatic cases (OR = 2.18, 95% confidence interval [CI] = 1.38–3.43, p = 0.001) (figure 2) and with the seven-day median of symptomatic cases (OR = 1.54, 95% CI = 1.03–2.30, p = 0.035). However, it was not associated with the number of daily cases (OR = 1.23, 95% CI = 0.53–2.90, p = 0.629) or their seven-day median (OR = 1.69, 95% CI = 0.21–13.87, p = 0.624).

The number of daily symptomatic cases differed significantly between days with (median = 11, range = 7–12, interquartile range [IQR] = 9–12) and days without (median = 8, range = 4–11, IQR = 7–10) mpox virus DNA detected in wastewater (p = 0.0024) (figure 3). However, newly reported case numbers did not differ significantly between days with (median = 0, range = 0–3, IQR = 0–1) and days without (median = 0, range = 0–2, IQR = 0–1) mpox virus DNA detected in wastewater (p = 0.877). Therefore, our results suggest an approximate median of 11 reported symptomatic cases (range = 7–12) may be needed to detect mpox virus DNA in wastewater from a catchment area of approximately 270,000 inhabitants.

Discussion

Using a wastewater-based surveillance system established for COVID-19, mpox virus DNA was detected in low copy numbers in 15 wastewater samples of a Central European city from 10 July to 21 August 2022. During this period, there were 0–3 newly reported mpox cases corresponding to 6–12 symptomatic patients. These numbers might be higher due to underreporting. No data on the actual shedding of mpox virus into wastewater has been published to date, which would allow for approximating the rate of underreporting. A comparison of the viral load in biological samples from 12 patients revealed mpox virus detection in all saliva and skin lesion samples and most semen, urine, and faeces samples [15]. Particularly low Ct values indicating high viral load were obtained for saliva, semen, faeces and skin lesions. A recent model-based theoretical evaluation by Chen and Bibby demonstrated that saliva, faeces, and urine contribute the most to the detectability of mpox virus in wastewater [16].

A daily shedding load per infected individual of 6×10^7 mpox virus genome copies was applied, which is at the lower end of the range assumed for SARS-CoV-2 [17]. Indeed, Wurtzer et al. suggested that wastewater loads of SARS-CoV-2 and mpox virus were within the same order of magnitude [10], which would match our data from a low COVID-19 incidence period (detection limit of 10–20 individuals infected with COVID-19 [13]) and this study (12 individuals with symptomatic mpox). Therefore, mpox virus DNA is detectable in wastewater, even when officially reported case numbers are low. Furthermore, our data confirm a high association between the detectability of mpox virus DNA in wastewater and the number of virus-shedding symptomatic cases but not with the number of newly diagnosed cases.

Our study was limited by its conduction in a single city with a limited population size and a limited number of diagnosed and reported mpox cases during the study period. Nevertheless, our findings support the utility of wastewater surveillance for detecting emerging infectious diseases, such as mpox. They should generalise to other settings with similar population sizes and catchment areas of associated wastewater treatment plants. Furthermore, while we cannot discount the possibility of false positive results, our negative findings for samples from a historic control period make them unlikely.

Conclusion

Mpox virus DNA is detectable in wastewater, even when officially reported case numbers are low. Detectability in wastewater is significantly associated with the number of symptomatic patients within a catchment area. These findings support the value of wastewater-based surveillance

Figure 2: Association between the probability of detecting mpox virus DNA in wastewater (sample positivity) and the number of symptomatic mpox cases based on logistic regression analyses. The significance level is indicated.

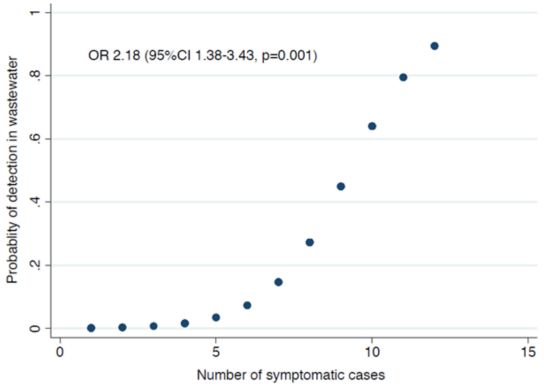


Figure 3: Box plots of the number of symptomatic mpox cases on days with and without mpox virus DNA detected in wastewater.

systems in assessing the prevalence of emerging and circulating infectious diseases.

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Potential competing interests
All authors have completed and submitted the International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest. No potential conflict of interest related to the content of this manuscript was disclosed.

