CORONASTEP Report 190
(2024 – Weeks 31 and 32)
SARS-CoV-2 Sewage Surveillance in Luxembourg

Summary

After a detailed analysis of the data collected to date, we demonstrated that it was possible to reduce the number of wastewater treatment plants (WWTPs) monitored without affecting the relevance and representativeness of the national data. The 8 WWTPs selected are as follows: Beggen, Schifflange, Bettembourg, Pétange, Mersch, Echternach, Ubersyren and Bleesbruck.
From now on, only the results of these eight analyses will be presented in the fortnightly reports.

This report 190 presents the results of SARS-CoV-2 contamination of wastewater at the inlet of the 8 wastewater treatment plants (WWTPs) analysed during weeks 31 and 32 of 2024. Following the increase in SARS-CoV-2 flows observed in the country's wastewater in recent weeks, the current trend is for the signal to decrease at national and regional levels. The measured national flux of SARS-CoV-2 ranged from approximately 8×10^{11} to 3×10^{11} RNA copies per day per 100,000 population equivalents.

Remark: Following the recent adoption of the Act amending the Act of 17 July 2020 on measures to combat the COVID-19 pandemic and the decisions of the Government Council regarding the surveillance of the COVID-19 epidemic, the data on clinical surveillance will no longer be reported on the national and regional charts. Indeed, the number of tests performed has already started to decrease drastically and is therefore no longer as representative of the virus circulation in the population as before.

Table 1 – National level of SARS-CoV-2 contamination of wastewater in Luxembourg.

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 national SARS-CoV-2 flux (RNA copies/day/100,000 equivalent inhabitants).

2020

Week	Week 3	Week 7	Week 9	Week 11	Week 14	Week 15	Week 16	Week 17	Week 18	Week 19	Week 20	Week 21	Week 22	Week 23	Week 24	Week 25	Week 26	Week 27	Week 28	Week 29	Week 30	Week 31	Week 32	Week 33	Week 34	Week 35	Week 36	Week 37	Week 38	Week 39	Week 40	Week 41	Week 42	Week 43	Week 44-1	Week 44-2	Week 45-1	Week 45-2	Week 45-3	Week 46-1	Week 46-2	Week 46-3	Week 47-1	Week 47-2	Week 48-1	Week 48-2	Week 48-3	Week 49-1	Week 49-2	Week 50-1	Week 50-2	Week 51-1	Week 51-2	Week 51-2	Week 52	Week 53

National SARS-CoV-2 Level

2021

Week	Week 01-1	Week 01-2	Week 02-1	Week 02-2	Week 03-1	Week 03-2	Week 04-1	Week 04-2	Week 05-1	Week 06-1	Week 06-2	Week 07-1	Week 07-2	Week 08-1	Week 08-2	Week 09-1	Week 09-2	Week 10-1	Week 10-2	Week 11-1	Week 11-2	Week 12-1	Week 12-2	Week 13-1	Week 13-2	Week 14-1	Week 14-2	Week 15-1	Week 15-2	Week 16-1	Week 16-2	Week 17-1	Week 17-2	Week 18-1	Week 18-2	Week 19	Week 20-1	Week 20-2	Week 21	Week 22-1	Week 22-2	Week 23-1	Week 23-2	Week 24-1	Week 24-2	Week 25	Week 26-1	Week 26-2	Week 27-1	Week 27-2	Week 28-1	Week 29-1	Week 29-2	Week 30-1	Week 30-2

National SARS-CoV-2 Level

2021 | **2022**

Week	Week 31-1	Week 31-2	Week 32-1	Week 32-2	Week 33-1	Week 33-2	Week 34-1	Week 34-2	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	Week 02-1	Week 02-2	Week 03-1	Week 03-2	Week 04-1	Week 04-2	Week 05-1	Week 05-2	Week 06-1	Week 06-2	Week 07-1	Week 07-2

