

CORONASTEP Report 111 (2022 - Week 01) SARS-CoV-2 Sewage Surveillance in Luxembourg

Summary

This report 111 presents the results of SARS-CoV-2 contamination of wastewater at the entrance of the 13 wastewater treatment plants (WWTPs) analysed during the week 01 of 2022. All WWTPs, excepted Hesperange and Boevange were analysed twice this week. However, only the results collected at the end of the week were presented in this report. The results obtained during the first sampling campaign are considered unrepresentative due to the very heavy rainfall in the country. The results obtained show abnormally low SARS-CoV-2 fluxes, most probably due to a dilution effect and sewage overflows.

The SARS-CoV-2 RNA flux measured in WWTPs at the end of this week continue to show a very high national prevalence of the virus, with a SARS-CoV-2 flux between 2 and 3 x 10¹² RNA copies per day per 100,000 equivalent-inhabitants. The current SARS-CoV-2 level in WWTPs is very similar to that observed in December 2021. The general trend over several weeks or even months is clearly upwards.

As a reminder, no sampling was carried out during the last week of 2021 (week 52).

Figure 1a – RT-qPCR quantification time-course monitoring of SARS-CoV-2 (E gene) in Luxembourgish wastewater samples from December 2019 to January 2022. Grey squares: daily-confirmed cases for Luxembourgish residents (<https://data.public.lu/fr/datasets/donnees-covid19/>), Blue dots: cumulative SARS-CoV-2 flux (RNA copies / day / 100 000 equivalent inhabitants)

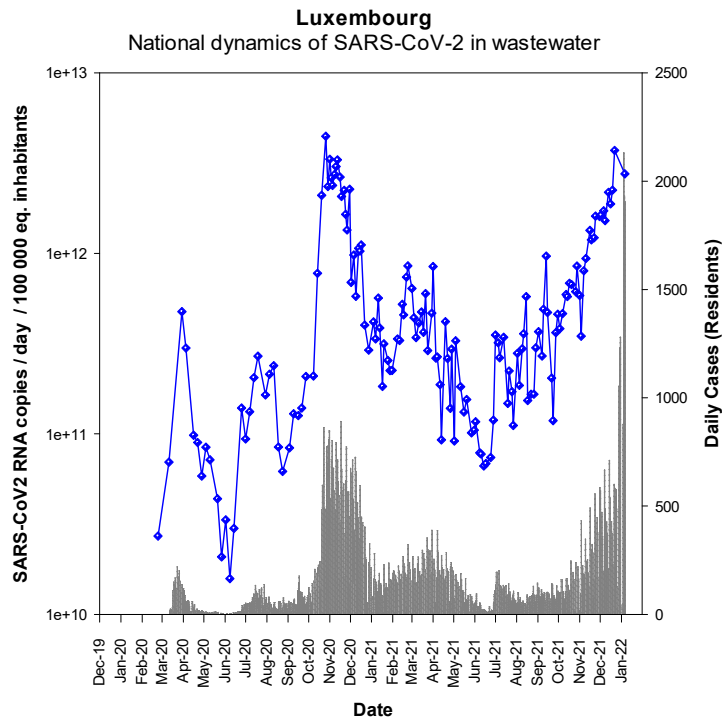


Figure 1b – Close-up of Figure 1a showing results from September 1st, 2020 on.

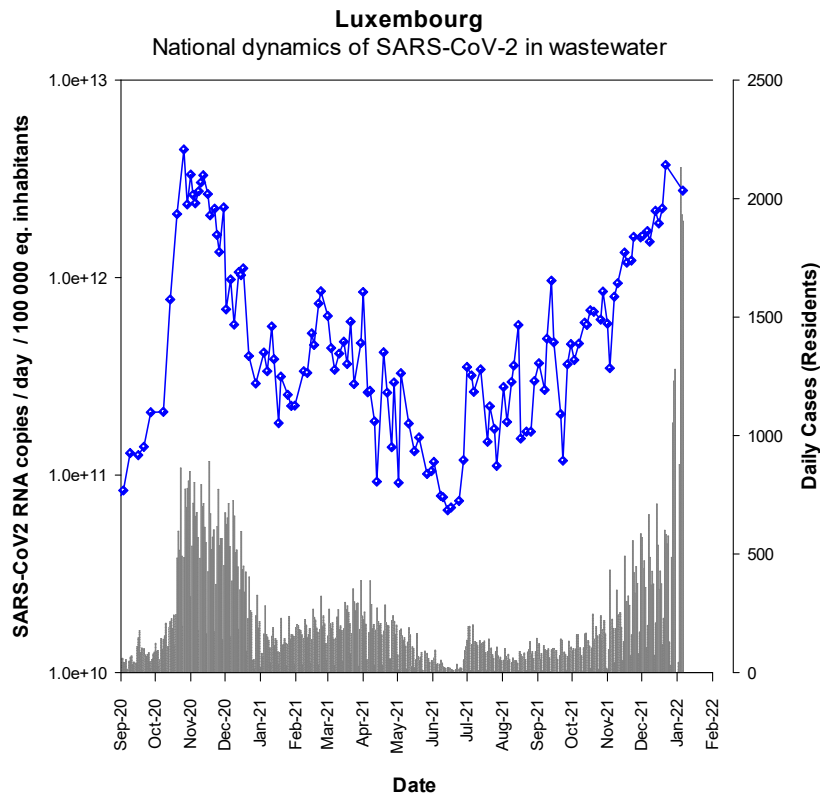


Table 2 - Level of SARS-CoV-2 contamination of each analyzed wastewater treatment plant in Luxembourg in 2021. BEG: Beggen, BET: Bettembourg, SCH: Schifflange, BLE: Blesbruck, MER: Mersch, PET: Pétange, HES: Hespérange, ECG: Echternach, UEB: Uebersyren, GRE: Grevenmacher, TRO: Troisvierges, BOE: Boevange sur Attert, WIL: Wiltz