References

1. European Centre for Disease Prevention and Control. Risk assessment: monkeypox multi-country outbreak. Stockholm: ECDC; 2022 23 May 2022. Available from: https://www.ecdc.europa.eu/en/publications-data/risk-assessment-monkeypox-multi-country-outbreak Last accessed 2023-07-29
2. Isidro J, Borges V, Pinto M, Sobral D, Santos JD, Nunes A, et al. Phylogenomic characterization and signs of microevolution in the 2022 multi-country outbreak of monkeypox virus. Nat Med. 2022 Aug;28(8):1569–72. [cited 2023 Jul 29] http://dx.doi.org/10.1038/s41591-022-01907-y.
3. Isidro J, Borges V, Pinto M, Sobral D, Santos JD, Nunes A, et al. Addendum: phylogenomic characterization and signs of microevolution in the 2022 multi-country outbreak of monkeypox virus. Nat Med. 2022 Oct;28(10):2220–1. [cited 2023 Jul 29] http://dx.doi.org/10.1038/s41591-022-02036-2.
4. Taylor L. Monkeypox: WHO to rename disease to prevent stigma. BMJ. 2022 Jun;377:o1489. [cited 2023 Jul 29] http://dx.doi.org/10.1136/bmj.o1489.
5. De Baetselier I, Van Dijck C, Kenyon C, Coppens J, Michiels J, de Block T, et al.; ITM Monkeypox study group. Retrospective detection of asymptomatic monkeypox virus infections among male sexual health clinic attendees in Belgium. Nat Med. 2022 Nov;28(11):2288–92. [cited 2023 Jul 29] http://dx.doi.org/10.1038/s41591-022-02004-w.
6. Miura F, van Ewijk CE, Backer JA, Xiridou M, Franz E, Op de Coul E, et al. Estimated incubation period for monkeypox cases confirmed in the Netherlands, May 2022. Euro Surveill. 2022 Jun;27(24):2200448. [cited 2023 Jul 29] http://dx.doi.org/10.2807/1560-7917.ES.2022.27.24.2200448.
7. Girón-Guzmán I, Díaz-Reolid A, Truchado P, Carcereny A, García-Pedemonte D, Hernáez B, et al. Spanish wastewater reveals the current spread of Monkeypox virus. Water Res. 2023 Mar;231:119621. [cited 2023 Jul 29] http://dx.doi.org/10.1016/j.watres.2023.119621.
8. Wolfe MK, Yu AT, Duong D, Rane MS, Hughes B, Chan-Herur V, et al. Use of Wastewater for Mpox Outbreak Surveillance in California. N Engl J Med. 2023 Feb;388(6):570–2. [cited 2023 Jul 29] http://dx.doi.org/10.1056/NEJMc2213882.
9. 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 Dec;852:158265. [cited 2023 Jul 29] http://dx.doi.org/10.1016/j.scitotenv.2022.158265.
10. Wurtzer S, Levert M, Dhenain E, Boni M, Tournier JN, Londinsky N, et al. First detection of Monkeypox virus genome in sewersheds in France. Environ Sci Technol Lett. 2022;9(11):991–6. [cited 2023 Jul 29] http://dx.doi.org/10.1021/acs.estlett.2c00693.
11. La Rosa G, Mancini P, Veneri C, Ferraro GB, Lucentini L, Iaconelli M, et al. Detection of Monkeypox virus DNA in the wastewater of an airport in Rome, Italy: expanding environmental surveillance to emerging threats. Emerg Infect Dis. 2023;29(1):193–6. [cited 2023 Jul 29] http://dx.doi.org/10.3201/eid2901.221311.
12. Mejia EM, Hizon NA, Dueck CE, Lidder R, Daigle J, Wonitowy Q, et al. Exploration of wastewater surveillance for Monkeypox virus. medRxiv. 2022:2022.11. http://dx.doi.org/10.22282091. 10.1101/2022.11.10.22282091. Last accessed 2023-07-29 http://dx.doi.org/10.1101/2022.11.10.22282091.
13. Bagutti C, Alt Hug M, Heim P, Maurer Pekerman L, Ilg Hampe E, Hübner P, et al. Wastewater monitoring of SARS-CoV-2 shows high correlation with COVID-19 case numbers and allowed early detection of the first confirmed B.1.1.529 infection in Switzerland: results of an observational surveillance study. Swiss Med Wkly. 2022 Jun;152(152):w30202. [cited 2023 Jul 29] http://dx.doi.org/10.4414/smw.2022.w30202. http://dx.doi.org/10.4414/SMW.2022.w30202.
14. Federal Office of Public Health. Reporting systems for infectious diseases requiring notication. Federal Office of Public Health FOPH Bern, Switzerland [https://www.bag.admin.ch/bag/de/home/krankheiten/infektionskrankheiten-bekaempfen/meldesysteme-infektionskrankheiten/meldepflichtige-ik/meldeformulare.html. Last accessed 2023-07-29
15. Suñer C, Ubals M, Tarín-Vicente EJ, Mendoza A, Alemany A, Hernández-Rodríguez Á, et al.; Movie Group. Viral dynamics in patients with monkeypox infection: a prospective cohort study in Spain. Lancet Infect Dis. 2023 Apr;23(4):445–53. [cited 2023 Jul 29] http://dx.doi.org/10.1016/S1473-3099(22)00794-0.
16. Chen W, Bibby K. Model-based theoretical evaluation of the feasibility of using wastewater-based epidemiology to monitor monkeypox [Last accessed]. Environ Sci Technol Lett. 2022;9(9):772–8. http://dx.doi.org/10.1021/acs.estlett.2c00496.
17. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virological assessment of hospitalized patients with COVID-2019. Nature. 2020 May;581(7809):465–9. [cited 2023 Jul 29] http://dx.doi.org/10.1038/s41586-020-2196-x.

