

Determinants and spatio-temporal structure of SARS-CoV-2 viral load in wastewater in Switzerland: key insights for future surveillance efforts

Julien Riou^{1✉*}, People from EAWAG? (Tim, Christoph...), People from BAG? (Rita, Anna, Moritz...), People from ETHZ? (James, Tanja...),

1 Department of Epidemiology and Health Systems, Unisanté, Center for Primary Care and Public Health & University of Lausanne, Lausanne, Switzerland

2

✉These authors contributed equally to this work.

✉Current Address: Route de la corniche 10, CH-1010 Lausanne, Switzerland

*Current email: julien.riou@unisanté.ch

Abstract

1 Introduction

Wastewater-based epidemiology (WBE) has now taken a central role in infectious disease surveillance. While the analysis of wastewater concentrations of drugs, pharmaceuticals, and other biomarkers at wastewater treatment plants (WWTPs) has been used for decades, the application of this method to assess the epidemiology of pathogens within communities (besides enteric bacteria) is a fairly recent development [1], fueled by the increased needs for surveillance during the SARS-CoV-2 pandemic. WBE is now being used in at least 34 countries [2]. This increase in popularity can be explained by several factors. As opposed to diagnosis-based approaches based on the notification of cases, hospitalizations and deaths with a positive laboratory test, WBE can be considered as a population-based approach that does not depend on testing, which is itself influenced by age, gender, and socio-economic position [3–5]. Because it is exempt from testing bias,

WBE can be considered as more representative of the entirety of the population living in the WWTP catchment areas, including people with no or mild symptoms, and people less likely to seek care with a health professional. WBE is also less costly and less intrusive than individual testing, and can be obtained in near real-time. However, its full potential and limitations are still being explored.

WBE relies upon the repeated collection of wastewater samples in a generally fixed set of WWTPs. For SARS-CoV-2, either quantitative Polymerase Chain Reaction (qPCR) or digital droplet PCR (dPCR) tests are then applied to obtain measurements of viral RNA concentrations (generally expressed in gene copies [gc] per liter). These measurements are then scaled based on the population living in the WWTP catchment area and on the flow of wastewater on the same day, resulting in a quantity called *viral load* (expressed in gc per day per 100,000 population). This normalization, by allowing for comparisons over time and space, is key in the use of these data to assess the dynamics of infection at the population level [6–8], and also allows for the computation of reproduction numbers [9]. But a deeper look into the mechanisms of generation of wastewater data reveals many uncertainties in the pathway leading from population prevalence to viral load. First, fecal shedding upon infection with SARS-CoV-2 dominates shedding routes into community-level wastewater, and is also highly heterogeneous. The probability of fecal shedding is about 40-50% on average, but is higher in case of gastro-intestinal symptoms [10, 11]. Fecal shedding lasts longer in symptomatic infections compared to asymptomatic, and in adults compared to children [12]. Virus titre in faeces can also vary widely according to individual characteristics [13]. Second, the population residing within WWTP catchment areas is not as well-defined as generally considered, with movements during the day due to commuting and work, and periodic changes within weeks and years depending on holiday periods. A third source of variability may be different laboratory methods (e.g., targeting N or S proteins) and protocols (e.g., RNA degradation over time until testing) that can influence measured values [13]. A direct consequence of these observations is that wastewater viral load data may suffer from increased levels of noise and bias depending on the local situation.

In this study, we take advantage of the dense network of WWTPs participating in SARS-CoV-2 surveillance in Switzerland to disentangle the different sources of

variability and bias, and characterize the local determinants and the space-time structure of measured SARS-CoV-2 viral load. We then use these insights to produce adjusted temporal trends at the national and regional levels, corresponding to the underlying dynamics of SARS-CoV-2 infection after removing all other known sources of variability and bias. We also consider the spatial structure of the data using clustering techniques, allowing to identify areas with similar profiles.

2 Methods

2.1 Data

2.1.1 Viral concentration in wastewater

This work is based on wastewater samples collected between 7 February 2022 and 21 November 2023 in WWTPs participating to the wastewater surveillance programme coordinated by the Swiss Federal Institute of Aquatic Science and Technology (EAWAG) and the Federal Office of Public Health (FOPH). A total of 118 WWTPs covering all 26 cantons participated in the programme at least once, including 6 WWTPs directly overseen by EAWAG that participated for the whole period (Table 1). The minimal length of participation for a WWTP was 106 days. The frequency of sampling varied between 1.7 and 6.5 times per week on average depending on the WWTP. Samples were stored on site at 4°C and transported in batches to 9 different laboratories for testing. Laboratories used slightly different methods for concentration, extraction and quantification with qPCR/dPCR, and two laboratories changed their protocol during the study period. Two separate quantifications were done, with the average being taken as the measurement. Measurements below the limit of quantification (LOQ) and below the limit of detection (LOD) were flagged as such. The flow of wastewater on the day of the sampling was also measured, and used for normalization. More details about the processing can be found elsewhere [14].