Dark green: negative samples for SARS-CoV-2 gene E (-), Green to red: positive samples for SARS-CoV-2 gene E. The intensity of the color is related to the RT-qPCR signal (Ct values) Grey boxes: no data

WWTP	2021																												2022									
	Week 35-1	Week 35-2	Week 36-1	Week 36-2	Week 37-1	Week 37-2	Week 38-1	Week 38-2	Week 39-1	Week 39-2	Week 40-1	Week 40-2	Week 41-1	Week 41-2	Week 42-1	Week 42-2	Week 43-1	Week 43-2	Week 44-1	Week 44-2	Week 45-1	Week 45-2	Week 46-1	Week 46-2	Week 47-1	Week 47-2	Week 48-1	Week 48-2		Week 49-1	Week 49-2	Week 50-1	Week 50-2	Week 51-1	Week 51-2	Week 01		
BEG	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange
BET	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange
SCH	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange
BLE	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange
MER	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange
PET	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange
HES	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange
ECH	Grey	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	
UEB	Grey	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange
GRE	Grey	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange
TRO	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange
BOE	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange
WIL	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange

Figure 2a – RT-qPCR quantification time-course monitoring of SARS-CoV-2 (E gene) in the four most impacted wastewater treatment plants from March 2020 to January 2022. Grey squares: daily-confirmed cases for the contributory area of each wastewater treatment plant, dots: SARS-CoV-2 flux (RNA copies / day / 10 000 equivalent inhabitants).

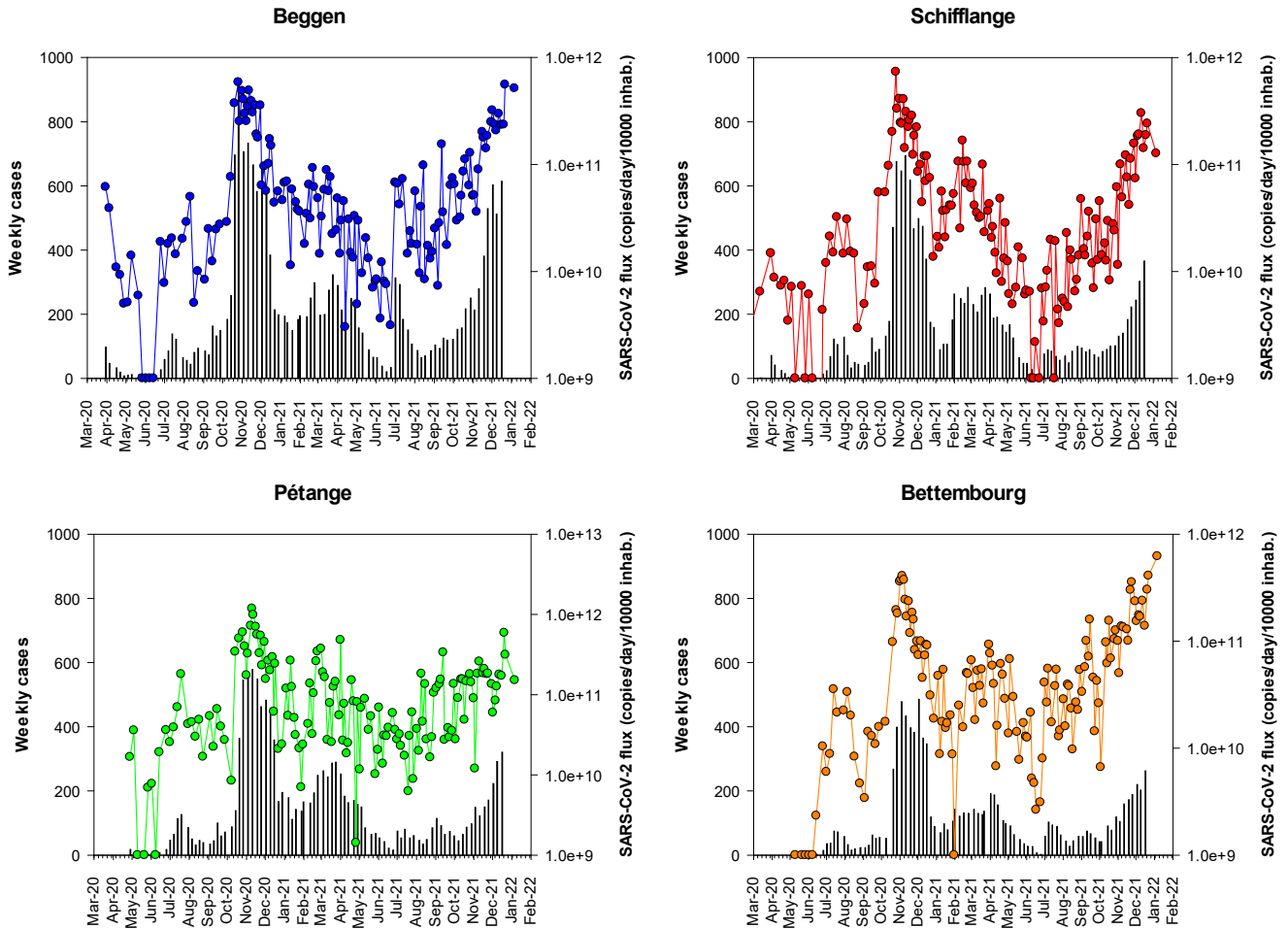


Figure 2b – Close-up of Figure 2a showing results from September 1st, 2020 on.

