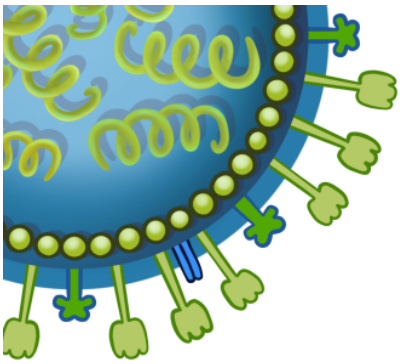
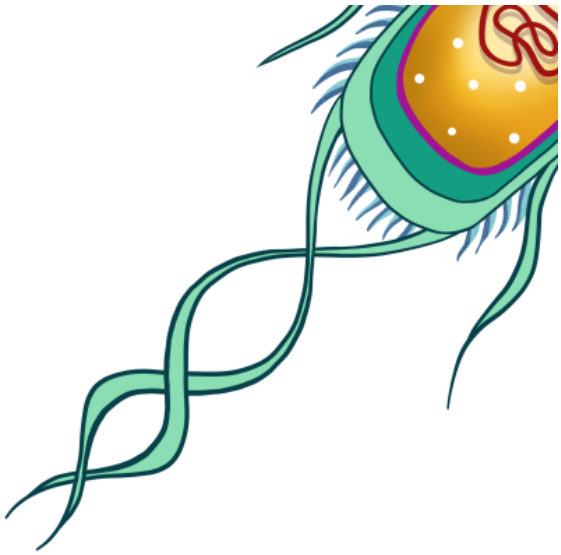


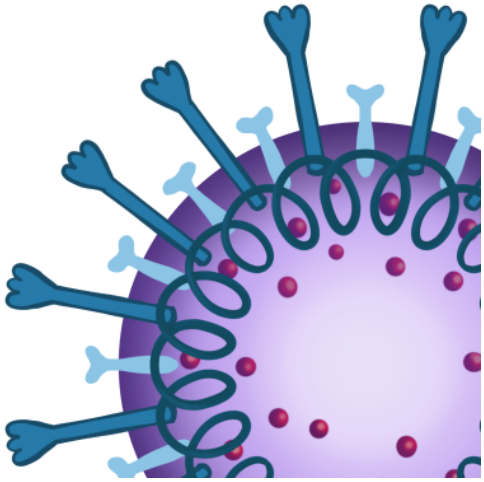


Wastewater-Based Infectious Disease Surveillance in Switzerland

Update: July 2023 - April 2024

Version 3 (13 September 2024)

Final



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Background

In February 2020, Eawag and EPFL began a collaboration on a project to develop wastewater-based testing of SARS-CoV-2. The project was initiated in response to the Covid-19 pandemic and was intended to determine if SARS-CoV-2 RNA loads in wastewater could provide insight into COVID-19 disease dynamics. The goal was to complement clinical testing, which in early 2020 was not yet fully established.

SARS-CoV-2 RNA was detected in those early samples, but it was in July 2020 that the sample collection, transport, and processing was sufficiently realized to allow data collection in real time. In October 2020, Eawag published an online dashboard sharing data generated from ARA Werdhoelzli (Zurich) with the public.

In February 2021, the Federal Office of Public Health expanded the program, funding the sample processing for wastewater from six sites, representing the catchments of over 1.2 million people, or 14% of the Swiss population (ARA Werdhoelzli (Zurich), ARA Chur, ARA Sensetal (Laupen), ARA Altenrhein, STEP Aïre (Geneva), and IDA CDA Lugano). Again, in May 2022, the program was expanded, eventually reaching over 100 wastewater treatment plants that actively participated in the Federal Office of Public Health's SARS-CoV-2 monitoring at the height of wastewater surveillance efforts. Contributing to this effort was not only the wastewater treatment plant operators, who collected, stored, and shipped samples across Switzerland, but also the effort of laboratory staff from a network of commercial and cantonal laboratories.

As the urgency and impact of the Covid-19 pandemic receded, so did the need for such an extensive sampling network. In July 2023, the FOPH-funded monitoring program was reduced to 14 wastewater treatment plants, while the number of targets was expanded beyond SARS-CoV-2 to also include Influenza A, Influenza B, and Respiratory Syncytial Virus. Monitoring for these pathogens occurs simultaneously using a novel sixplex digital PCR assay developed at Eawag. The assay targets five genomic regions of four respiratory viral pathogens (IAV, IBV, RSV, and SARS-CoV-2 N1 and N2) as well as additionally targeting Murine Hepatitis Virus (MHV), which is not naturally present in wastewater. MHV is targeted because it is used, in our laboratory, in quality control and assurance. By monitoring recovery of MHV, we ensure consistency in the application of our laboratory methods. The methods and results of monitoring from July 2023 through April 2024 are included in this report.

The report also includes data on the presence and proportion of culturable antibiotic bacteria in a subset of six wastewater treatments across Switzerland. Antimicrobial resistance (AMR) is among the top ten public health threats today. In 2019, nearly five million deaths were linked to bacterial infections related to AMR (Antimicrobial Resistance Collaborators 2022). Surveillance for antimicrobial resistance is overwhelmingly driven by reports on resistance from clinics, limiting available information on resistance circulating in communities. In a pilot program during this reporting period, we tracked culturable resistance of a subset of priority pathogens, namely extended-spectrum beta-lactamase producing *Escherichia coli* (ESBL- *E. coli*), carbapenemase-producing *E. coli* (CP-*E. coli*), vancomycin-resistant *Enterococcus faecium/fecalis* (VRE), ESBL-KESC (*Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Citrobacter* spp.), carbapenemase-producing KESC (CP-KESC), and methicillin-resistant *Staphylococcus aureus* (MRSA). The antimicrobial resistant bacteria were chosen based on the World Health Organization's list of bacterial priority pathogens (which includes a list of bacterial pathogens of particular public health importance), availability of culture-based methods, and likelihood of quantification in wastewater (World Health Organization 2024, *WHO Integrated Global Surveillance on ESBL-Producing E. Coli Using a "One Health" Approach: Implementation and Opportunities* 2021). The data provide insights into the extent to which wastewater-derived data can support understanding of antibiotic resistance epidemiology.

Wastewater surveillance is a burgeoning scientific field, with potential promise to expand the depth and breadth of its impact on understanding public health. In late 2021, the Swiss National Science Foundation, through the Sinergia funding scheme, provided a consortium of researchers at ETH Zurich, EPFL, and Eawag a four-year research grant to advance the field of wastewater-based surveillance scientifically. The funding supports the monitoring effort by providing opportunities to investigate novel research to improve characterization and understanding of wastewater as a resource for infectious disease epidemiology.

The scope of this report is on quantifying target pathogens and antimicrobial resistance in wastewater treatment plants across Switzerland. This is only one type of public health data currently being derived from wastewater in Switzerland. Other projects include DroMedAria[1], which monitors pharmaceuticals, illicit drugs,

[1] DroMedAria project available at: https://www.dromedario.ch/ (Accessed 30 June 2023)

and other substances; monitoring SARS-CoV-2 variants through sequencing wastewater extracts[2]; and estimating the effective reproduction number for SARS-CoV-2, Influenza A and B, and Respiratory Syncytial Virus[3].

Sampling Sites

For the reporting period, we processed wastewater from 14 wastewater treatment plants across Switzerland serving approximately 2.3 million people, or 27% of the total Swiss population (Table 1, Figure 1).

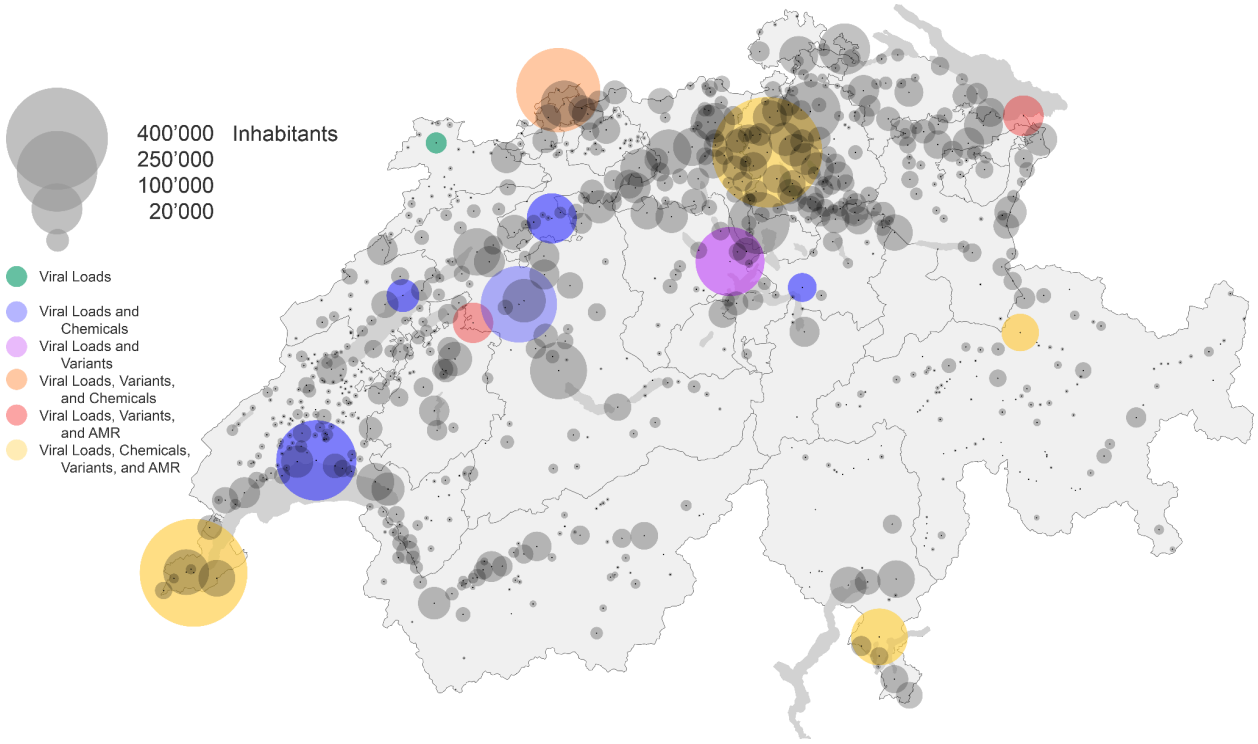


Figure 1: Map of the locations of all wastewater treatment plants in Switzerland, with the locations of treatment plants providing samples for processing for respiratory viruses highlighted in color. Color coding is based on the other analyses occurring at the location, including for monitoring of viral loads, SARS-CoV-2 variants, antimicrobial resistance, and chemicals as part of theDroMedAario[4] project.

[2] CovSpectrum wastewater data available at: https://cov-spectrum.org/stories/wastewater-in-switzerland; (Accessed 30 June 2023)

[3] Effective Reproduction Number estimates available at https://wise.ethz.ch (accessed 30 June 2024).
[4] https://www.dromedario.ch/ (Accessed 28 June 2024).

Table 1: Names, locations, and estimated populations served by the wastewater treatment plants enrolled in the monitoring of respiratory pathogens. Wastewater treatment plants that are also monitored for variants, antimicrobial resistance, and chemicals are noted in the additional columns.

Kanton	**WWTP Name**	**City/Region**	**Est. Pop.**	**Variants**	**Antimicrobial Resistance**	**Chemicals**
ZH	ARA Werdhoelzli	Zürich	471000	x	x	x
GE	STEP Aïre	Geneva	451000	x	x	x
BE	ARA Region Bern	Bern	225000			x
BS	ARA Basel /ProRheno	Basel	273000	x		x
VD	STEP Vidy	Lausanne	248000			x
LU	ARA Buholz	Luzern	184000	x		
TI	IDA CDA Lugano	Lugano	124000	x	x	x
GR	ARA Chur	Chur	55000	x	x	x
BE	ARA Sensetal	Laupen BE	62000	x	x	
NE	STEP Neuchatel	Neuchâtel	41000			x
JU	ARA Porrentruy	Porrentruy	16500			
SG	ARA Altenrhein	Altenrhein	64000	x	x	
SZ	ARA Schwyz	Schwyz	31000			x
SO	ARA Zuchwil	Solothurn	96000			x

Methods

From July 2023 until June 2024, 3’465 wastewater samples were processed for respiratory virus pathogens from 14 wastewater treatment plants (see Sampling Sites). The total number of processed wastewater samples highlights the effectiveness of the program logistics, as it is equivalent to approximately 4.75 samples per week per treatment plant. Samples were shipped on ice weekly from the wastewater treatment plants to Eawag (Duebendorf). To quantify loads of respiratory pathogens, samples were processed within 24 hours of receipt. To quantify culturable antimicrobial resistance, only the most recent samples collected were processed from six wastewater treatment plants (Figure 1), resulting in total storage and transport times of up to 48 hours. The number of samples processed for antimicrobial resistance varied by target and are reported in the results.