Appendix: supplementary tables

Table S1:
Sampling days in July and August 2022, composite sampling type, sample analysis (n= 39), and qualitative real-time PCR results for mpox virus DNA.

July 2022			**August 2022**		
Sampling days	Sample type*	Sample analysis and result**	Sampling days	Sample type	Sample analysis and result
2022-07-01	24 hrs	Negative	2022-08-01	48 hrs	Positive
2022-07-02	48 hrs	na	2022-08-02		
2022-07-03			2022-08-03	24 hrs	Positive
2022-07-04	24 hrs	Negative	2022-08-04	24 hrs	Negative
2022-07-05	24 hrs	na	2022-08-05	48 hrs***	Negative
2022-07-06	24 hrs	Negative	2022-08-07		
2022-07-07	24 hrs	na	2022-08-06	24 hrs	Positive
2022-07-08	24 hrs	Negative	2022-08-08	24 hrs	Positive
2022-07-09	48 hrs	Positive	2022-08-09	24 hrs	Positive
2022-07-10			2022-08-10	24 hrs	Negative
2022-07-11	24 hrs	na	2022-08-11	24 hrs	Positive
2022-07-12	24 hrs	Negative	2022-08-12	24 hrs	Positive
2022-07-13	24 hrs	na	2022-08-13	48 hrs	Positive
2022-07-14	24 hrs	Negative	2022-08-14		
2022-07-15	48 hrs	Positive	2022-08-15	24 hrs	Negative
2022-07-16			2022-08-16	24 hrs	Negative
2022-07-17	24 hrs	Positive	2022-08-17	24 hrs	Negative
2022-07-18	24 hrs	Positive	2022-08-18	24 hrs	Negative
2022-07-19	24 hrs	na	2022-08-19	24 hrs	Negative
2022-07-20	24 hrs	Positive	2022-08-20	48 hrs	Positive
2022-07-21	24 hrs	na	2022-08-21		
2022-07-22	24 hrs	Positive	2022-08-22	48 hrs	Negative
2022-07-23	48 hrs	Negative	2022-08-23	24 hrs	na
2022-07-24			2022-08-24	24 hrs	Negative
2022-07-25	24 hrs	Negative	2022-08-25	24 hrs	na
2022-07-26	24 hrs	Negative	2022-08-26	24 hrs	Negative
2022-07-27	24 hrs	Negative	2022-08-27	48 hrs	Negative
2022-07-28	24 hrs	Negative	2022-08-28		
2022-07-29	72 hrs	Negative			
2022-07-30					
2022-07-31					

* 24-, 48-, or 72-hour composite samples are indicated. The pooling at the wastewater treatment plant generally affected consecutive days with one exception (48-hr sample on 2022-08-05 and 2022-08-07). In figure 1, pooled sample results were attributed to the corresponding later date.
** Samples were either not analysed (na) or positive or negative for mpox virus DNA detected by real-time PCR.
*** Two non-consecutive days of wastewater pooling.

Table S2 is available for download as a separate file at https://doi.org/10.57187/s.3706.