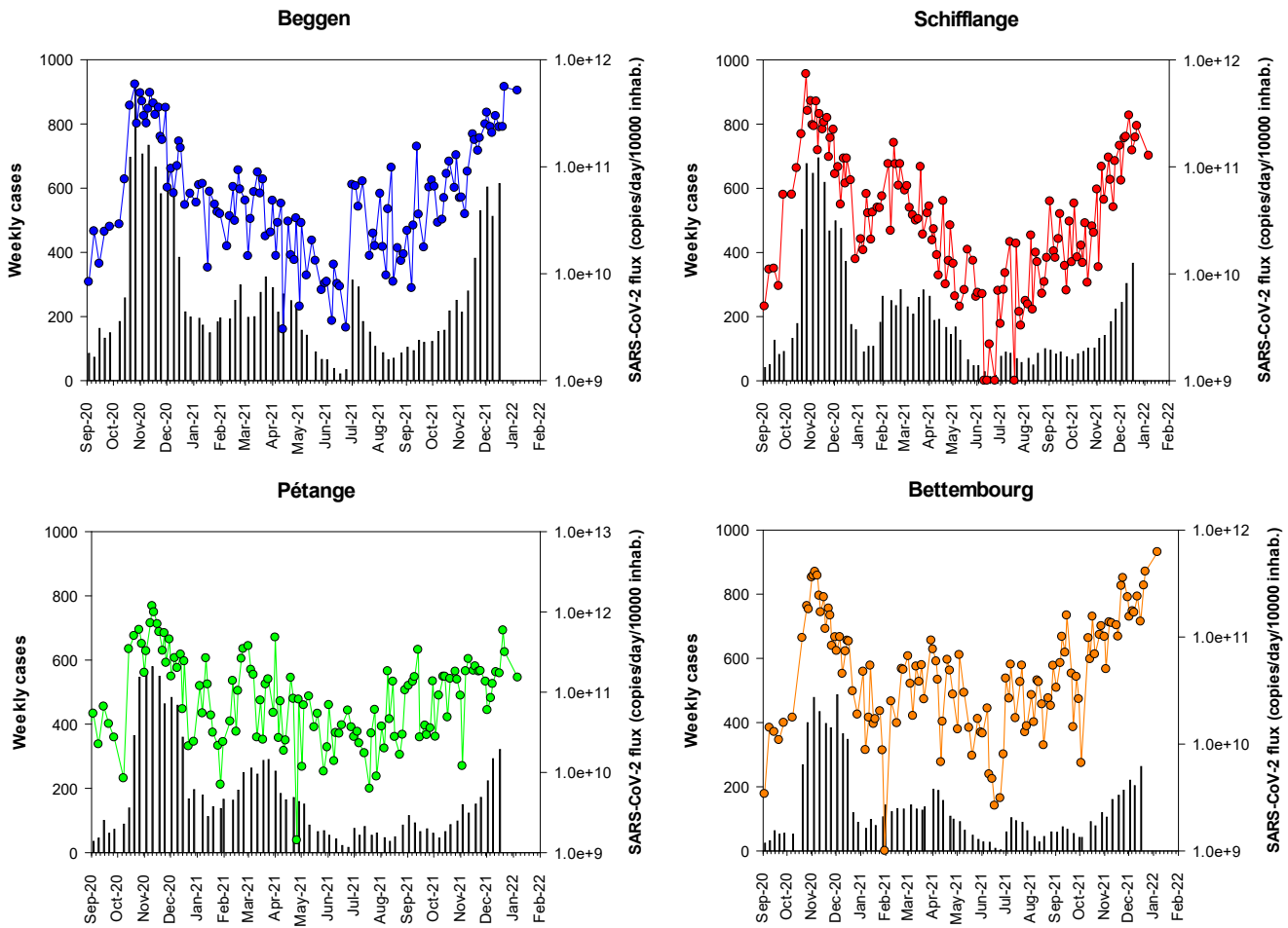


Figure 3a – RT-qPCR quantification time-course monitoring of SARS-CoV-2 (E gene) in Hespérange, Mersch and Boevange-sur-Attert wastewater treatment plants from March 2020 to January 2022. Grey squares: daily-confirmed cases for the contributory area of each wastewater treatment plant, dots: SARS-CoV-2 flux (RNA copies / day / 10 000 equivalent inhabitants).