2.1.2 Local data

We used publicly available data from the Federal Statistical Office (FSO) to

2.1.3 Data analysis

72

Measurements of viral concentration (C , unit: gene copies [gc] per liter) were transformed into viral load (V ; unit: gc per day per 100,000) using the flow of wastewater on the same day (F) and the size of the population of the WWTP catchment area (P):

$$V = \frac{C \times F}{P/100,000}.$$

Table 1. Description of wastewater data used for SARS-CoV-2 surveillance in Switzerland (2022-2023).

	Value (count or median and range)
Number of WWTPs	118
Number of laboratories	9
Number of laboratory methods	11
Total measurements	23,025
Measurements below LOQ	675
Measurements below LOD	110
Date of first measurement	2022-02-07
Date of last measurement	2023-11-21
Viral concentration [gc/L]	94,350 (range: 0 to 18,162,400)
Wastewater flow [m3/day]	14,343 (range: 291 to 913,343)
Viral load [gc/day/100,000]	3.7e+12 (range: 1.0e+00 to 7.4e+14)
Population covered	37,507 (range: 2,095 to 475,198)
Proportion of population under 20	0.24 (range: 0.18 to 0.35)
Proportion of population over 65	0.36 (range: 0.22 to 0.69)
Proportion of population non-Swiss	0.15 (range: 0.05 to 0.30)
Median of Swiss index of socio-economic position (SEP)	63 (range: 54 to 78)

3	Results	73
3.1	Description, table, maps (TBR)	74
3.2	local determinants (TBR)	75
3.3	spatial structure (TBR)	76
3.4	single temporal trend (TBR)	77
3.5	spatial clustering (TBR)	78
4	Discussion	79
	Focus on old, symptomatic is actually an advantage if you want to anticipate on the burden	80 81

Supporting information

S1 Text. .

Acknowledgments

We gratefully acknowledge all data contributors...

Funding

Data and Code Availability

References

- [1] Phil M. Choi, Ben J. Tschärke, Erica Donner, Jake W. O’Brien, Sharon C. Grant, Sarit L. Kaserzon, Rachel Mackie, Elissa O’Malley, Nicholas D. Crosbie, Kevin V. Thomas, and Jochen F. Mueller. Wastewater-based epidemiology biomarkers: Past, present and future. *TrAC Trends in Analytical Chemistry*, 105:453–469, August 2018.

- [2] Shimoni Shah, Sylvia Xiao Wei Gwee, Jamie Qiao Xin Ng, Nicholas Lau, Jiayun Koh, and Junxiong Pang. Wastewater surveillance to infer COVID-19 transmission: A systematic review. *Science of The Total Environment*, 804:150060, January 2022.
- [3] Eileen P. Scully, Grant Schumock, Martina Fu, Guido Massaccesi, John Muschelli, Joshua Betz, Eili Y. Klein, Natalie E. West, Matthew Robinson, Brian T. Garibaldi, Karen Bandeen-Roche, Scott Zeger, Sabra L. Klein, and Amita Gupta. Sex and Gender Differences in Testing, Hospital Admission, Clinical Presentation, and Drivers of Severe Outcomes From COVID-19. *Open Forum Infectious Diseases*, 8(9):ofab448, September 2021.
- [4] Nathan M. Stall, Wei Wu, Lauren Lapointe-Shaw, David N. Fisman, Vasily Giannakeas, Michael P. Hillmer, and Paula A. Rochon. Sex- and Age-Specific Differences in COVID-19 Testing, Cases, and Outcomes: A Population-Wide Study in Ontario, Canada. *Journal of the American Geriatrics Society*, 68(10):2188–2191, 2020.
- [5] Julien Riou, Radoslaw Panczak, Christian L. Althaus, Christoph Junker, Damir Perisa, Katrin Schneider, Nicola G. Criscuolo, Nicola Low, and Matthias Egger. Socioeconomic position and the COVID-19 care cascade from testing to mortality in Switzerland: A population-based analysis. *The Lancet Public Health*, 6(9):e683–e691, September 2021.
- [6] Amy E. Kirby, Maroya Spalding Walters, Wiley C. Jennings, Rebecca Fugitt, Nathan LaCross, Mia Mattioli, Zachary A. Marsh, Virginia A. Roberts, Jeffrey W. Mercante, Jonathan Yoder, and Vincent R. Hill. Using Wastewater Surveillance Data to Support the COVID-19 Response — United States, 2020–2021. *MMWR. Morbidity and Mortality Weekly Report*, 70(36):1242–1244, September 2021.
- [7] Michiel van Boven, Wouter A. Hetebrij, Arno Swart, Erwin Nagelkerke, Rudolf FHJ van der Beek, Sjors Stouten, Rudolf T. Hoozeveen, Fuminari Miura, Astrid Kloosterman, Anne-Merel R. van der Drift, Anne Welling, Willemijn J. Lodder, and Ana Maria de Roda Husman. Patterns of SARS-CoV-2 circulation revealed by a nationwide sewage surveillance programme, the Netherlands, August 2020 to February 2022. *Eurosurveillance*, 28(25):2200700, June 2023.