Respiratory Pathogens

Wastewater sample processing and nucleic acid extraction.

Wastewater influent (24-hour composite) samples were routinely collected by WWTP personnel, stored at 4°C and transported on ice within one week to our laboratory at Eawag, Swiss Federal Institute of Aquatic Science and Technology in Dübendorf. Before 1 June 2024, five samples from each WWTP were processed weekly; after this date four samples per week were processed. Nucleic acids were extracted from 40 ml of wastewater using a modified version of the Wizard Enviro Total Nucleic Acid Kit (Promega Corporation, USA), as previously described (Nadeau et al. 2024). Nucleic acids were eluted in 80 µl RNAse-free water and then purified using the OneStep PCR Inhibitor Removal Kit (Zymo Research, USA). Extracts were analyzed using dPCR immediately following their preparation on the same day, and additional aliquots were stored at -80°C.

Digital PCR assays and PCR inhibition

All dPCR assays were developed on the Naica® system 6-color Crystal Digital PCR Prism-6 (Stilla Technologies, France). For this reporting period, we relied on a six-plex digital PCR assay, referred to as RESPV6, which measures the presence of SARS-CoV-2, Influenza A, Influenza B, and Respiratory Syncytial Virus. RESPV6 was created by merging two different assays previously used in the Swiss monitoring program: i) a duplex assay (targeting the SARS-CoV-2 nucleoprotein gene locus 1 [N1] and MHV), and ii) a previously described four-plex assay, known as RESPV4, with some modifications (Nadeau et al. 2024).

The RESPV6 assay was prepared using a total 27 µl pre-reaction volume, which consisted of 21.6 µl of mastermix (qScript XLT One-Step RT-qPCR ToughMix, QuantaBio) and 5.4 µl of template. The reaction volume (25 µl) was loaded into Sapphire chips (Stilla Technologies). Chips were loaded into a Geode (Stilla Technologies) which partitions the mastermix into droplets (12 min at 40°C) before thermocycling using the following conditions: reverse transcription (50°C for 1 h), enzyme activation (95°C for 5 min), and 40 cycles of denaturation (95°C for 30 s) and annealing/extension (57.5°C for 1 min).

PCR inhibition in the RESPV6 assay was evaluated for a subset of samples, as previously described (Nadeau et al. 2024; Huisman et al. 2022). When inhibition was detected above our specified threshold of 60% or more inhibition, samples from the corresponding wastewater treatment plant were processed again on digital PCR with increased dilution. In this way, inhibition measurements were used as in quality control, to ensure measurements were not impacted substantially by inhibitory substances.

RNA extraction efficiency quality control.

To determine the efficiency of RNA extraction, a known amount of viral control (Murine Hepatitis Virus strain MHV-A59, or MHV) was spiked into 40 ml of wastewater prior to processing. Measurement of MHV RNA simultaneous to the other respiratory pathogens in six-plex dPCR assay allowed estimation of recovery efficiency by dividing the measured concentration by the estimated spiked concentration and expressing the result as a percentage. Recovery efficiency was used to monitor consistency in lab processing, but was not used to adjust viral concentration measurements.

Positive and negative controls.

Positive controls used in dPCR assays were prepared by combining nucleic acid templates to achieve approximately 500 gene copies per µl for each target. Synthetic viral RNA (SARS-CoV-2 RNA reference material; EURM-019, Joint Research Center) was used for SARS-CoV-2, whereas gBlocks (Integrated DNA Technologies, USA) were used for Influenza A and B, and RSV. Positive material for MHV was obtained by extraction.

Negative controls included one full process control per week, which consists of a sampling bottle pre-filled with sterile water and shipped to one of the wastewater treatment plants. The bottle is returned with the samples, and then processed alongside the samples from that treatment plant. Additionally, every thermocycler included one no template control (NTC). The full process controls consistently had 2 or fewer positive fluorescent droplets, consistent with our definition of a non-detect. When the NTC had 3 or more positive fluorescent droplets, all samples in that thermocycler were measured a second time.

Comparison with clinical data from Sentinella.

The Swiss Federal Office of Public Health (FOPH) collects clinical data about respiratory viruses mainly through two survey systems: the mandatory reporting system and the Swiss Sentinel[5]. In this report, we refer only to data collected via the Swiss Sentinel System (Sentinella), which is a voluntary reporting system involving around 160 to 180 family physicians and pediatricians and provides data for the four pathogens targeted for quantification covered with our RESPV6 assay. Data is presented as it is publicly available[6], aggregated to the total number of cases reported per week across all of Switzerland.

Data and statistical analysis.

All dPCR data were analyzed using the Crystal Miner Software version 4.0 (Stilla Technologies), which provides RNA quantities expressed as gene copies per microliter of reaction (gc/µl of reaction). Wastewater samples were defined as positive when the concentration was above the limit of detection (LoD), which was set to a threshold of at least three positive partitions per well, corresponding to approximately 5 gc/reaction. Values were transformed to gene copies per liter of wastewater (gc/Lww). Viral RNA concentrations were then converted to total daily loads per treatment plant by multiplying by total daily wastewater flow. A per person viral RNA load (expressed as gc/person-day) was calculated by dividing the total daily load by the average population in the wastewater treatment plant catchment area.

[5] https://idd.bag.admin.ch/survey-systems/overview (Accessed 5 September 2024)
[6] https://www.idd.bag.admin.ch/survey-systems/sentinella (Accessed 15 June 2024)

Antimicrobial Resistance

Sample Collection and Processing

For antimicrobial resistance monitoring, wastewater samples were collected from six WWTPs in Switzerland (Figure 1). As this is the first report covering antimicrobial resistance surveillance in wastewater, we include all samples processed through April 2024. Specifically, samples were collected weekly since November 2021 for ESBL-*E. coli* and since December 2022 for CP-*E. coli*, CP-KESC, ESBL-KESC, MRSA, VRE.

Enumeration of total and ESBL-producing *E. coli*

To enumerate total *E. coli*, wastewater samples were serially diluted in 2 steps of 10-fold dilution with sterile 0.9% NaCl. 100 µl of the resulting 100-fold dilution was plated on CHROMagar™ Orientation chromogenic media (CHROMagar, France). To enumerate ESBL-*E. coli*, 100 µl of undiluted wastewater samples were plated on CHROMagar™ ESBL chromogenic media (CHROMagar, France). Samples were plated in single replicates until February 08, 2022, and afterward in duplicates. Plates were incubated at 37°C for 24 hours. Colony counts were determined by manually counting the dark pink to reddish colonies on the CHROMagar™ Orientation for total *E. coli* and CHROMagarTM ESBL for ESBL- *E. coli.*

Enumeration of total and ESBL-producing KESC

The classification of KESC in microbiology refers to a set of bacteria capable of growth on the same media: *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., and *Citrobacter* spp. In this study, we report the group of KESC as further resolution to the species level would require additional resources. To enumerate total KESC, wastewater samples were serially diluted in three steps of 10-fold dilutions with sterile 0.9% NaCl, and 100 µl of the resulting 1000-fold dilution were plated on CHROMagar™ Orientation chromogenic media (CHROMagar, France). ESBL-KESC were enumerated from the CHROMagar™ ESBL plates used for counting ESBL-E. coli (see above). Colony counts were then determined by manually counting the dark blue to metallic blue colonies for total KESC on CHROMagar™ Orientation, and the respective colored colonies for ESBL-KESC on the CHROMagar™ ESBL plates.

Enumeration of carbapenemase-producing *E. coli* (CP-*E. coli*) and KESC

To enumerate carbapenemase-producing *E. coli* (CP-E. coli) and KESC, 100 µl of undiluted wastewater samples were plated on CHROMagar™ mSuperCARBA™

chromogenic media (CHROMagar, France). Plates were incubated at 37°C for 24 hours. Colony counts were then determined by manually counting the respective colored colonies for CP-*E. coli* (dark pink to reddish) and CP-KESC (dark blue to metallic blue) on the CHROMagar™ mSuperCARBA™ plates. Total *E. coli* and total KESC were enumerated as described in the paragraphs above.

Enumeration of total and vancomycin resistant *Enterococcus faecium/fecalis* (VRE)

To enumerate total *Enterococcus faecium/fecalis*, wastewater samples were serially diluted in three steps of 10-fold dilution steps with sterile 0.9% NaCl until January 2024, and in two steps of 10-fold dilutions thereafter. 100 µl of the diluted samples were plated on CHROMagar™ VRE chromogenic media (CHROMagar, France) without supplement. To enumerate VRE, 100 µl of undiluted wastewater samples were plated on CHROMagar™ VRE chromogenic media (CHROMagar, France) which contains 6 mg/L vancomycin. Plates were incubated at 37°C for 24 hours. Colony counts were then determined by manually counting the pink to mauve colonies on the CHROMagar™ VRE without supplement for total *Enterococcus faecium/fecalis* and on the CHROMagar™ VRE for VRE.

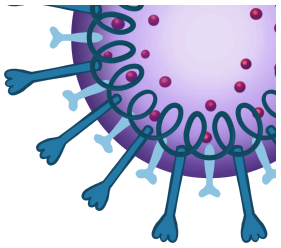
Enumeration of total and methicillin-resistant *Staphylococcus aureus* (MRSA)

To enumerate total *Staphylococcus aureus*, 100 µl of undiluted wastewater samples were plated on CHROMagar™ Staphylococcus chromogenic media (CHROMagar, France). To enumerate MRSA, 100 µl of undiluted wastewater samples were plated on CHROMagar™ MRSA chromogenic media (CHROMagar, France). Plates were incubated at 37°C for 24 hours. Colony counts were then determined by manually counting the pink to mauve colonies on the CHROMagar™ Staphylococcus for total *Staphylococcus aureus* and on the CHROMagar™ MRSA for MRSA.

Statistical analyses

All analyses were conducted using R (v4.1.1) and R Studio (v2022.12.0.353). For each sample, the percentage of resistant bacteria was calculated by dividing the number of resistant colonies by the number of total colonies. When sample duplicates were available, the average percentage of resistant bacteria was computed for both display and analysis purposes.

Results: SARS-CoV-2



SARS-CoV-2 is the causative agent of Covid-19. We monitor two targets for SARS-CoV-2, the N1 and N2 gene targets. These gene targets are widely used in clinical diagnostics and wastewater monitoring. The decision to use two targets was driven by mutations in the N1 gene region, causing concern that counts in wastewater may be underestimated due to reduced amplification efficiency (Lesbon et al. 2021).

Throughout the monitoring period, we detected SARS-CoV-2 in almost all of our samples (98% of the samples). From this finding, we assume SARS-CoV-2 continues to circulate in Switzerland throughout the year at all sites. This high detectability is distinct from the detectability of the other three viruses we look for in wastewater, which are characterized by less frequent detection. We also observe differences in variation in loads, with some sites (ARA Werdhoelzli in Zurich) being more stable than others (STEP Vidy in Lausanne) (Figure 2).

SARS-CoV-2 loads are substantially higher than loads from other respiratory pathogens we track (more than one order of magnitude higher). The reasons are unclear, but may be driven by a combination of: higher viral loads in people during infections (leading to higher viral counts in wastewater per infected person), more pronounced fecal shedding, longer duration of shedding, and higher infection prevalence rates (a higher number of people infected).

SARS-CoV-2 dynamics showed outbreaks occurring at all sites. A considerable wave of SARS-CoV-2 loads occurred nationally between October 2023 and February 2024, appearing in all fourteen sites monitored (Figures 2-4). Notably, the loads at many of the sites tracked during this period exceeded the highest loads we had ever recorded. A second wave is observed toward the end of the study period (April 2024), coinciding with the timing of the introduction of the KP.2. variant of SARS-CoV-2. This wave is noticeable at STEP Vidy (Figure 2).

The samples processed at Eawag are also sequenced to determine the presence and relative abundance of circulating SARS-CoV-2 variants. This work is conducted by the group of Niko Beerenwinkel (ETH Zurich), Functional Genomics Center Zurich, and NEXUS (ETH Zurich). Results are reported on covSpectrum[7].