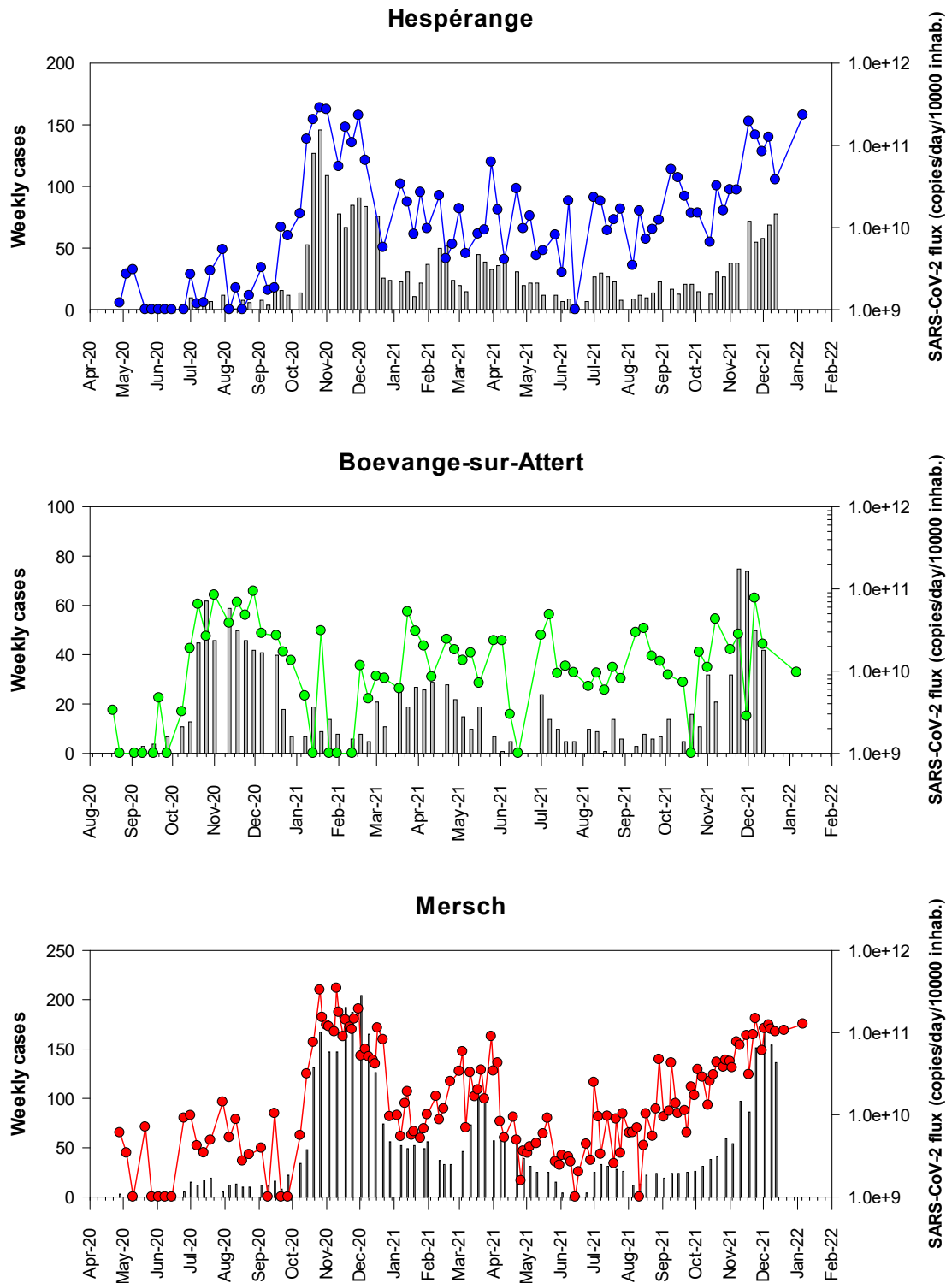


Figure 3b – Close-up of Figure 3a showing results from September 1st, 2020 on.

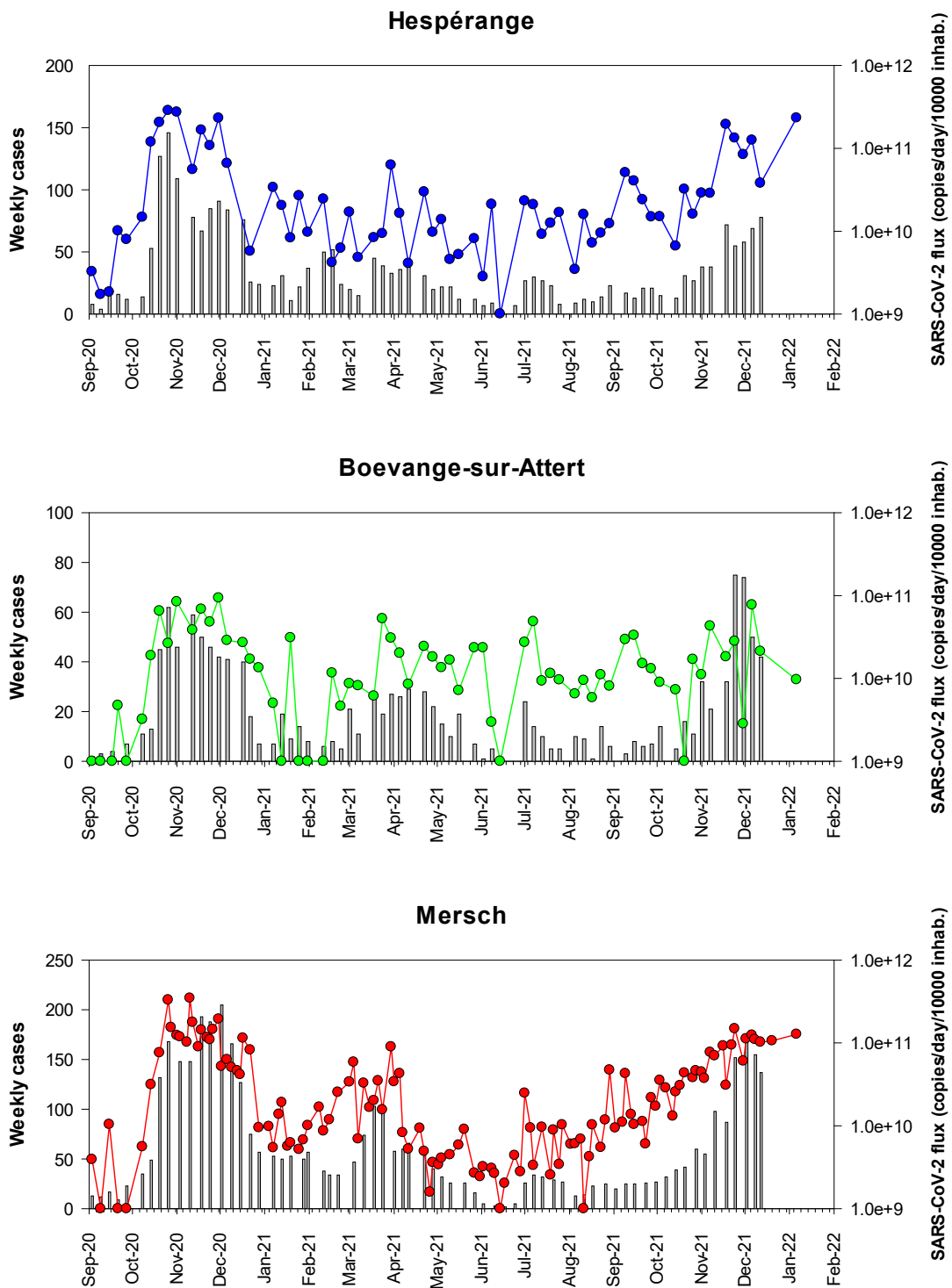


Figure 4a – RT-qPCR quantification time-course monitoring of SARS-CoV-2 (E gene) in SIDEST wastewater treatment plants from March 2020 to January 2022. Grey squares: daily-confirmed cases for the contributory area of each wastewater treatment plant, dots: SARS-CoV-2 flux (RNA copies / day / 10 000 equivalent inhabitants).