National SARS-CoV-2 Level

2022

Week	Week 08-1	Week 08-2	Week 09-1	Week 09-2	Week 10-1	Week 10-2	Week 11-1	Week 11-2	Week 12-1	Week 12-2	Week 13-1	Week 13-2	Week 14-1	Week 14-2	Week 15-1	Week 15-2	Week 16-1	Week 16-2	Week 17-1	Week 17-2	Week 18-1	Week 18-2	Week 19-1	Week 19-2	Week 20-1	Week 20-2	Week 21-1	Week 21-2	Week 22-1	Week 22-2	Week 23-1	Week 23-2	Week 24-1	Week 24-2	Week 25-1	Week 25-2	Week 26-1	Week 26-2	Week 27	Week 28	Week 29	Week 30	Week 31	Week 32	Week 33	Week 34	Week 35	Week 36	Week 37	Week 38	Week 39	Week 40	Week 41	Week 42	Week 43

National SARS-CoV-2 Level

	2022							2023																																																	
Week	Week 44	Week 45	Week 46	Week 47	Week 48	Week 49	Week 50	Week 51	Week 01	Week 02	Week 03	Week 04	Week 05	Week 06	Week 07	Week 08	Week 09	Week 10	Week 11	Week 12	Week 13	Week 14	Week 15	Week 16	Week 17	Week 18	Week 19	Week 20	Week 21	Week 22	Week 23	Week 24	Week 25	Week 26	Week 27	Week 28	Week 29	Week 30	Week 31	Week 32	Week 33	Week 34	Week 35	Week 36	Week 37	Week 38	Week 39	Week 40	Week 41	Week 42	Week 43	Week 44	Week 45	Week 46	Week 47
National SARS-CoV-2 Level																																																								

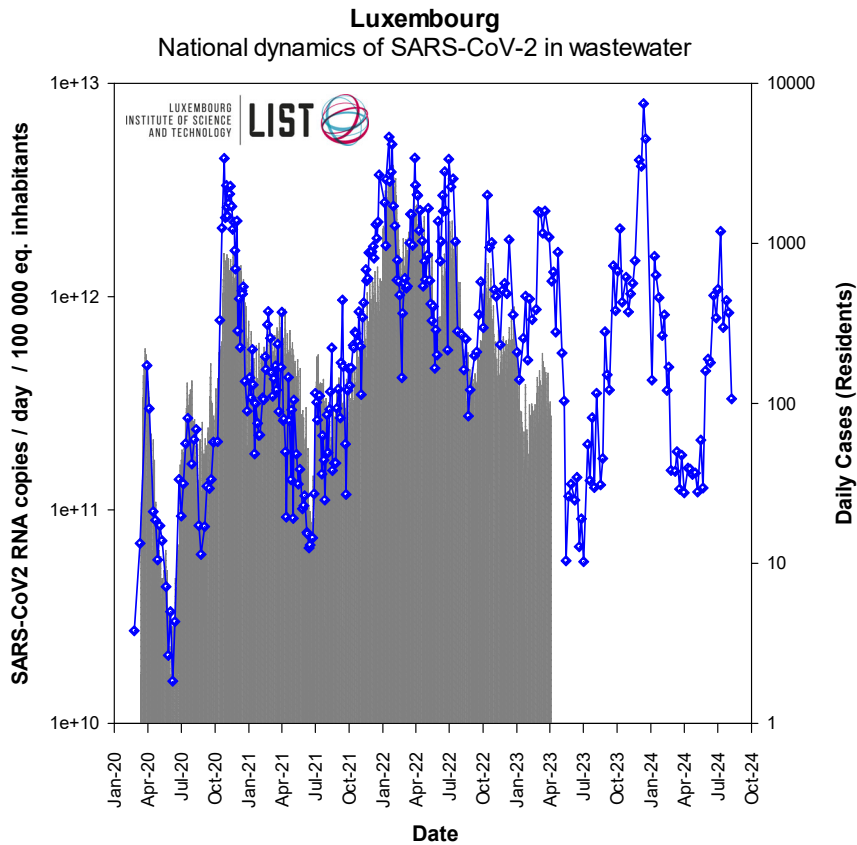| | 2023 | | | | 2024 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Week** | Week 48 | Week 49 | Week 50 | Week 51 | Week 01 | Week 02 | Week 03 | Week 04 | Week 05 | Week 06 | Week 07 | Week 08 | Week 09 | Week 10 | Week 11 | Week 12 | Week 13 | Week 14 | Week 15 | Week 16 | Week 17 | Week 18 | Week 19 | Week 20 | Week 21 | Week 22 | Week 23 | Week 24 | Week 25 | Week 26 | Week 27 | Week 28 | Week 29 | Week 30 | Week 31 | Week 32 | | | | | | | | | | | | | | |
| **National SARS-CoV-2 Level** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Figure 1 – RT-qPCR quantification time-course monitoring of SARS-CoV-2 (E gene) in Luxembourgish wastewater samples from December 2019 to August 2024. 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).