- [8] Colleen C Naughton, Fernando A Roman, Jr, Ana Grace F Alvarado, Arianna Q Tariqi, Matthew A Deeming, Krystin F Kadonsky, Kyle Bibby, Aaron Bivins, Gertjan Medema, Warish Ahmed, Panagis Katsivelis, Vajra Allan, Ryan Sinclair, and Joan B Rose. Show us the data: Global COVID-19 wastewater monitoring efforts, equity, and gaps. *FEMS Microbes*, 4:xtad003, January 2023.
- [9] Jana S. Huisman, Jérémie Scire, Lea Caduff, Xavier Fernandez-Cassi, Pravin Ganesanandamoorthy, Anina Kull, Andreas Scheidegger, Elyse Stachler, Alexandria B. Boehm, Bridgette Hughes, Alisha Knudson, Aaron Topol, Krista R. Wigginton, Marlene K. Wolfe, Tamar Kohn, Christoph Ort, Tanja Stadler, and Timothy R. Julian. Wastewater-Based Estimation of the Effective Reproductive Number of SARS-CoV-2. *Environmental Health Perspectives*, 130(5):057011, May 2022.
- [10] Yawen Zhang, Mengsha Cen, Mengjia Hu, Lijun Du, Weiling Hu, John J. Kim, and Ning Dai. Prevalence and Persistent Shedding of Fecal SARS-CoV-2 RNA in Patients With COVID-19 Infection: A Systematic Review and Meta-analysis. *Clinical and Translational Gastroenterology*, 12(4):e00343, April 2021.
- [11] Aravind Natarajan, Soumaya Zlitni, Erin F. Brooks, Summer E. Vance, Alex Dahlen, Haley Hedlin, Ryan M. Park, Alvin Han, Danica T. Schmidtke, Renu Verma, Karen B. Jacobson, Julie Parsonnet, Hector F. Bonilla, Upinder Singh, Benjamin A. Pinsky, Jason R. Andrews, Prasanna Jagannathan, and Ami S. Bhatt. Gastrointestinal symptoms and fecal shedding of SARS-CoV-2 RNA suggest prolonged gastrointestinal infection. *Med*, 3(6):371–387.e9, June 2022.
- [12] Danying Yan, Xiaobao Zhang, Can Chen, Daixi Jiang, Xiaoxiao Liu, Yuqing Zhou, Chenyang Huang, Yiyi Zhou, Zhou Guan, Cheng Ding, Lu Chen, Lei Lan, Xiaofang Fu, Jie Wu, Lanjuan Li, and Shigui Yang. Characteristics of Viral Shedding Time in SARS-CoV-2 Infections: A Systematic Review and Meta-Analysis. *Frontiers in Public Health*, 9, March 2021.
- [13] Paola Foladori, Francesca Cutrupi, Nicola Segata, Serena Manara, Federica Pinto, Francesca Malpei, Laura Bruni, and Giuseppina La Rosa. SARS-CoV-2 from faeces

to wastewater treatment: What do we know? A review. *Science of The Total Environment*, 743:140444, November 2020.

