Executive Summary

Aim. To follow the dynamics of the Covid-19 pandemic independently of reported cases, the Federal Office of Public Health supported the development and application of wastewater-based epidemiology (WBE) through this research project *AbwasSARS-CoV-2*.

Monitoring period and locations. From 1 February 2021 to 30 April 2023, Eawag analyzed daily raw wastewater samples for SARS-CoV-2 RNA from six locations. These encompassed catchments of the six wastewater treatment plants Altenrhein, Chur, Geneva, Laupen, Lugano and Zurich, covering together approximately 14% of the Swiss population. Across all locations, SARS-CoV-2 RNA was detected in almost all samples¹. The results were typically published once per week on a publically accessible online dashboard.

Comparison of wastewater and case data. During most of the monitoring period, Switzerland invested substantially in clinical case surveillance, allowing robust comparisons between wastewater and clinical data. The 7-day medians of wastewater data and reported positive cases showed similar trends until January 2023, when free clinical testing was abandoned. Consequently, the number of reported cases decreased substantially, while wastewater data was unaffected. The correlation between wastewater data and case data remained high, however, at a different scale. Although only every second infected person sheds SARS-CoV-2 RNA in feces, SARS-CoV-2 was detected almost all days except during a few days in summer 2021 when incidence was low. The findings highlight the potential of wastewater to provide objective insights into COVID-19 disease dynamics.

Effective reproductive number. Alignment between wastewater data and clinical case reporting was further assessed through comparison of the effective reproductive number R_e . R_e indicates the efficiency of SARS-CoV-2 spread in a given population. Throughout the study, the R_e estimated from clinical cases and wastewater largely agreed. Notably, during periods with low clinical surveillance – indicated by high test positivity rates or very low numbers of reported cases – the uncertainty of R_e based on case data increased substantially, while the uncertainty based on wastewater remained low.

Open challenges. Wastewater shows SARS-CoV-2 dynamics but cannot yet be used to estimate an absolute number of infected individuals. This is due to uncertainty in: i) variation in the virus shedding rates among individuals, ii) fate of viral RNA during in-sewer transport, and iii) impacts of lab methodology such as efficiency of RNA extraction. Establishment of empirical relationships between RNA loads in wastewater and case numbers suffers from potential bias in case numbers due to the unknown fraction of unreported cases and varying testing regimes, which depended on regional capacity of testing facilities and willingness to test.

Wastewater as lead indicator. Whether or not wastewater data can serve as an early indicator of Covid-19 is highly dependent on the investments in clinical case surveillance. In Switzerland, clinical sample processing and reporting was sufficiently efficient such that wastewater data dynamics were coincident to clinical case dynamics. However, when there was limited or insufficient testing (high positivity rates), wastewater still provided timely information about the extent of circulation of SARS-CoV-2 in the population.

Variants of concern. The wastewater extracts obtained in this project were sequenced and the sequencing data was used to estimate the prevalence of (emerging) variants [sequencing and analysis was part of another contract]. Typically, new variants could be detected earlier – up to several weeks – in wastewater compared to sequencing clinical samples.

Outlook. In view of the <u>suggested institutionalization of WBE</u> by Swiss parliament² the experience from this project can inform future activities: to guarantee high quality data, it is recommended to take and analyze at least five wastewater samples per week. This will allow estimating reliably the effective reproductive number R_e and facilitate detecting the introduction and prevalence of emerging variants. Approximately 25% of the Swiss population could be covered when sampling ten large wastewater treatment plants. What has been shown successfully for SARS-CoV-2 in this project can be extended to other pathogens. When there is already investment in the infrastructure to collect, transport, and process samples, the surveillance can be extended to other pathogens at little additional cost, e.g. to respiratory viruses such as RSV or Influenza A and B. An additional beneficial potential is the analysis of wastewater for chemicals, e.g., pharmaceuticals with abuse potential, (il)licit drugs, antihistamines and other exogenous and endogenous health indicators.

... on average in 88% of samples [Feb - Nov 2021, laboratory protocol based on centrifugal filter concentration] ... in over 99.4% of samples [Nov 2021 to Apr 2023, laboratory protocol based on direct total nucleic acid extraction] ² <u>https://www.parlament.ch/de/ratsbetrieb/amtliches-bulletin/amtliches-bulletin-die-verhandlungen?SubjectId=60611</u>

¹ Detection of N-gene targeting the N1-region ...