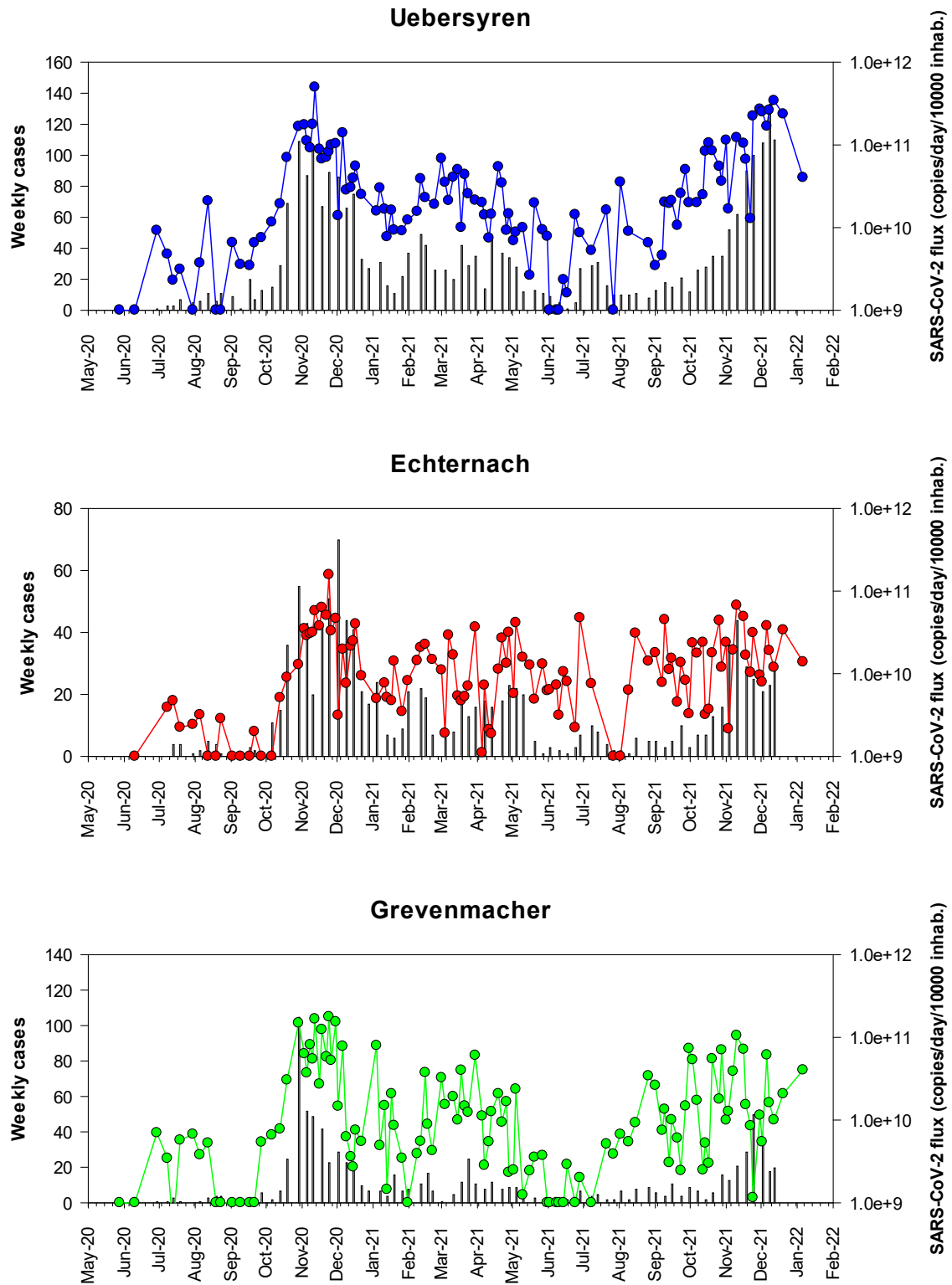


Figure 4b – Close-up of Figure 4a showing results from September 1st, 2020 on

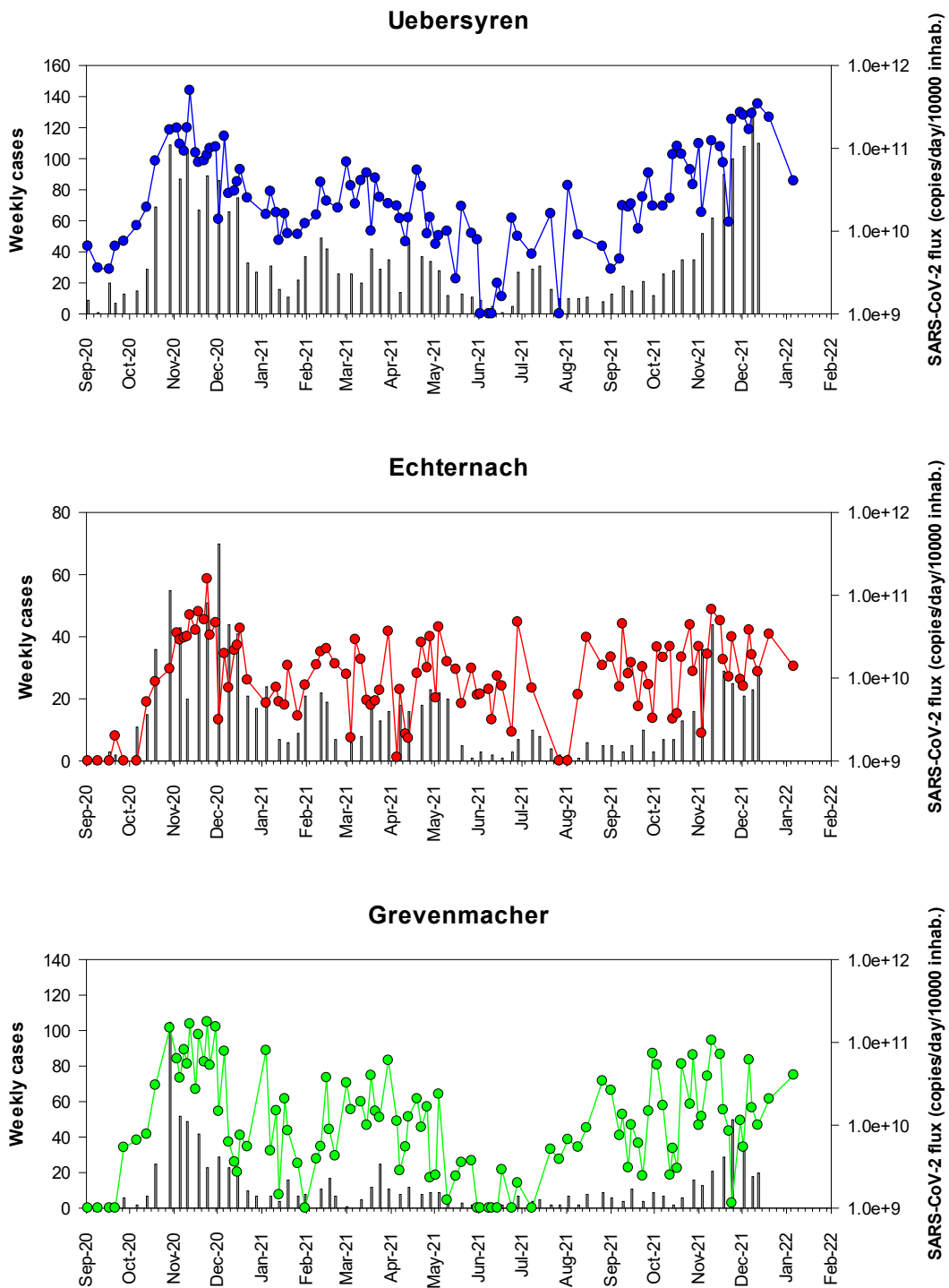


Figure 5a – RT-qPCR quantification time-course monitoring of SARS-CoV-2 (E gene) in SIDEN wastewater treatment plants from March 2020 to January 2022. Grey squares: daily-confirmed cases for the contributory area of each wastewater treatment plant, dots: SARS-CoV-2 flux (RNA copies / day / 10 000 equivalent inhabitants)