[7] https://cov-spectrum.org/stories/wastewater-in-switzerland (Accessed June 28, 2024)

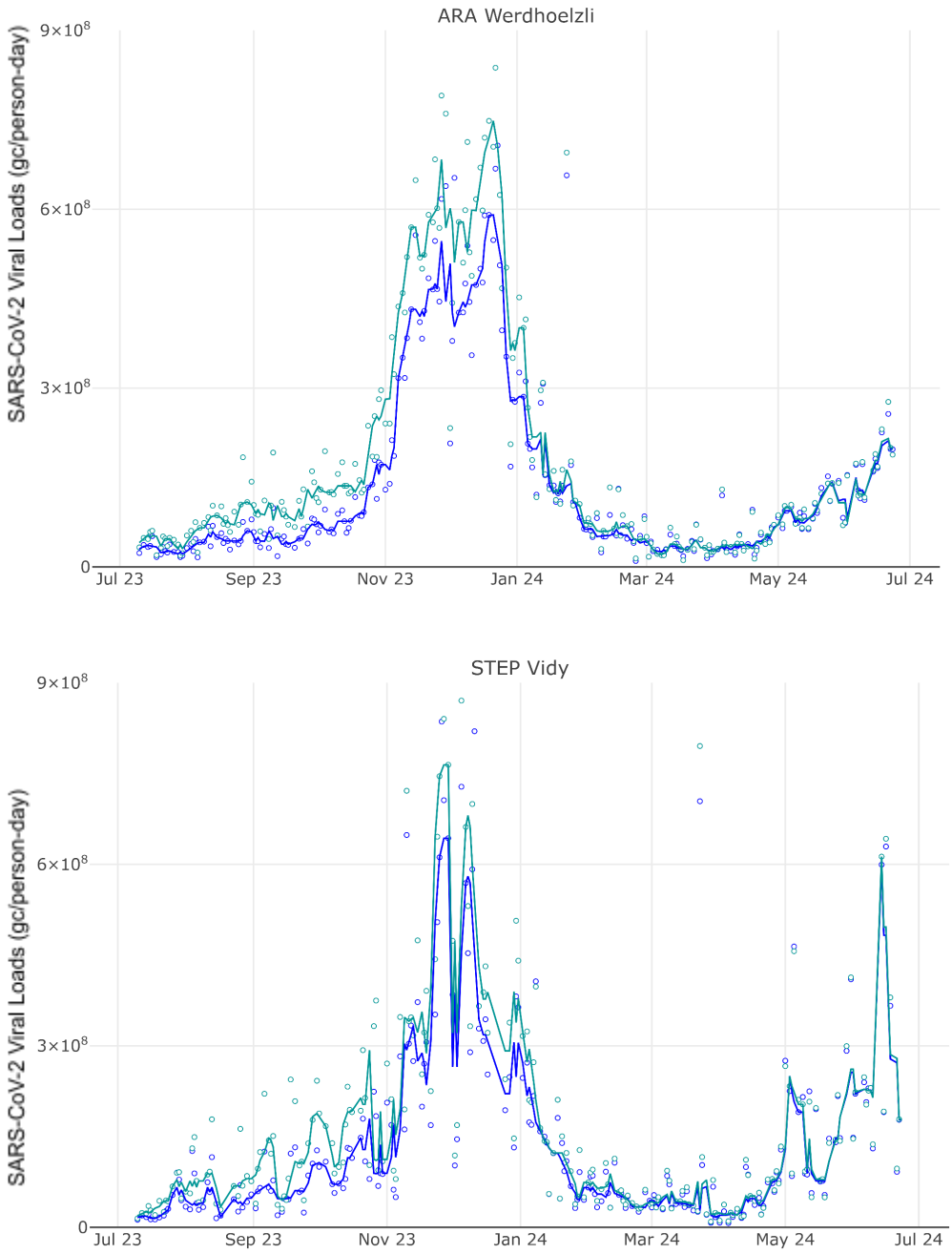


Figure 2: SARS-CoV-2 loads for ARA Werdhoelzli in Zurich (top) and STEP Vidy in Lausanne (bottom) over the reporting period. Sample loads (open circles) and 7-day rolling median (lines) are presented for two SARS-CoV-2 targets N1 (dark blue) and N2 (light green) monitored throughout the study period.

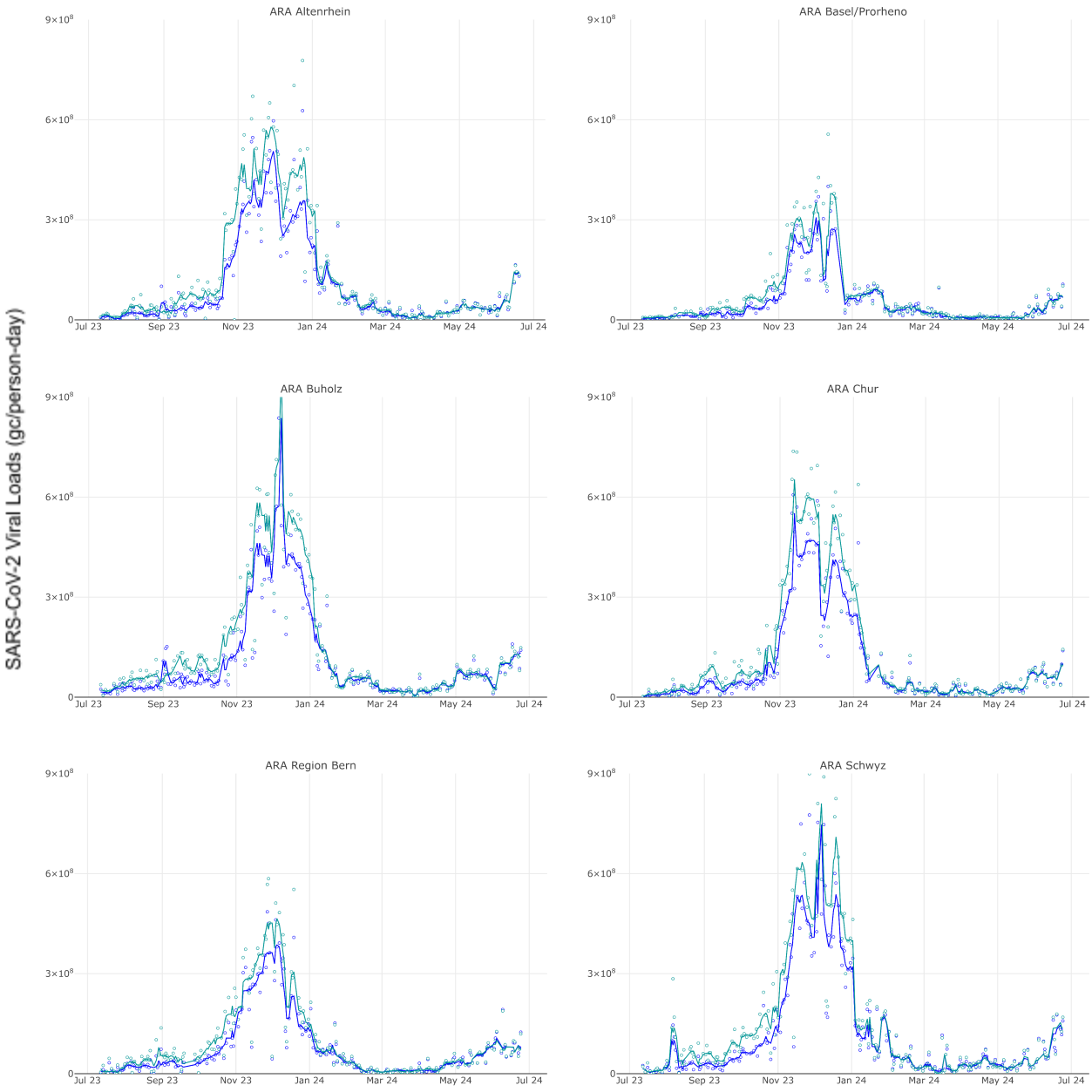


Figure 3: SARS-CoV-2 loads for ARA Altenrhein, ARA Basel, ARA Buholz in Emmen, ARA Chur, ARA Region Bern, and ARA Schwyz over the reporting period. Sample loads (open circles) and 7-day rolling median (lines) are presented for two SARS-CoV-2 targets N1 (dark blue) and N2 (light blue) monitored throughout the study period.

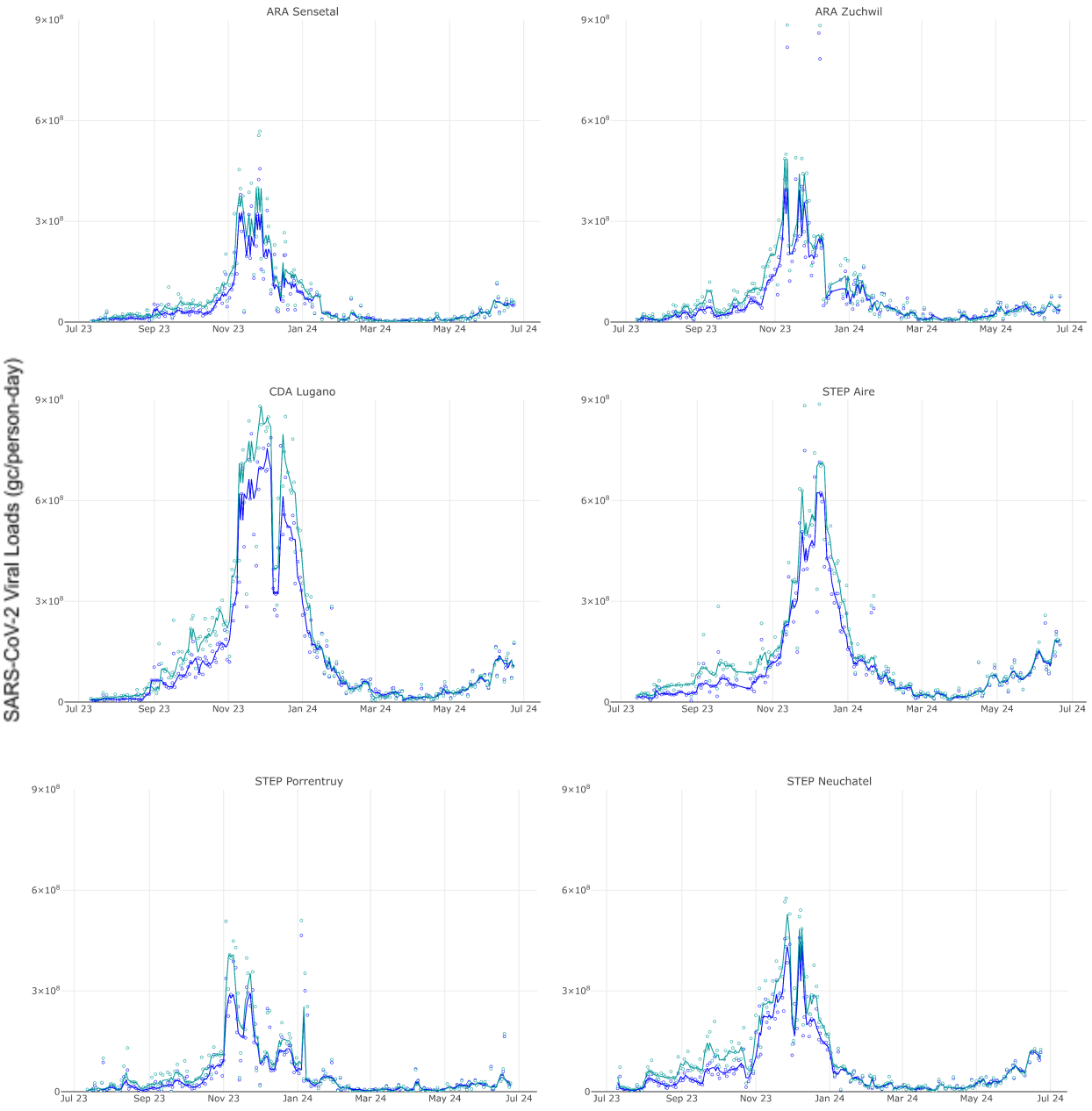


Figure 4: SARS-CoV-2 loads for ARA Sensetal in Laupen, ARA Zuchwil, IDA CDA Lugano, STEP Aire in Geneva, STEP Porrentruy, and STEP Neuchatel over the reporting period. Sample loads (open circles) and 7-day rolling median (lines) are presented for two SARS-CoV-2 targets N1 (dark blue) and N2 (light blue) monitored throughout the study period.

National Alignment of SARS-CoV-2 with Clinical Cases

SARS-CoV-2 concentrations observed in wastewater throughout the study period show alignment with the publicly available data on the number of positive tested cases among reported cases with acute respiratory infection in the Sentinella surveillance system (Figure 5). Spearman's rank correlation coefficient[8],) between the nationally-averaged viral RNA loads and the Sentilla surveillance systems is 0.77 ($p < 0.001$).