- [14] Sarah Nadeau, Alexander J. Devaux, Claudia Bagutti, Monica Alt, Evelyn Ilg Hampe, Melanie Kraus, Eva Würfel, Katrin N. Koch, Simon Fuchs, Sarah Tschudin-Sutter, Aurélie Holschneider, Christoph Ort, Chaoran Chen, Jana S. Huisman, Timothy R. Julian, and Tanja Stadler. Influenza transmission dynamics quantified from RNA in wastewater in Switzerland. *Swiss Medical Weekly*, 154(1):3503–3503, January 2024.

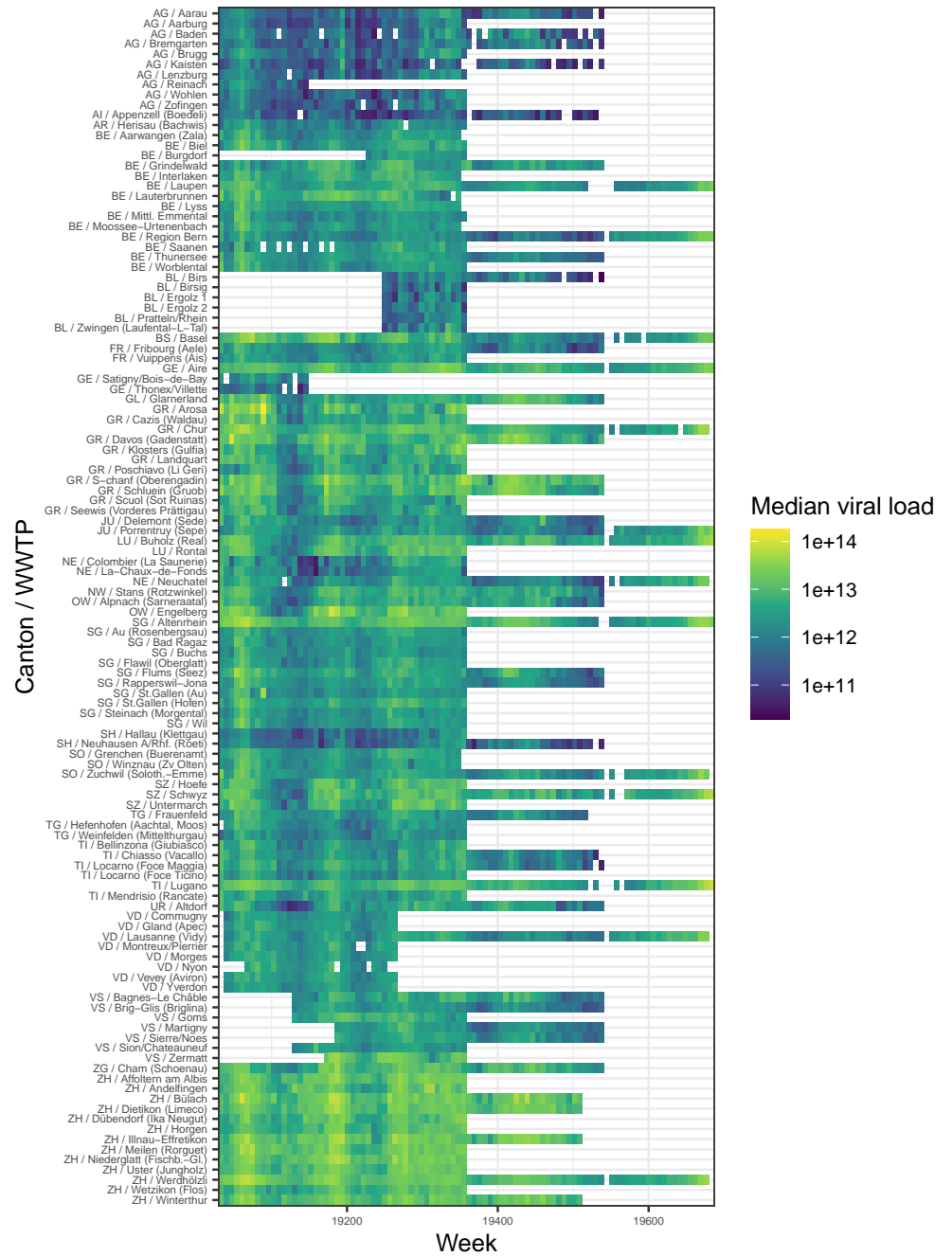


Fig 1. Weekly median SARS-CoV-2 viral load in wastewater by wastewater treatment plant [WWTP] (values below the limit of detection or of quantification are removed).