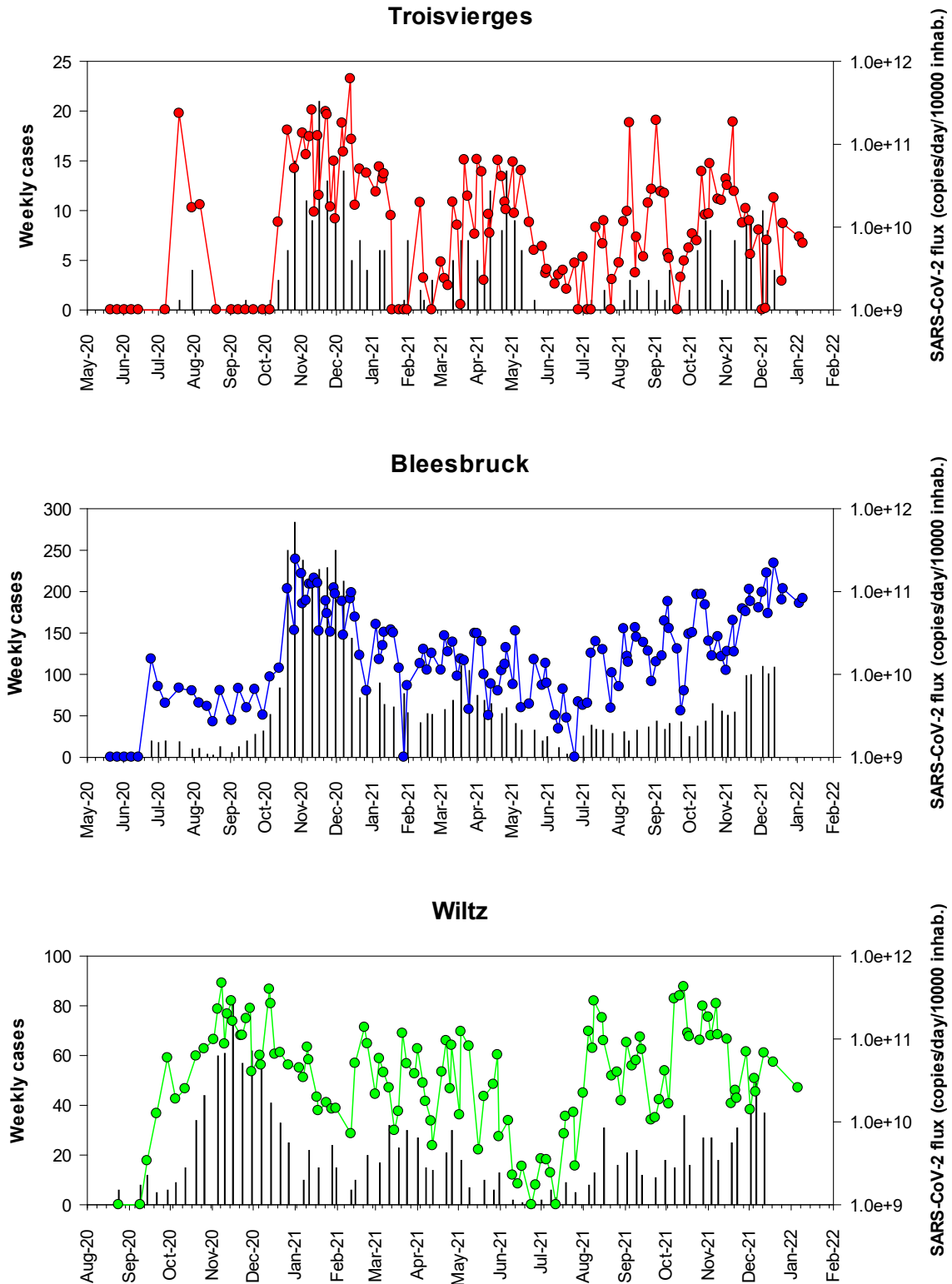
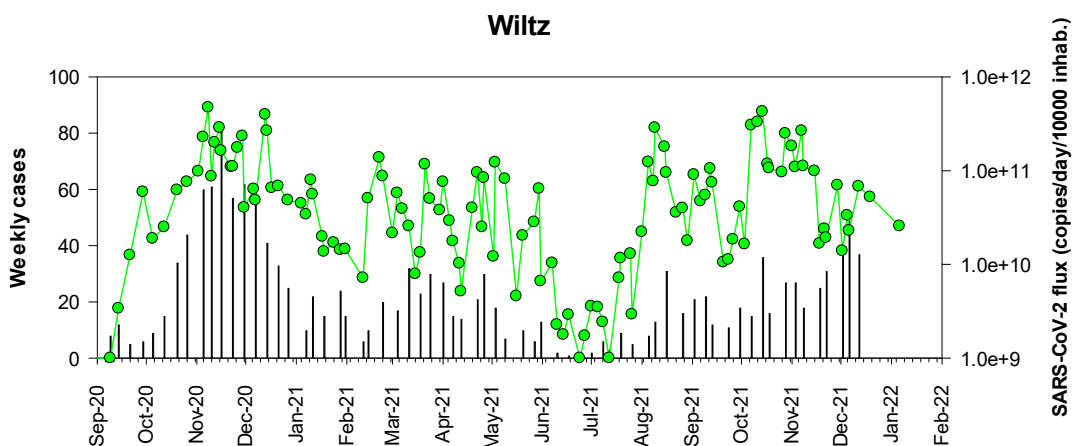
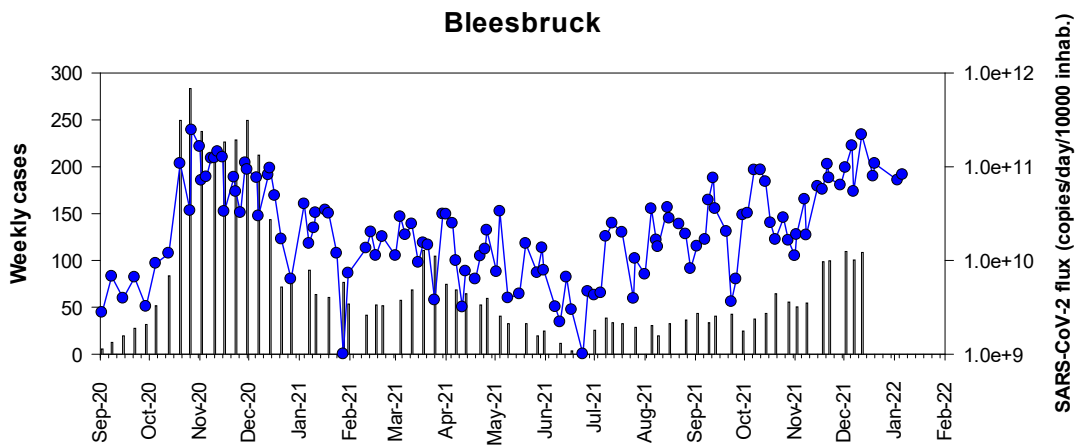
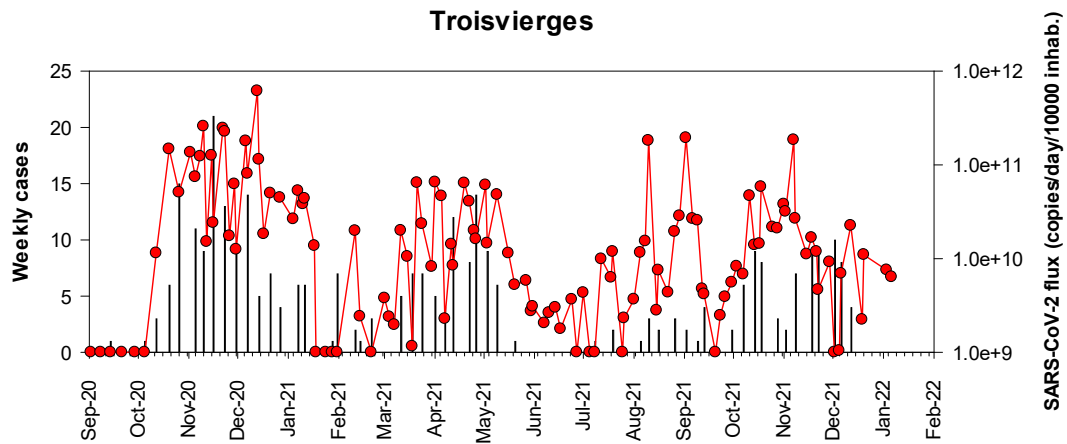


Figure 5b – Close-up of Figure 5a showing results from September 1st, 2020 on.



Materials and Methods

Sewage samples

From March 2020 to January 2022, up to thirteen wastewater treatment plants (WWTPs) were sampled at their inlet according to the planning presented in Table 3. The operators of the WWTPs collected a 24-h composite sample according to their routine sampling procedure. Composite sample was stored at 4°C until sample processing.

Sample processing

The samples were transported to the laboratory at 4°C and viral RNA was isolated on the day of sampling. Larger particles (debris, bacteria) were removed from the samples by centrifugation at 2,400 x g for 20 min at 4°C. A volume of 120 mL of supernatant was filtered through Amicon® Plus-15 centrifugal ultrafilter with a cut-off of 10 kDa (Millipore) by centrifugation at 3,220 x g for 25 min at 4°C. The resulting concentrate was collected and 140 µL of each concentrate was then processed to extract viral RNA using the QIAamp Viral RNA mini kit (Qiagen) according to the manufacturer's protocol. Elution of RNA was done in 60 µL of elution buffer.