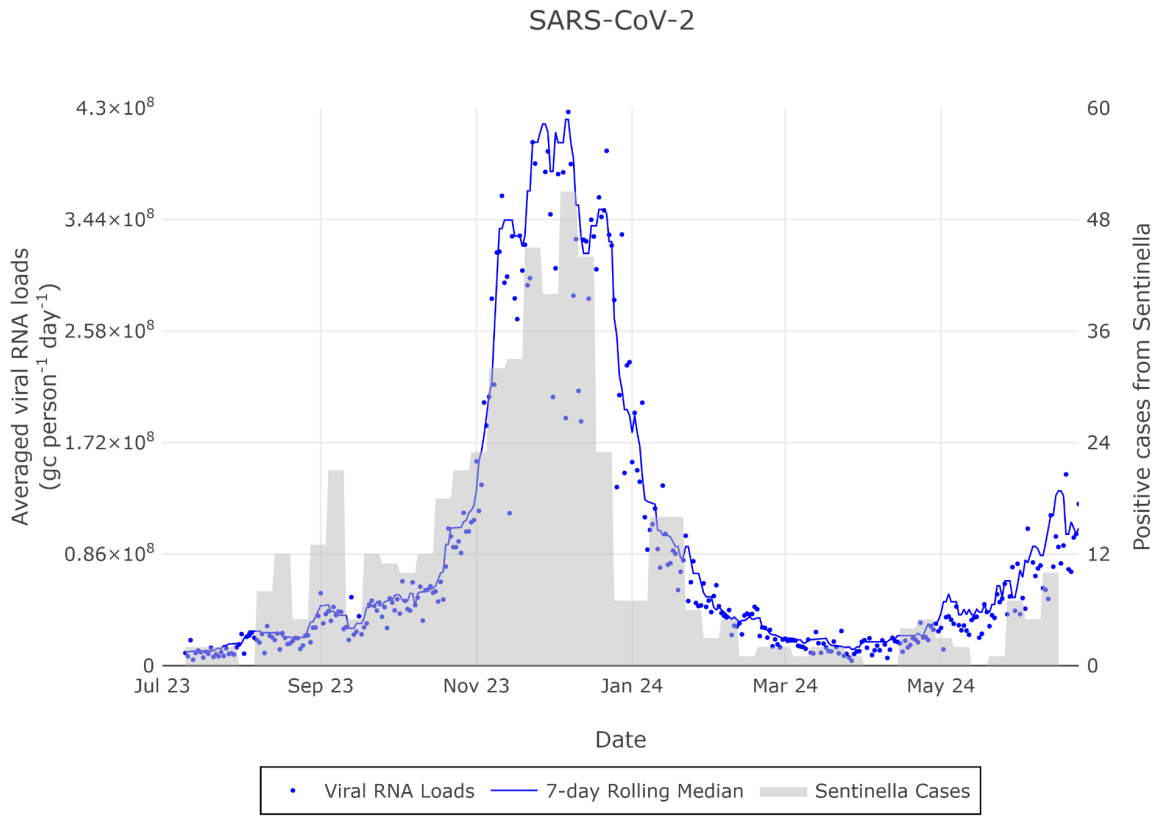
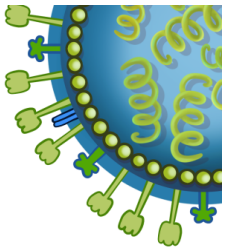


Figure 5: Comparison of averaged SARS-CoV-2 viral loads per person per day (blue dots) and corresponding 7-day rolling median (blue line) to the weekly number of positive tested cases among reported cases with acute respiratory infection in the Sentinella surveillance system for the whole of Switzerland (grey bars). Data are contemporary to data access date.

[8] Spearman's rank correlation is an indication of the strength and direction of an association of two variables and variables between -1 and 1. Positive values show a direct relationship, negative values show an inverse relationship. Higher absolute values (closer to -1 or 1) show stronger correlations than lower absolute values (closer to 0).

Results for Influenza A, Influenza B, and Respiratory Syncytial Virus



Seasonal flu (influenza) is transmitted by the Influenza A and Influenza B viruses.[9] Influenza viruses are included within the set of infectious diseases requiring notification in Switzerland.[10] Respiratory Syncytial Virus is also a respiratory virus[11], but notification is not required in Switzerland when detected in humans.

During the monitoring period, all three viruses were regularly detected in wastewater across all fourteen sites. Influenza A was detected in 25% (n = 801) of all samples processed, Influenza B was detected in 30% (n = 963), and Respiratory Syncytial Virus was also detected in 30% (n = 989). The lower frequency of detection of these pathogens relative to SARS-CoV-2 may be attributable to a combination of potential factors, including: 1) lower prevalence rates in the community (fewer of people infected), 2) lower shedding loads (fewer viruses shed into wastewater per infected person), 3) shorter durations of shedding (infected people shed into wastewater for shorter periods) relative to SARS-CoV-2, and 4) differential stability of the viral RNA in the wastewater stream (i.e., SARS-CoV-2 RNA is more stable than the other viruses tested). Indeed, we observed lower concentrations and corresponding loads of Influenza A, influenza B, and Respiratory Syncytial Virus compared to SARS-CoV-2 throughout the period (Figures 3-8), which is similar to historic observations.

Influenza A viral loads followed a clear seasonal pattern at all sites. For most sites (n = 13) including STEP Aïre in Geneva, the seasonal outbreak was observed between December 2023 and March 2024 (Figure 6). Notably distinct was the pattern observed in Lugano, where the peak occurred earlier than at the other sites (Figure 6) and with a higher per person per day peak load.

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[9] For more information, please see website maintained by FOPH at https://www.bag.admin.ch/bag/en/home/krankheiten/krankheiten-im-ueberblick/grippe.html (Accessed 27 June 2024)

[10] For more information, please see website maintained by FOPH at: https://www.bag.admin.ch/bag/en/home/krankheiten/infektionskrankheiten-bekaempfen/meldesysteme-infektionskrankheiten/meldepflichtige-ik.html (Accessed 27 June 2024).

[11] For more information, please see website maintained by FOPH at https://www.bag.admin.ch/bag/en/home/krankheiten/ausbrueche-epidemien-pandemien/aktuelle-ausbrueche-epidemien/respiratorische-viren.html (Accessed 27 June 2024).

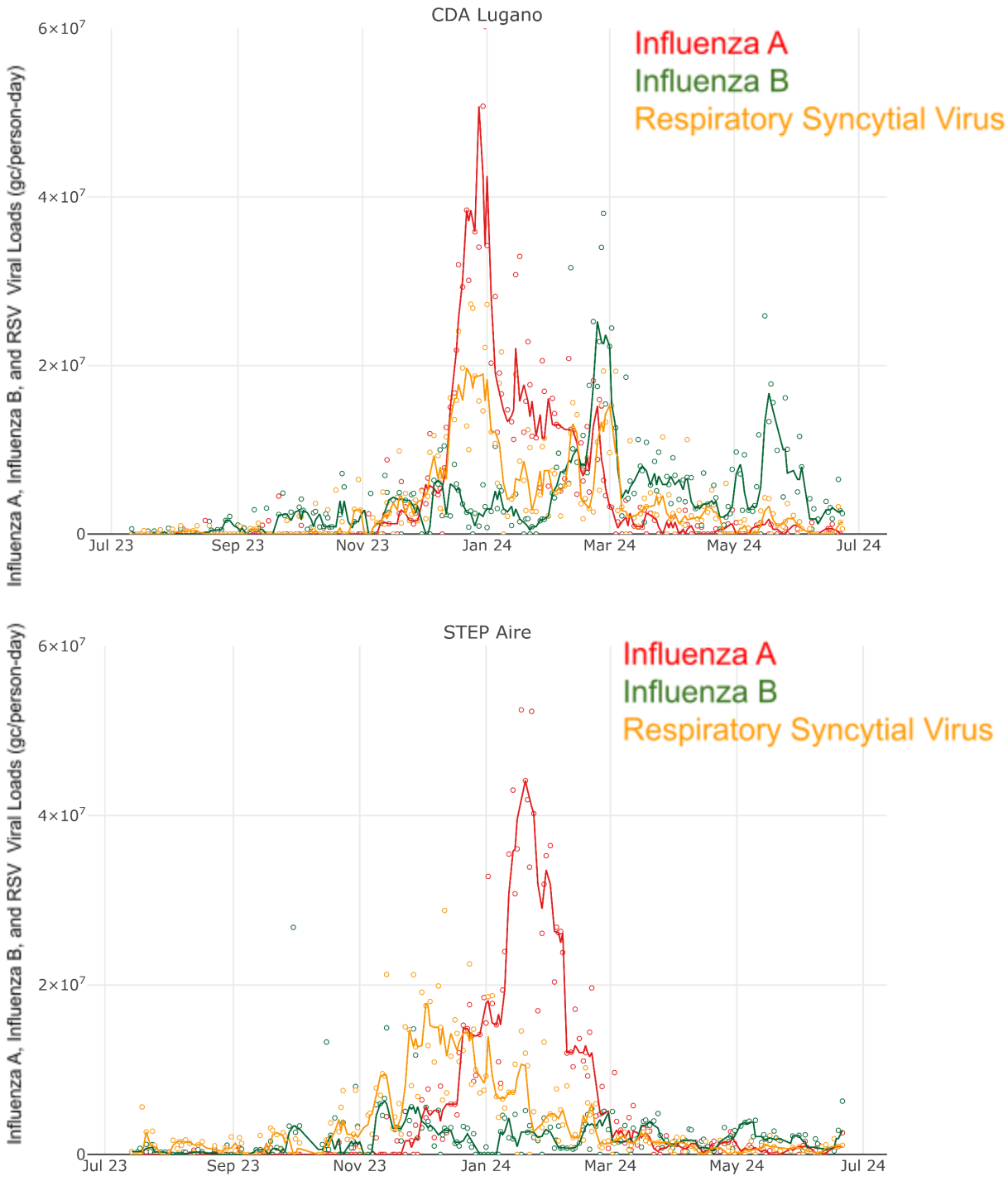

Figure 6: Influenza A (red), Influenza B (green), and Respiratory Syncytial Virus (orange) viral loads for Lugano (top) and Geneva (bottom) over the reporting period. Sample loads (open circles) and 7-day rolling median (lines) are presented.

We observed low and intermittent levels of Influenza B at most sites, with the seasonality less clear than observed for Influenza A (Figures 7-8). The lower peak loads, and lack of a clear seasonal signal, suggests that Influenza B circulation was lower than Influenza A circulation in Switzerland during the 2023-2024 winter. This finding aligns with clinical data from the Sentinella surveillance system showing more Influenza A cases than Influenza B cases (Figures 9-10).

Notably, two distinct sites but with adjacent catchments (ARA Region Bern, ARA Sensetal in Laupen) were characterized by lower peak loads of Influenza A, Influenza B, and Respiratory Syncytial Virus than the other sites. The cause of this is unclear, and may be attributable to lower prevalence of the pathogens circulating in these communities. Speculatively, it may alternatively be a consequence of the wastewater catchment area properties, such as potential dilution or increased decay of signals due to fate and transport processes in the sewer network. Evidence in support of lower prevalence in the community is that these two sites are distinct treatment plants but geographically co-located. Additionally, although peak loads of SARS-CoV-2 were also lower at these sites, the difference in peak loads was not as large as the difference observed for Influenza A, Influenza B, and Respiratory Syncytial Virus. Planned future monitoring at these sites, relative to others, may elucidate whether this phenomenon repeats next season.

Respiratory Syncytial Virus also circulated at lower levels than peak Influenza A at all sites (Figures 7-8). Respiratory Syncytial Virus loads followed a typical seasonal pattern with increased concentrations at many, but not all sites. Contributing to the lack of a clear trend is the intermittent detection of RSV throughout the entire monitoring period, which suggests ongoing, but low, prevalence of RSV throughout the year. Peak RSV loads were lower than than the previously observed season. When a national average load is calculated, the seasonal pattern of RSV across all sites becomes clear, aligning with dynamics observed from data collected within the Sentinella surveillance system (Figure 11).