Real-time One-Step RT-PCR

Samples were screened for the presence of *Sarbecovirus* (*Coronaviridae*, *Betacoronaviruses*) and/or SARS-CoV-2 virus RNA by two distinct real-time one-step RT-PCR assays, targeting the E gene (Envelope small membrane protein) and the N gene (nucleoprotein). The E gene real-time RT-PCR can detect *Sarbecoviruses*, i.e. SARS-CoV, SARS-CoV-2 and closely related bat viruses. In the context of the COVID19 pandemic, it can be assumed that only SARS-CoV-2 strains will be detected by this assay given that SARS-CoV virus has been eradicated and other bat viruses do not commonly circulate in the human population. The E gene assay is adapted from Corman et al. [17]. The N gene real-time RT-PCR assay (N1 assay) specifically detects SARS-CoV-2 virus. It is adapted from the CDC protocol¹. The two primers/probe sets are presented in Table 3. The RT-qPCR protocols and reagents were all provided by the LIH.

Table 4 – RT-qPCR primer-probe sets

Target	Primer name	Primer sequence (5' to 3')	References
E gene	E_Sarbeco_F1	5-ACAGGTACGTTAATAGTTAATAGCGT-3	Corman et al., 2020
	E_Sarbeco_R2	5-ATATTGCAGCAGTACGCACACA-3	
	E_Sarbeco_P1	5'-FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ1	
N gene	2019-nCoV_N1_Fw	5'-GAC CCC AAA ATC AGC GAA AT-3'	CDC, 2019
	2019-nCoV_N1_Rv	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'	
	2019-nCoV_N1 Probe	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3'	

Each reaction contained 5 µL of RNA template, 5 µL of TaqPath 1-step RT-qPCR MasterMix (A15299, Life Technologies), 0.5 µL of each primer (20 µM) and probe (5 µM) and the reaction volume was adjusted to a final volume of 20 µL with molecular biology grade water. Thermal cycling reactions were carried out at 50 °C for 15 min, followed by 95 °C for 2 min and 45 cycles of 95 °C for 3 sec and 58°C (E gene) or 55°C (N gene) for 30 sec using a Vii7 Real-Time PCR Detection System (Life Technologies). Reactions were considered positive (limit of detection – LOD) if the cycle threshold (Ct value) was below 40 cycles.

¹ <https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-primer-probes.pdf>

Controls

A non-target RNA fragment commercially available (VetMAX™ Xeno™ IPC and VetMAX™ Xeno™ IPC Assay, ThermoFischer Scientific) was added to the viral RNA extract from sewage concentrates as an internal positive control (IPC). This IPC-RNA is used to control the performance of the RT-qPCR (E gene) and to detect the presence of RT-qPCR inhibitors.

Viral RNA copies quantification of both targeting genes in wastewater samples was performed using RT-qPCR standard curves generated using EDX SARS-CoV-2 Standard (Biorad). This standard is manufactured with synthetic RNA transcripts containing 5 targets (E, N, S, ORF1a, and RdRP genes of SARS-CoV-2, 200,000 copies/mL each). Using such a standard, the limits of quantification (LOQ) of both RT-qPCR assays were estimated to 1 RNA copy per reaction (Figure 6).



Figure 6 – RT-qPCR standard curves established for both target genes (E gene and N gene) of SARS-CoV-2 using a commercially available standard (Biorad).

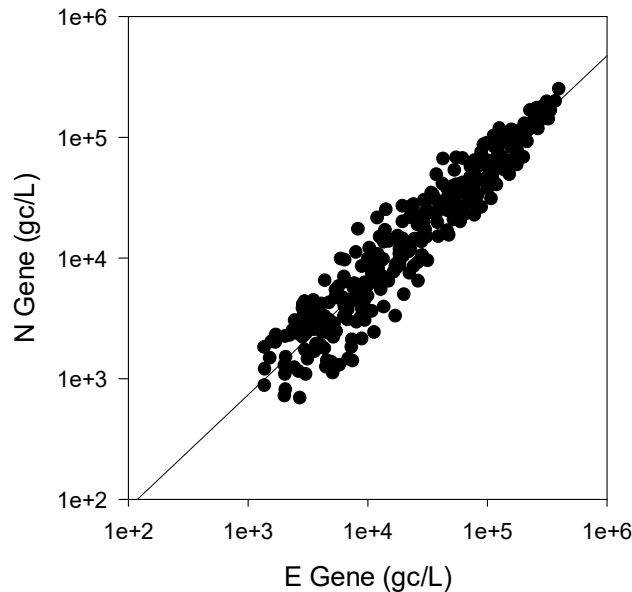
Data interpretation

A sample is declared positive for the presence of SARS-CoV-2 if both targets (E and N gene) are detected with Ct values less than or equal to the LOQ. If only one target is detected or if target genes are detected with Ct values between the LOD and the LOQ, samples are reported as presumptive positive (+/-). A sample is declared negative when no target genes are detected (Ct values superior to the LOD).

In case of presumptive positive, sample is tested again using another RT-qPCR detection assay (Allplex 2019-nCoV Assay, Seegene). This commercially available detection kit is a multiplex real-time RT-PCR assay for simultaneous detection of three target genes of SARS-CoV-2 in a single tube. The assay is designed to detect RdRP and N genes specific for SARS-CoV-2, and E gene specific for all *Sarbecovirus* including SARS-CoV-2.

As shown in Figure 7, a highly significant correlation (Pearson Correlation, $R^2=0.964$, $p = 5.979 \cdot 10^{-24}$) was obtained between the SARS-CoV-2 RNA concentrations estimated using the E gene and the N gene, respectively. Therefore, only the E gene results were presented in this report.

Figure 7 - Relationship between the SARS-CoV-2 RNA concentration (RNA copies / L of wastewater) estimated by the both distinct RT-qPCR systems targeting the E and N gene, respectively (n=415),



Acknowledgments

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