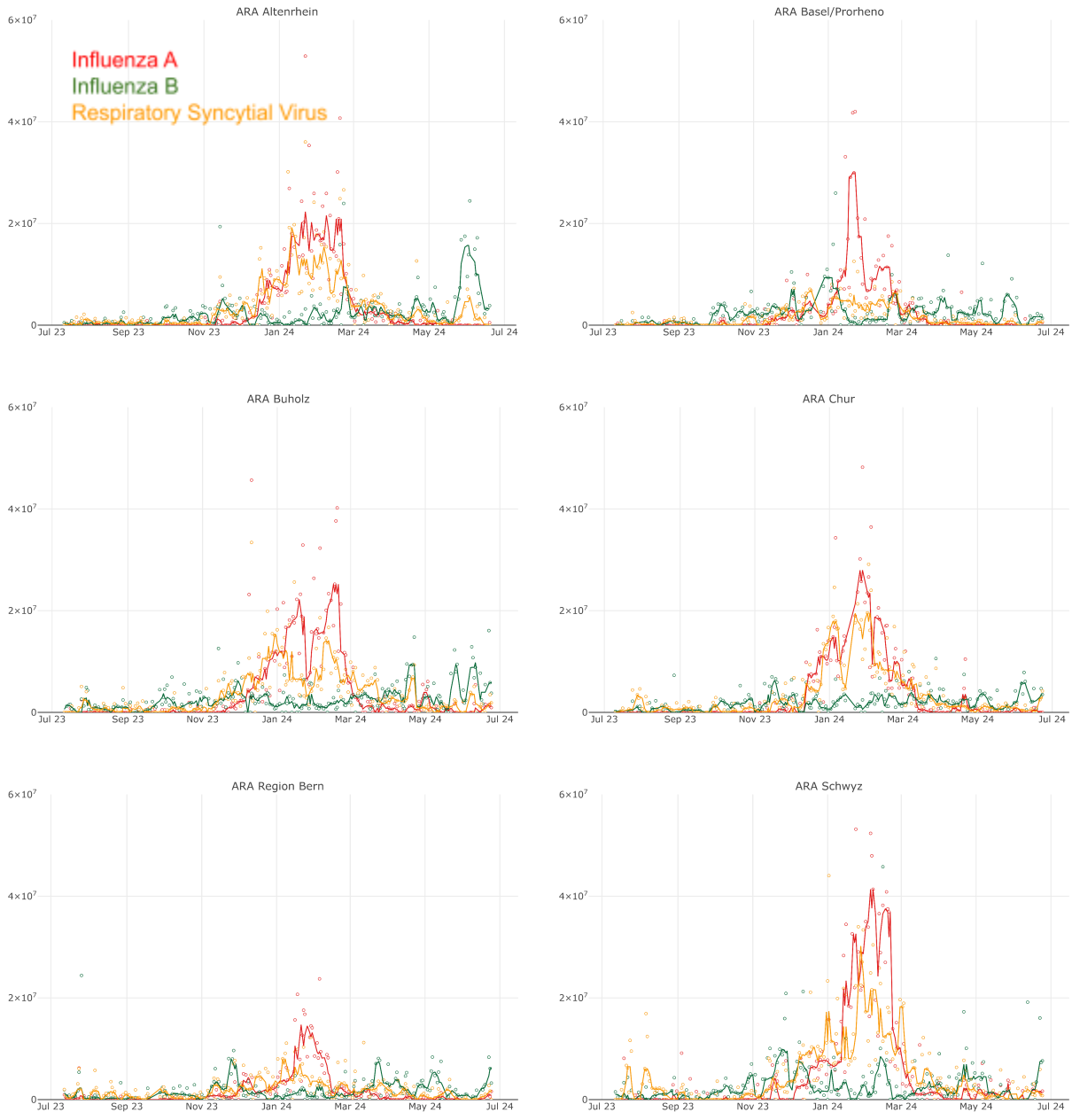

Figure 7: Influenza A (red), Influenza B (green), and Respiratory Syncytial Virus (yellow) viral loads for ARA Altenrhein, ARA Basel/ProRheno, ARA Buholz in Emmen, ARA Chur, ARA Region Bern, and ARA Schwyz over the reporting period. Sample loads (open circles) and 7-day rolling median (lines) are presented.

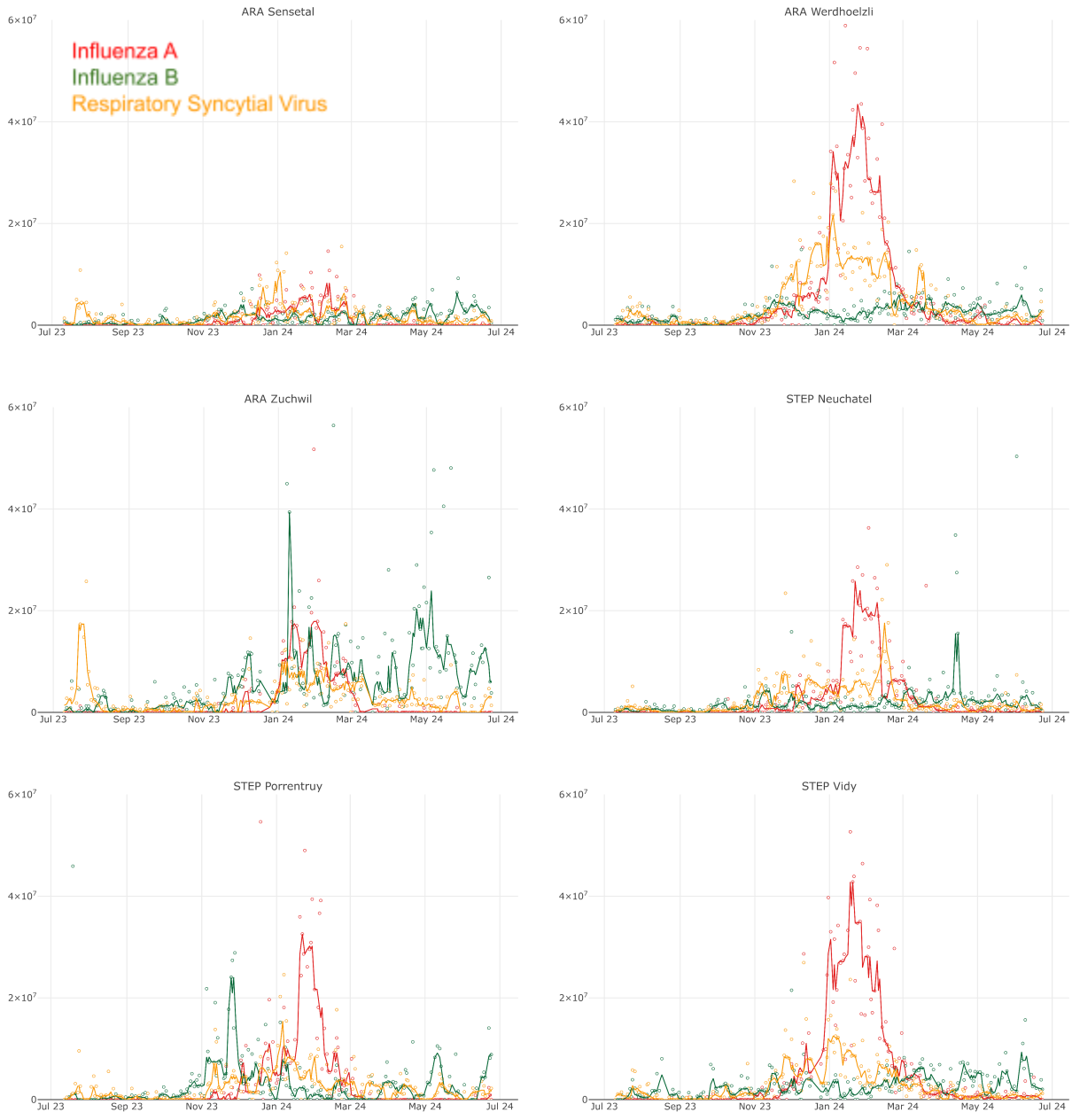

Figure 8: Influenza A (red), Influenza B (green), and Respiratory Syncytial Virus (yellow) viral loads for ARA Sensetal in Laupen, ARA Werdhoelzli in Zurich, ARA Zuchwil, STEP Neuchatel, STEP Porrentruy, and STEP Vidy in Lausanne over the reporting period. Sample loads (open circles) and 7-day rolling median (lines) are presented.

National Alignment with Clinical Cases

Influenza A

The averaged viral RNA loads for Influenza A aligned very well with the number of positive tested cases among reported cases with acute respiratory infection from the Sentinella surveillance system (Figure 9). Similar to SARS-CoV-2, the Spearman rank correlation coefficient was high (0.75, $p < 0.001$).

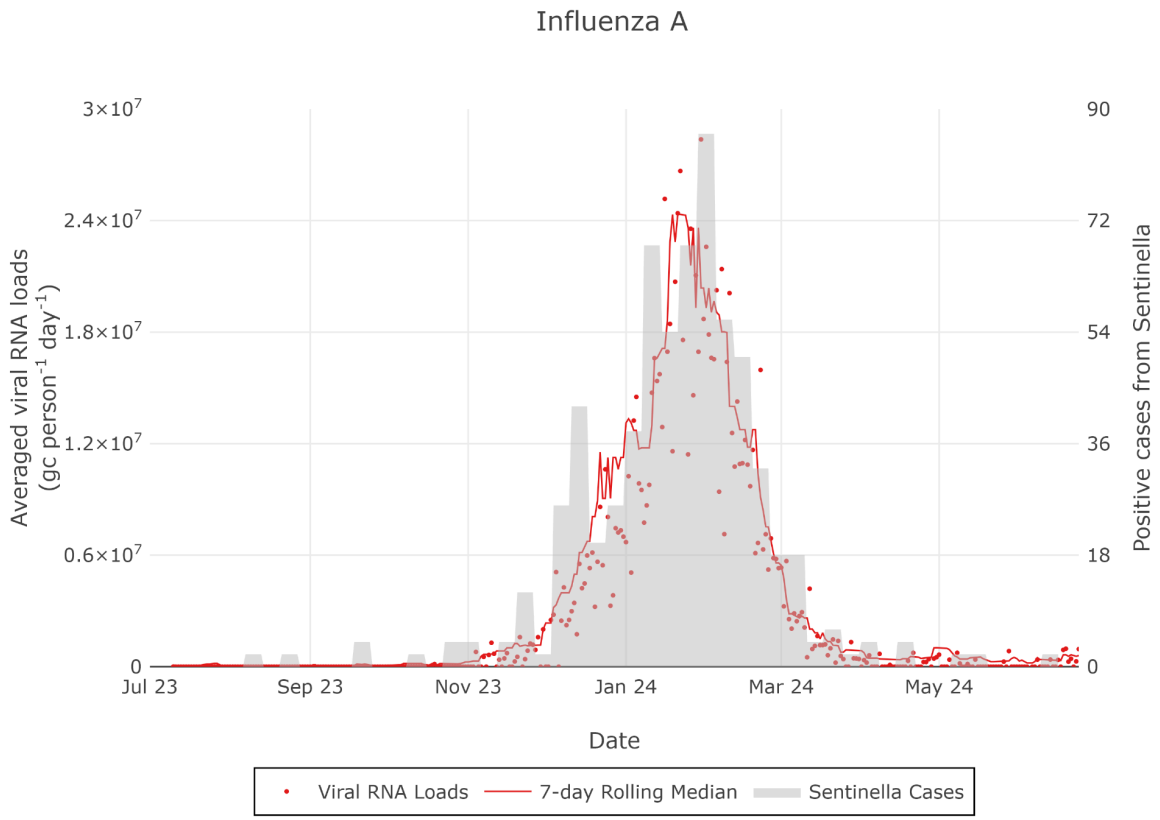

Figure 9: Comparison of averaged Influenza A viral loads per person per day (red dots) and corresponding 7-day rolling median (red line) to the weekly number of positive tested cases among reported cases with acute respiratory infection in the Sentinella surveillance system for the whole of Switzerland (grey bars). Data are contemporary to data access date.

Influenza B

The averaged viral RNA loads for Influenza B showed moderate alignment with the number of positive tested cases among reported cases with acute respiratory infection from the Sentinella surveillance system (Figure 10). This is highlighted by the visualization (Figure 10), as well as the relatively weak, but still significant, Spearman rank correlation coefficient (0.40, $p < 0.001$).

The national average loads appeared to increase throughout the late fall 2023 and then remained at approximately 4 x 10^6 gc per person per day with high levels of variation throughout the rest of the reporting period. In contrast to the viral loads, the number of positive tested cases among reported cases with acute respiratory infection were intermittent with two clusters of cases, one in February-March 2024 and a second in late April-May 2024. The low but stable detected loads throughout the study period suggests shedding in the community occurred throughout most of the study period, which is different than suggested by clinical cases who requested outpatient treatment. The cause of this difference is unknown, and may be a reflection of methodology or epidemiology. In the next reporting period, we will further monitor the specificity and accuracy of the assay.

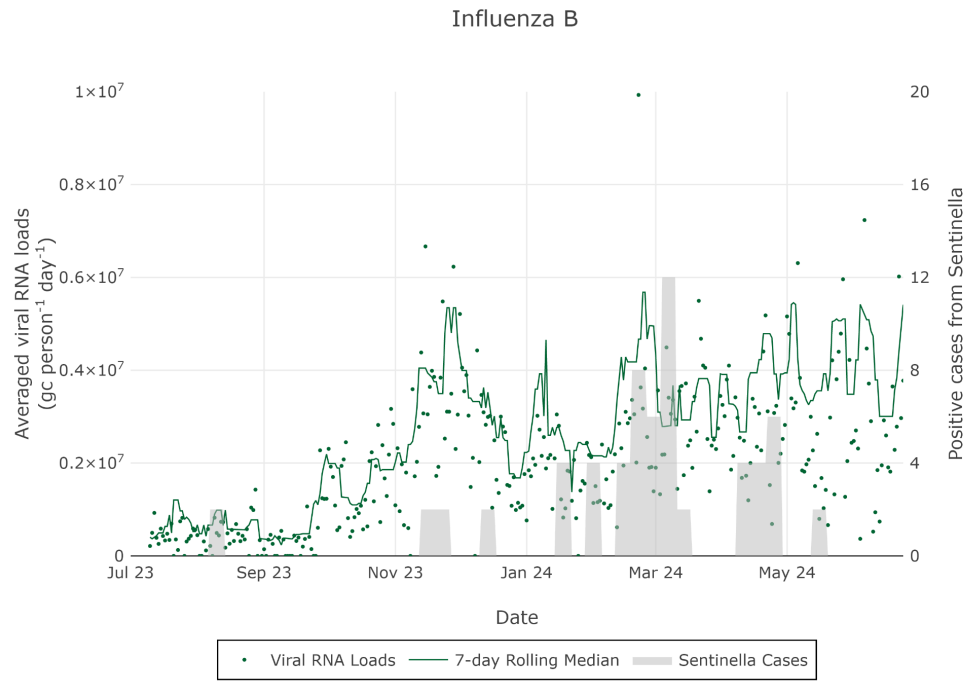

Figure 10: Comparison of averaged Influenza B viral loads per person per day (green dots) and corresponding 7-day rolling median (green line) to the weekly number of positive tested cases among reported cases with acute respiratory infection in the Sentinella surveillance system for the whole of Switzerland (grey bars). Data are contemporary to data access date.

Respiratory Syncytial Virus

The averaged viral RNA loads for Respiratory Syncytial Virus aligned well with the number of positive tested cases among reported cases with acute respiratory infection from the Sentinella surveillance system (Figure 11). The Spearman rank correlation coefficient was high (0.66, $p < 0.001$), with deviations from clinical cases early in the reporting period (summer 2023) when Respiratory Syncytial Virus was detected in wastewater, but there were no clinical cases reported.

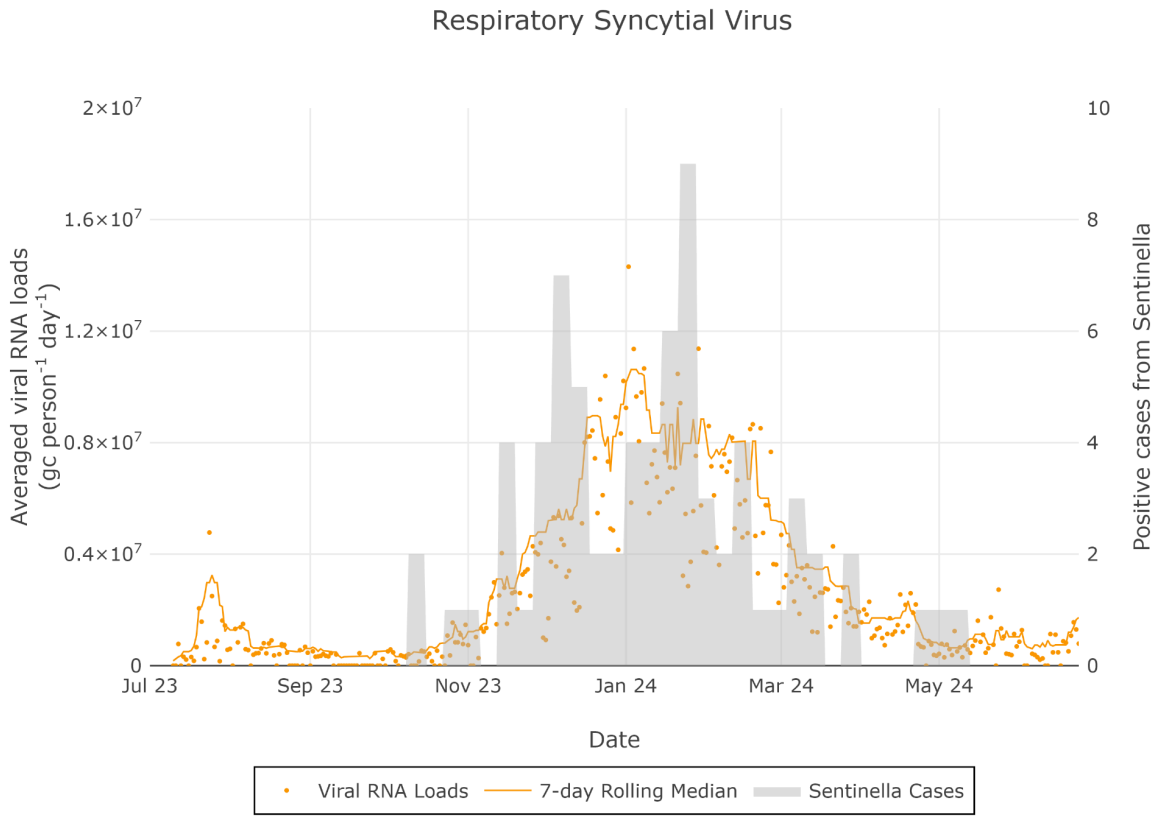

Figure 11: Comparison of averaged Respiratory Syncytial Virus viral loads per person per day (orange dots) and corresponding 7-day rolling median (orange line) to the weekly number of positive tested cases among reported cases with acute respiratory infection in the Sentinella surveillance system for the whole of Switzerland (grey bars). Data are contemporary to data access date.

Results for Antimicrobial Resistance

ESBL-producing *E. coli*

ESBL-producing *E. coli* was detected at all time points, at all sites throughout the period in the 683 samples analyzed. The median (interquartile range) proportion of total *E. coli* that is ESBL-*E. coli* was 1.7% (1.2%, 2.2%) across all sites. The relative proportions were consistent across all sites with minor temporal variation.

ESBL-producing *E. coli* data for the first year (November 2021- November 2022) was further analyzed and reported (Conforti et al. 2024). In this publication, proportions of ESBL-producing *E. coli* in wastewater were linked conceptually to community carriage of ESBL-producing *E. coli*, suggesting changes in wastewater may indicate changes in community carriage.

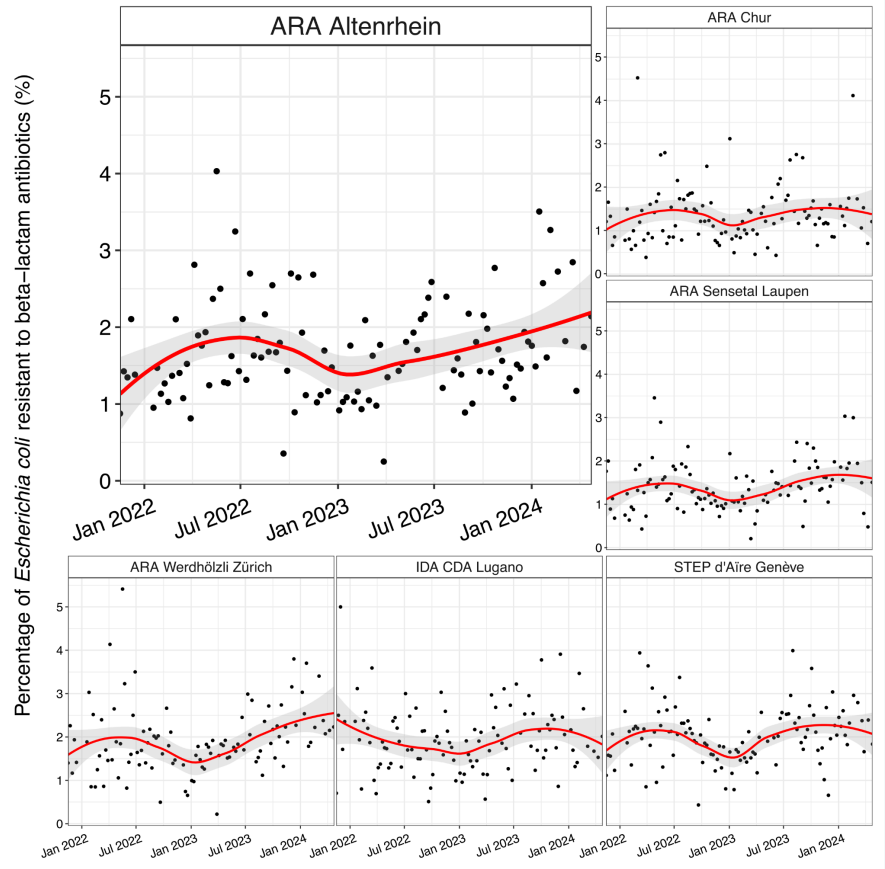

Figure 12: Percentage of extended-spectrum -lactamase-producing *E. coli* (ESBL-*E. coli*) in wastewater samples from six locations in Switzerland from November 2021 to April 2024. Black circles represent measured values, red line is the LOESS regression, with 95% confidence interval for predictions as grey band.

Carbepenemase-producing *E. coli.*

Carbapenemase-producing *E. coli* were detected throughout the year at all sites and in 81% of the 392 samples analyzed. The median (interquartile range) of the proportion of Carbapenemase-producing *E. coli* out of total *E. coli* was 0.04% (0.01%, 0.10%). Temporal patterns in the proportion of Carbapenemase-producing *E. coli* over the monitoring period varied by site. Generally, samples from wastewater treatment plants serving larger populations (STEP Geneva, IDA CDA Lugano, and ARA-Werdhoelzli Zurich) appeared consistent throughout the year, whereas smaller sites (ARA Altenrhein, ARA Chur, ARA Laupen) showed increased proportions in March and April 2023 and lower concentrations in September and October 2024.

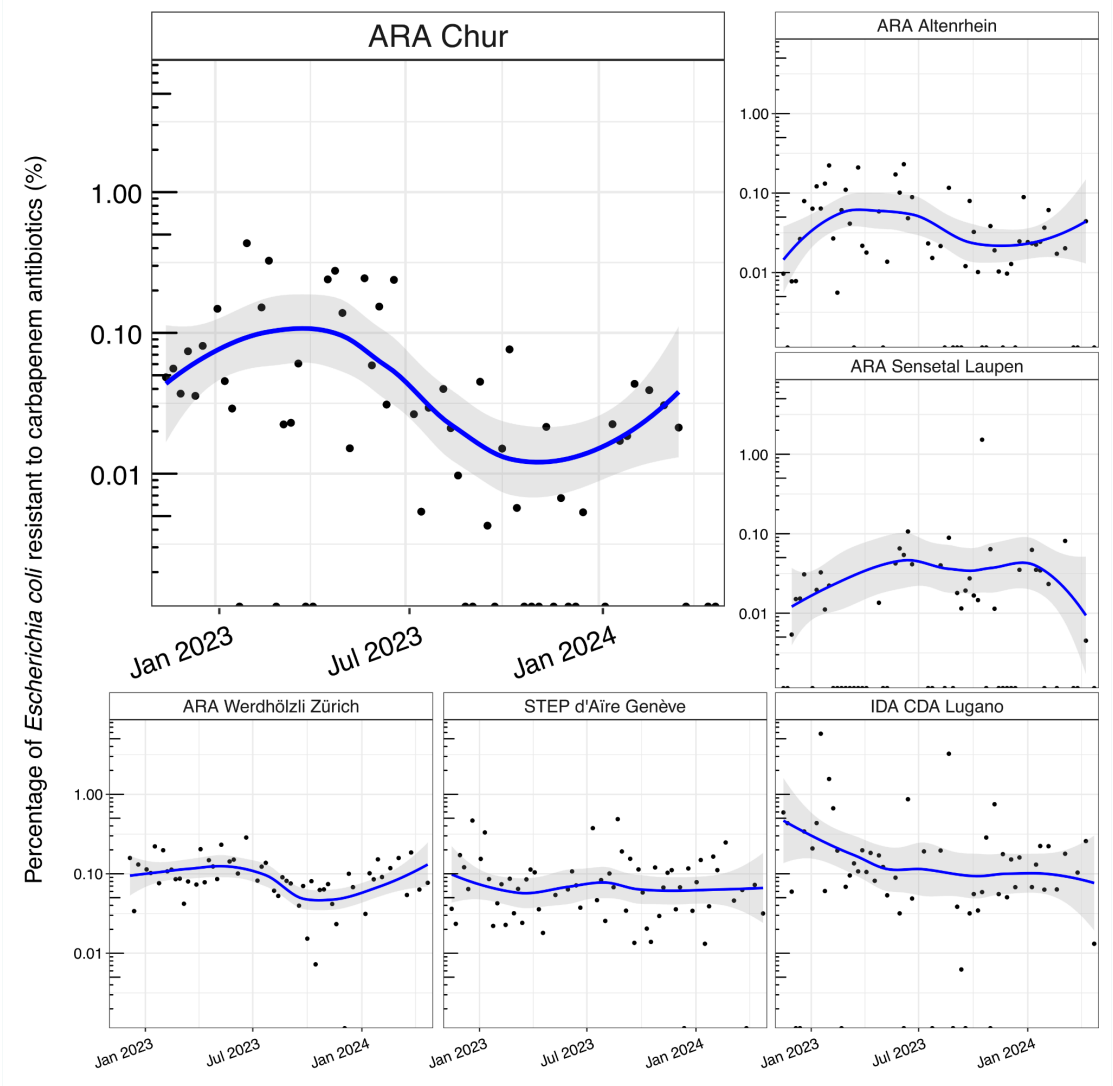

Figure 13: Percentage of carbapenemase-producing *E. coli* (CP-*E. coli*) in wastewater samples from six locations in Switzerland from December 2022 to April 2024. Black circles represent measured values, blue line is the LOESS regression, with 95% confidence interval for predictions as grey band.

ESBL-producing KESC.

ESBL-producing KESC, representing the phenotypically similar set of microorganisms that are co-cultured on the same selective agar, includes *Klebsiella* spp., *Enterobacter* spp., *Seratia* spp., and *Citrobacter* spp. ESBL-producing KESC were detected in all 381 samples processed. The median (interquartile range) proportion of all KESC that are ESBL-producing was 0.2% [0.3%, 0.6%]. The temporal variation was consistent across all sites, with reduced proportions observed in Spring 2023 and increased proportions in Fall 2023.

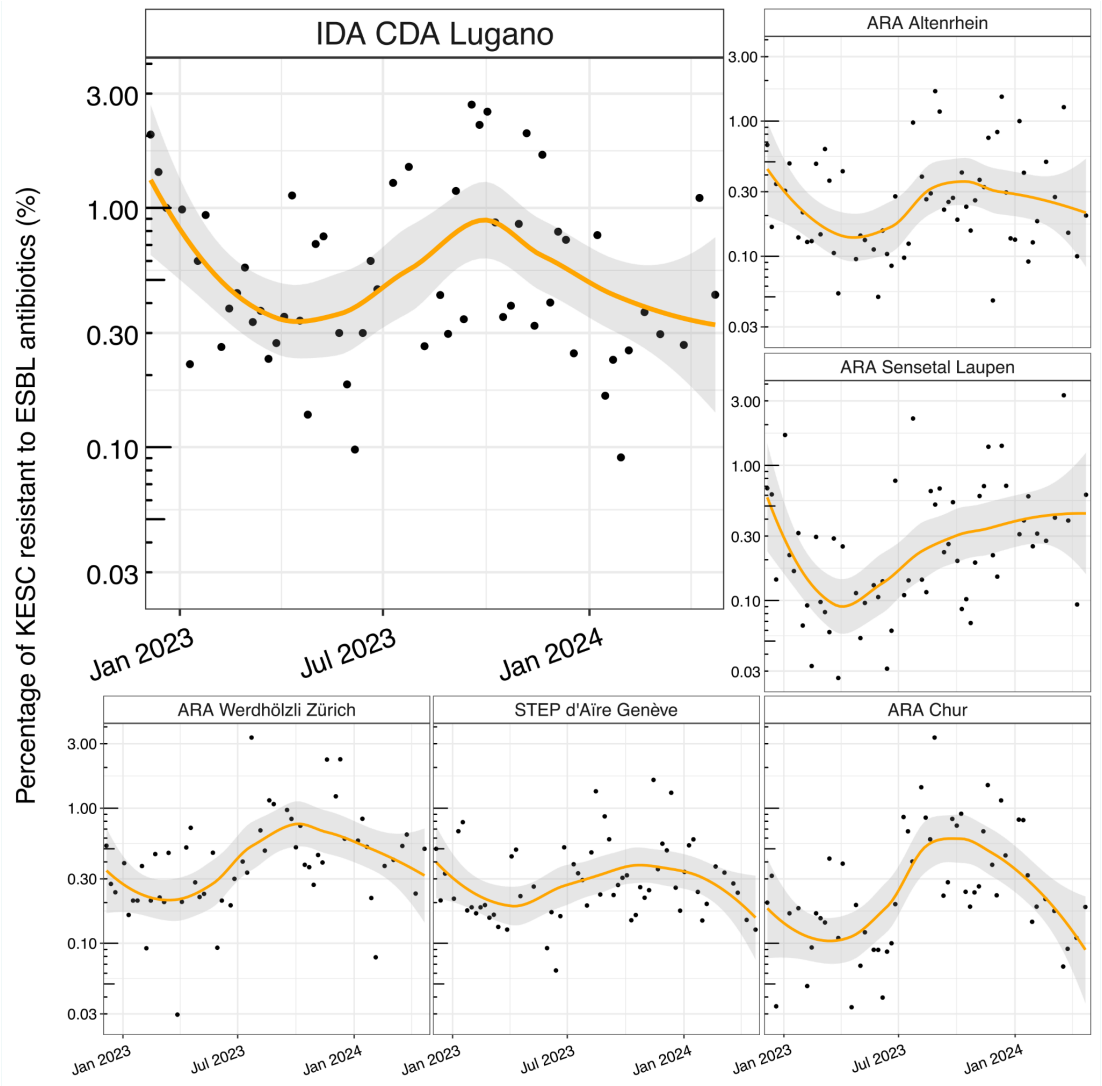

Figure 14: Percentage of extended-spectrum b-lactamase-producing KESC (ESBL-KESC) in wastewater samples from six locations in Switzerland from December 2022 to April 2024. The y-axis is log10 transformed to facilitate visualization of the data. The group KESC refers to the set of microorganisms with similar culturability, including: *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp. Black circles represent measured values, orange line is the LOESS regression, with 95% confidence interval for predictions as grey band.

Carbepenemase-producing KESC.

Carbepenemase-producing KESC, were detected in 96% of the 380 samples processed. The median (interquartile range) proportion of all KESC that are carbepenamase-producing was 0.06% [0.02%, 0.22%]. The relative proportions of carbepenamase-producing KESC were notably higher in some sites (Lugano, Zurich) than in others (Altenrhein, Sensetal Laupen).

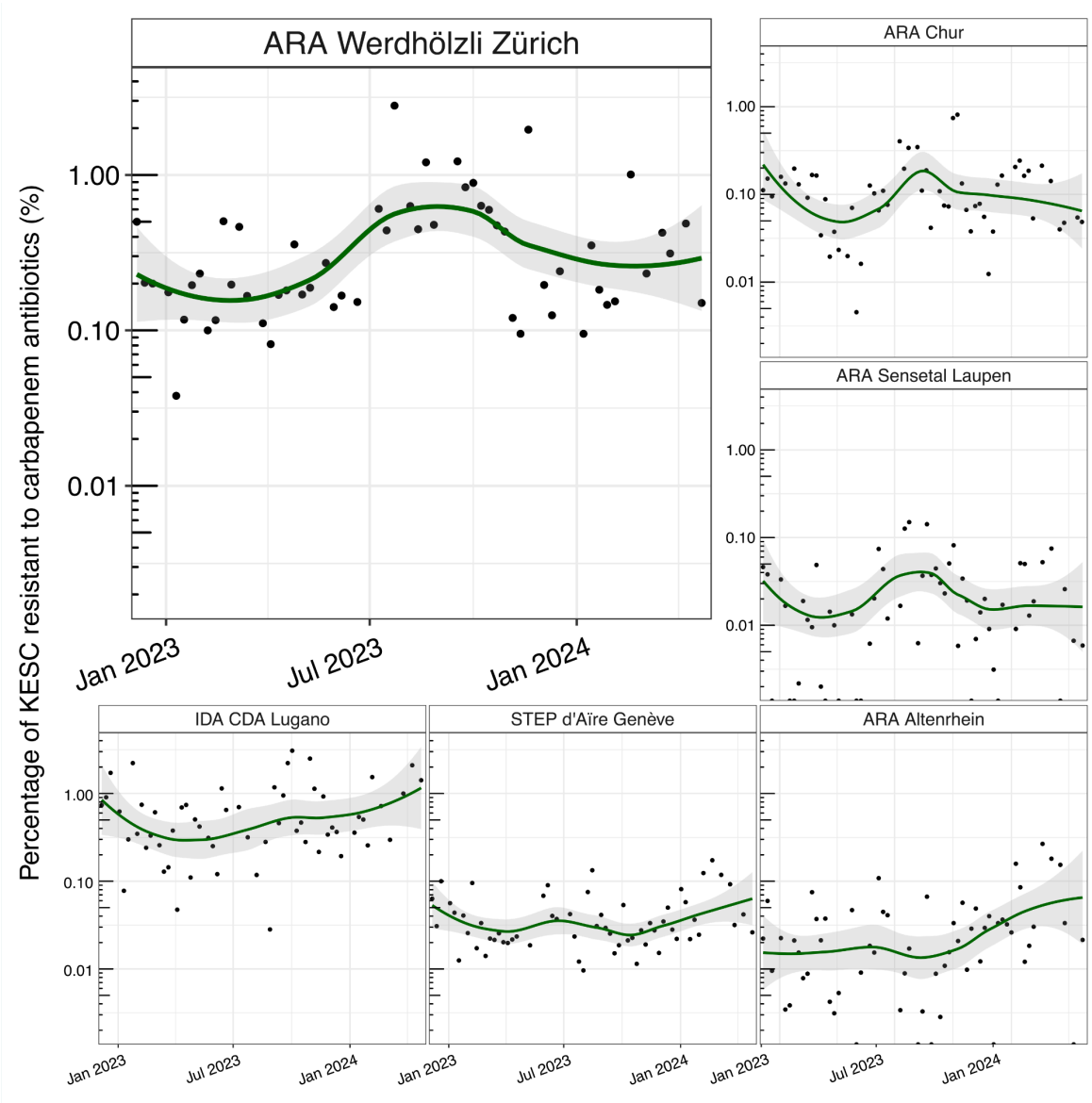

Figure 15: Percentage of carbapenemase-producing KESC (CP-KESC) in wastewater samples from six locations in Switzerland from December 2022 to April 2024. The y-axis is log10 transformed to facilitate visualization of the data. The group KESC refers to the set of microorganisms with similar culturability, including: *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp. Black circles represent measured values, green line is the LOESS regression, with 95% confidence interval for predictions as grey band.

Vancomycin-resistant *Enterococcus faecium/fecalis*

Vancomycin-resistant *Enterococcus faecium/fecalis* (VRE) were detected in all sites across Switzerland, and in 98% of the samples that were processed. Of the 391 samples with detectable VRE, the median (interquartile range) proportion of VRE out of total Enterococci was 11% (5%, 26%) showing relatively high variation throughout the study period. Proportions of VRE were notably higher in Zurich, Lugano, and, towards the end of the study period, Geneva, relative to other sites, suggesting increased VRE in these catchments.

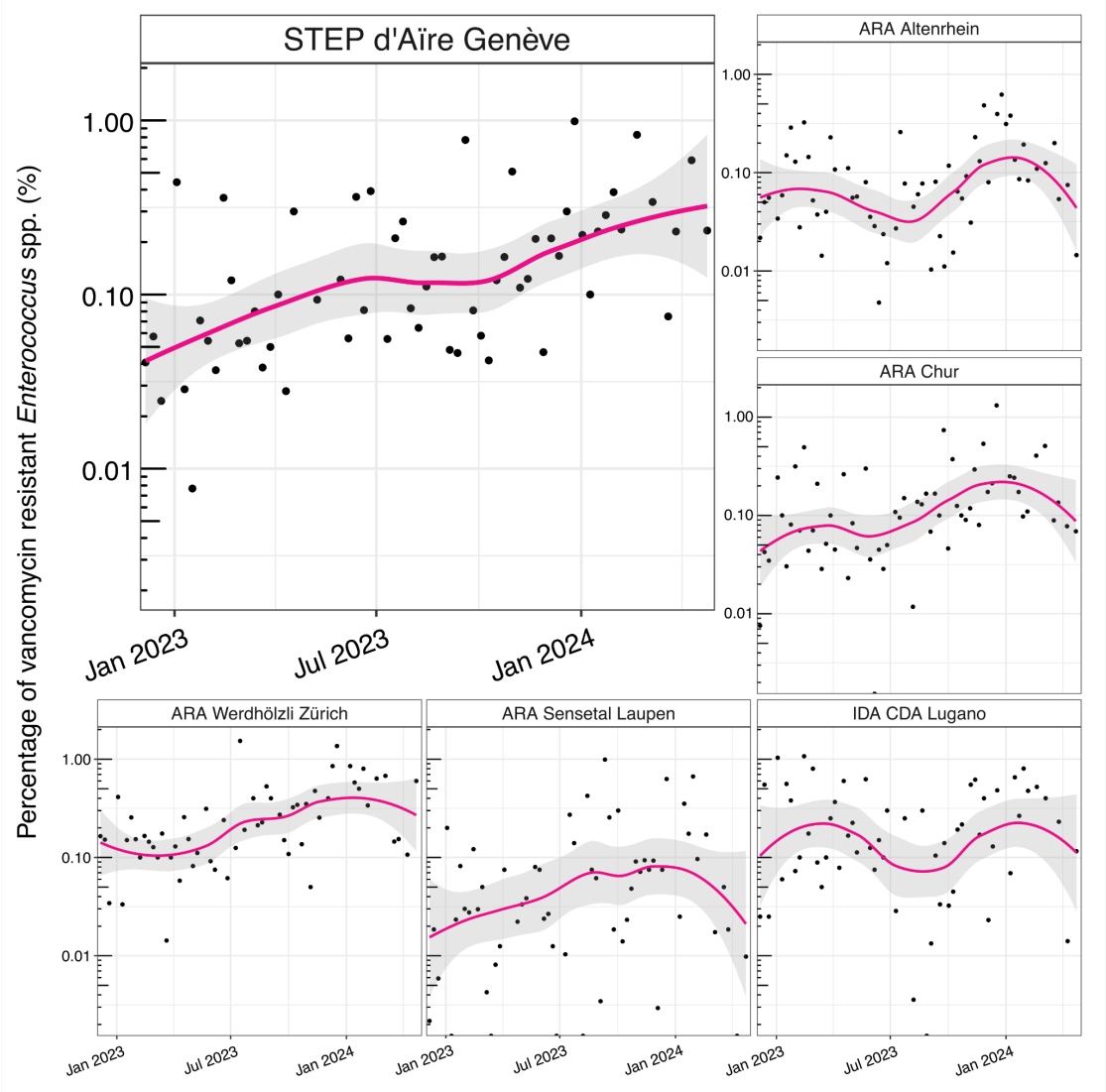

Figure 16: Percentage of vancomycin-resistant *Enterococcus faecium/fecalis* in wastewater samples from six locations in Switzerland from December 2022 to April 2024. The y-axis is log10 transformed to facilitate visualization of the data. Black circles represent measured values, magenta line is the LOESS regression, with 95% confidence interval for predictions as grey band.

Methicillin-resistant *Staphylococcus aureus*

Methicillin-resistant *Staphylocuccus aureus* (MRSA) were presumptively detected in 92% of the samples collected with a median (interquartile range) proportion of 16% (4%, 26%). Notably, subsequent steps to confirm the identity of MRSA based on the phenotype exhibited in the culture-based assay suggests the agar may lack specificity for applications to wastewater. Further confirmation, including whole genome sequencing, is ongoing for a subset of isolates. Results showed higher variability in the proportions of MRSA out of total *Staphylocccccus aureus* than for other targets. This high heterogeneity may be a consequence of using a method that is not sufficiently specific for *Staphylocuccus aureus*. Further interpretation of this data should await insights on specificity testing results.

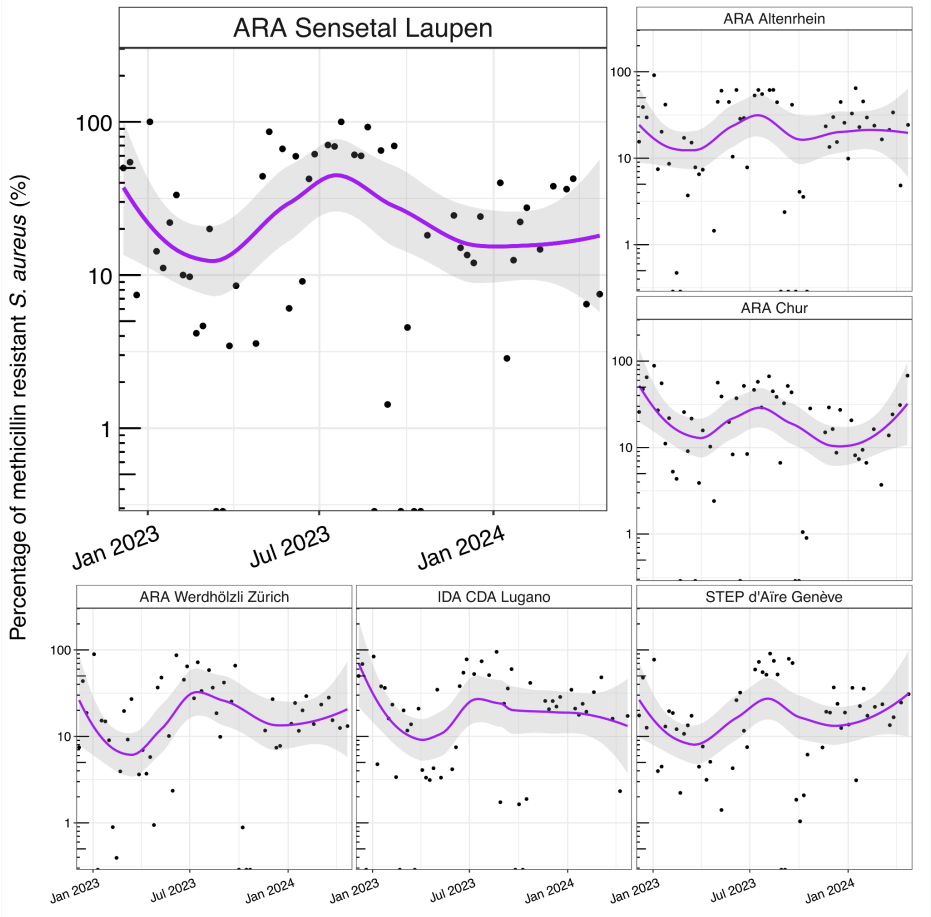

Figure 17: Percentage of presumptive methicillin-resistant *Staphylococcus aureus* (MRSA) in wastewater samples from six locations in Switzerland from December 2022 to April 2024. The y-axis is log10 transformed to facilitate visualization of the data. Black circles represent measured values, purple line is the LOESS regression, with 95% confidence interval for predictions as grey band.

Conclusions and Outlook

Wastewater-based surveillance of respiratory viruses and antimicrobial resistant bacteria over the study period provided insights into carriage of transmissible microorganisms in the Swiss population. The continued monitoring of SARS-CoV-2, Influenza A, Influenza B, and Respiratory Syncytial Virus for two years at six sites across Switzerland (and now, for one year at an additional eight sites), demonstrates clear temporal and geographic differences in the magnitude, timing, and duration of respiratory pathogen outbreaks. As shown through comparison with the publicly available data on clinical infections for the pathogens, wastewater-based detection recapitulates clinically observed trends, particularly for pathogens with clear seasonal patterns. Further, wastewater-based surveillance offers opportunities to support clinical surveillance by providing a geographically-resolved and consistent method for tracking outbreaks. As methods for wastewater-based surveillance have remained largely fixed, peak loads in a given year can be meaningfully compared to previously observed peak loads, allowing insight into the relative magnitude of outbreaks.

Other aspects of wastewater-based surveillance in Switzerland are not included in the scope of this report, which is limited to laboratory-based surveillance and associated viral loads of respiratory pathogens and antimicrobial resistance. Examples of additional innovation already implemented includes estimating the effective reproduction number (Re)[12] of the respiratory pathogens (Nadeau et al. 2024; Huisman et al. 2022), tracking SARS-CoV-2 variants[13] through sequencing extracts from wastewater (Jahn et al. 2022), and monitoring for pharmaceuticals, opioids, and other medications[14].

Wastewater-based surveillance holds substantial promise as an approach to support public health, and continues to receive substantial attention

[12] Effective reproduction number estimates are available at https://wise.ethz.ch/ (accessed 28 June 2024)
[13] SARS-CoV-2 variants are available at https://cov-spectrum.org/stories/wastewater-in-switzerland (accessed 28 June 2024)
[14] DroMedArio project website and associated data is available at https://www.dromedario.ch/ (accessed 28 June 2024)

internationally. The European Union[15][16] and the United States[17] both support broad initiatives for wastewater-based surveillance as well as innovation in the field. Switzerland remains at the forefront of surveillance and innovation. In addition to the monitoring work supported by the FOPH here, funding from the Swiss National Science Foundation *Sinergia* funding mechanism supports a consortium of researchers through the WISE: Wastewater Infectious Disease Surveillance and Epidemiology in Switzerland grant. Innovation through WISE links to, and complements, on-going surveillance efforts. Innovations include extending methodologies to the quantification and sequencing of other priority pathogens, identifying processes influencing data quality to improve wastewater-derived insights, and leveraging wastewater-derived data to track and inform disease epidemiology in Switzerland.

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Contributors

The work was made possible through the extensive contributions of a large and interdisciplinary team. Specific acknowledgement goes out to members of the Wastewater Monitoring Laboratory at Eawag responsible for managing logistics, data collection and analysis, visualization and reporting. Members during the period of reporting are: Lea Caduff, Sheena Conforti, Ayazhan Dauletova, Jolinda de Korne, Charlie Gan, Camille Halblützel, Aurélie Holschneider, Seju Kang, Guy

[15] EU Wastewater Observatory for Public health available at: https://wastewater-observatory.jrc.ec.europa.eu/ (accessed 30 June 2024).
[16] GLOWACON: A global initiative for wastewater surveillance for public health: https://health.ec.europa.eu/latest-updates/launching-glowacon-global-initiative-wastewater-surveillance-public-health-2024-03-21_en (accessed 9 September 2024).
[17] National Wastewater Surveillance System available at: https://www.cdc.gov/nwss/wastewater-surveillance.html (accessed 30 June 2024).

Loustalot, Rachel McLeod, Melissa Pitton, Patrick Schmidhalter, Linda Schneider, Anna Wettlaufer, and Daniela Yordanova.

Members of the network of wastewater treatment plants are acknowledged for their efforts organizing the logistics of collecting and shipping wastewater samples weekly. Also acknowledged is the network of experts Eawag has engaged with at the Federal Offices, cantonal laboratories, private companies, and academia both in Switzerland and internationally.

Members of the Swiss National Science Foundation *Sinergia*-funded WISE: Wastewater-based Infectious Surveillance[18] consortium provided guidance throughout the monitoring period. WISE is led by Prof. Dr. Tanja Stadler (ETH Zurich), with co-applicants Prof. Dr. Niko Beerenwinkel (ETH Zurich), Dr. Christoph Ort (Eawag), Timothy R. Julian (Eawag), and project partner Tamar Kohn (EPFL).

The report was prepared by Melissa Pitton, Sheena Conforti, Rachel McLeod, Christoph Ort, and Timothy R. Julian. A first draft was reviewed by Rita Born, Mortiz Wagner, and Helmut Bürgmann (Internal Review). Images of viruses and *E. coli* are artistic interpretations created by Paola Galinova. For further information or clarification about the contents of the report, please contact covid.abwasser@eawag.ch.

Data Availability

Data on respiratory pathogens is submitted regularly to the Federal Office of Public Health, which maintains the Infectious Disease Dashboard at https://www.idd.bag.admin.ch/ (accessed 27 June 2024).

The WISE consortium maintains an independent dashboard with respiratory pathogen data available at: https://wise.ethz.ch/ (accessed 27 June 2024).

Data on antimicrobial resistance is available on request to covid.abwasser@eawag.ch. Although there are plans to integrate this data in the WISE dashboard, it has not yet been integrated at the time of this report.

[18] https://data.snf.ch/grants/grant/205933

Version History

Version 1 was submitted for review on 30 July 2024.
Version 2 was submitted as final on 10 September 2024.
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References:

Antimicrobial Resistance Collaborators. 2022. “Global Burden of Bacterial Antimicrobial Resistance in 2019: A Systematic Analysis.” *The Lancet* 399 (10325): 629–55.

Conforti, Sheena, Aurélie Holschneider, Émile Sylvestre, and Timothy R. Julian. 2024. “Monitoring ESBL- in Swiss Wastewater between November 2021 and November 2022: Insights into Population Carriage.” *mSphere* 9 (5): e0076023.

Huisman, Jana S., Jérémie Scire, Lea Caduff, Xavier Fernandez-Cassi, Pravin Ganesanandamoorthy, Anina Kull, Andreas Scheidegger, et al. 2022. “Wastewater-Based Estimation of the Effective Reproductive Number of SARS-CoV-2.” *Environmental Health Perspectives* 130 (5): 57011.

Jahn, Katharina, David Dreifuss, Ivan Topolsky, Anina Kull, Pravin Ganesanandamoorthy, Xavier Fernandez-Cassi, Carola Bänziger, et al. 2022. “Early Detection and Surveillance of SARS-CoV-2 Genomic Variants in Wastewater Using COJAC.” *Nature Microbiology* 7 (8): 1151–60.

Lesbon, Jéssika Cristina Chagas, Mirele Daiana Poleti, Elisângela Chicaroni de Mattos Oliveira, José Salvatore Leister Patané, Luan Gaspar Clemente, Vincent Louis Viala, Gabriela Ribeiro, et al. 2021. “Nucleocapsid (N) Gene Mutations of SARS-CoV-2 Can Affect Real-Time RT-PCR Diagnostic and Impact False-Negative Results.” *Viruses* 13 (12). https://doi.org/10.3390/v13122474.

Nadeau, Sarah, Alexander J. Devaux, Claudia Bagutti, Monica Alt, Evelyn Ilg Hampe, Melanie Kraus, Eva Würfel, et al. 2024. “Influenza Transmission Dynamics Quantified from RNA in Wastewater in Switzerland.” *Swiss Medical Weekly* 154 (January):3503.

WHO Integrated Global Surveillance on ESBL-Producing E. Coli Using a “One Health” Approach: Implementation and Opportunities. 2021. World Health Organization.

World Health Organization. 2024. *WHO Bacterial Priority Pathogens List, 2024: Bacterial Pathogens of Public Health Importance, to Guide Research, Development, and Strategies to Prevent and Control Antimicrobial Resistance*. World Health Organization.