a) *Linear scale*

b) *Log$_{10}$ scale*

*Table 2 - Level of SARS-CoV-2 contamination of each analysed wastewater treatment plant in Luxembourg in 2023 and 2024. BEG: Beggen, BET: Bettembourg, SCH: Schifflange, BLE: Bleesbruck, MER: Mersch, PET: Pétange, ECG: Echternach, UEB: Uebersyren*

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

Figure 2 – RT-qPCR quantification time-course monitoring of SARS-CoV-2 (E gene) in the eight selected wastewater treatment plants from March 2020 to August 2024. Grey squares: daily confirmed cases for the contributory area of each wastewater treatment plant, coloured dots: SARS-CoV-2 flux (RNA copies/day/10,000 equivalent inhabitants).
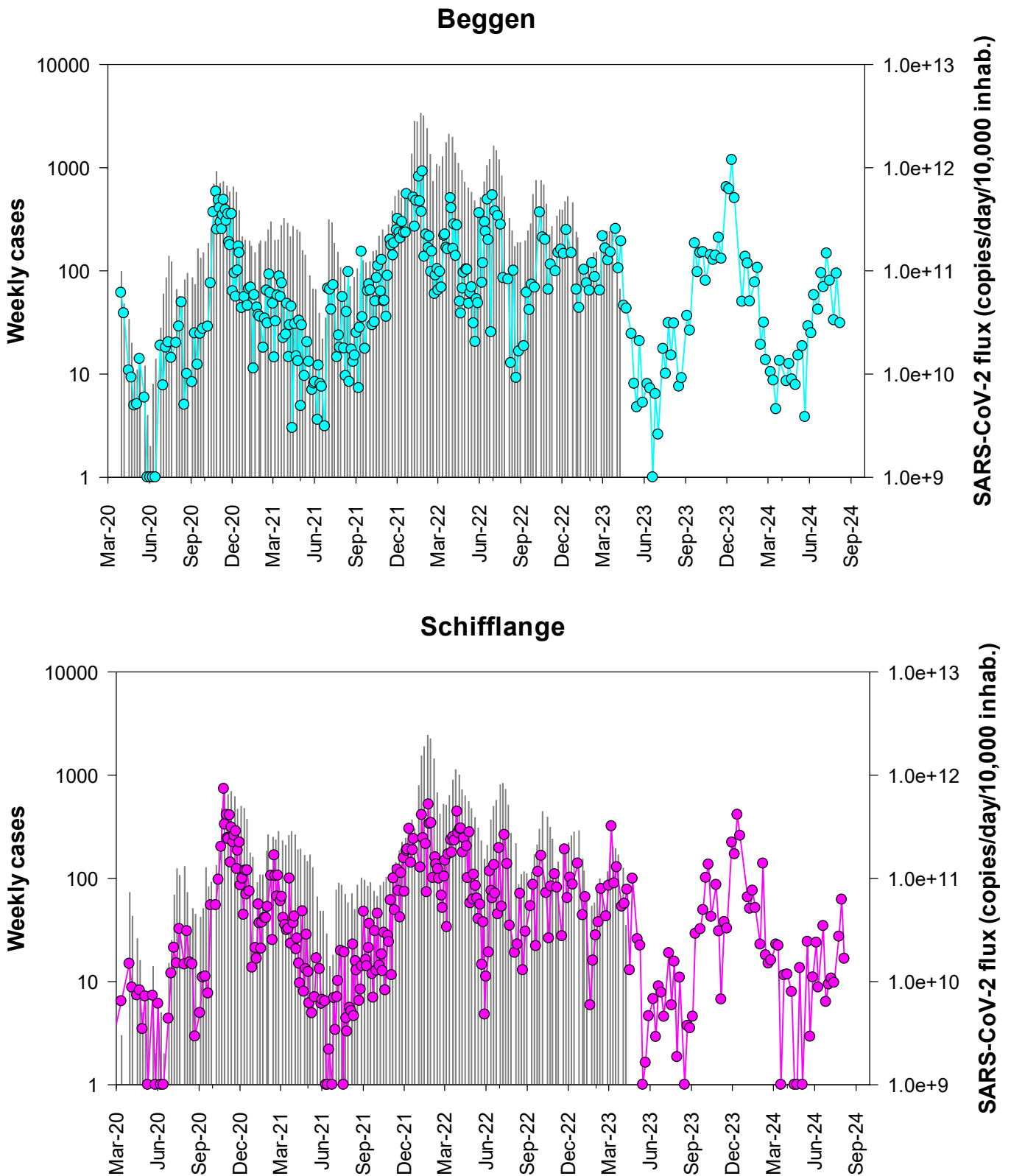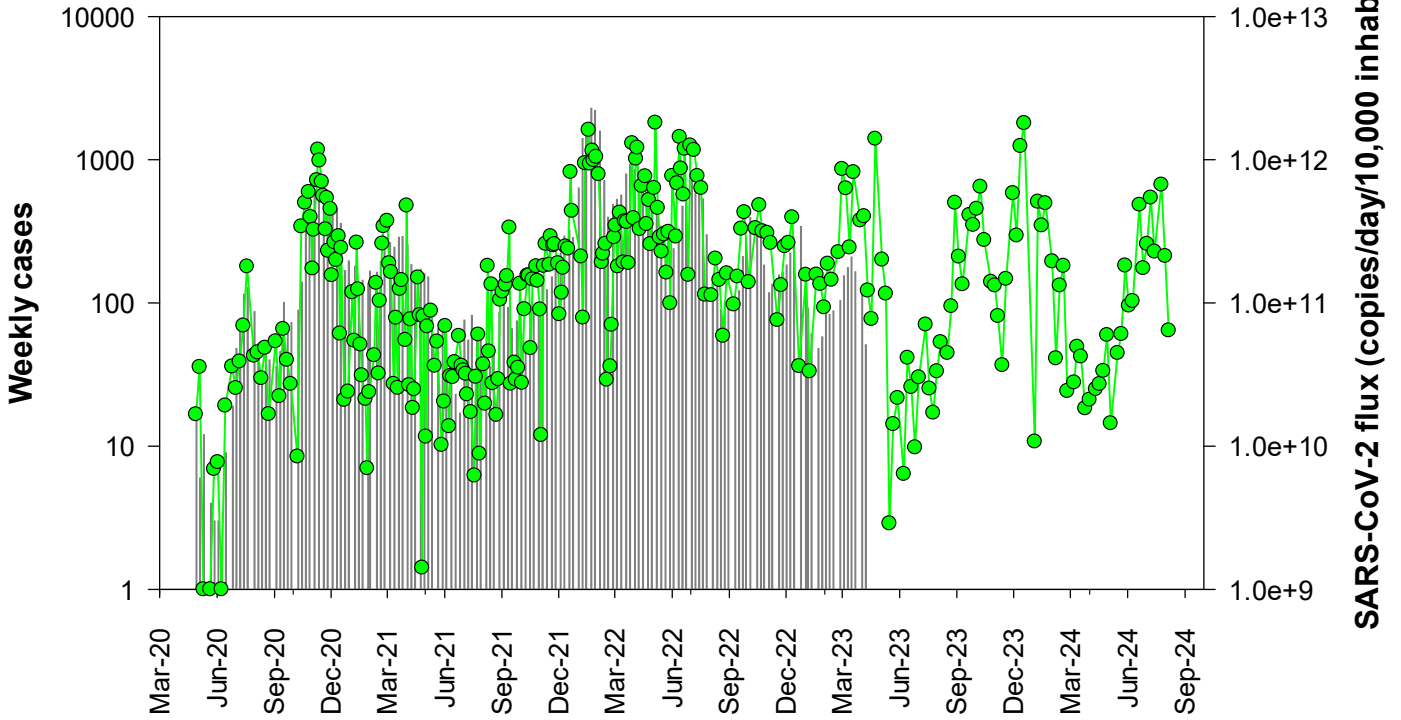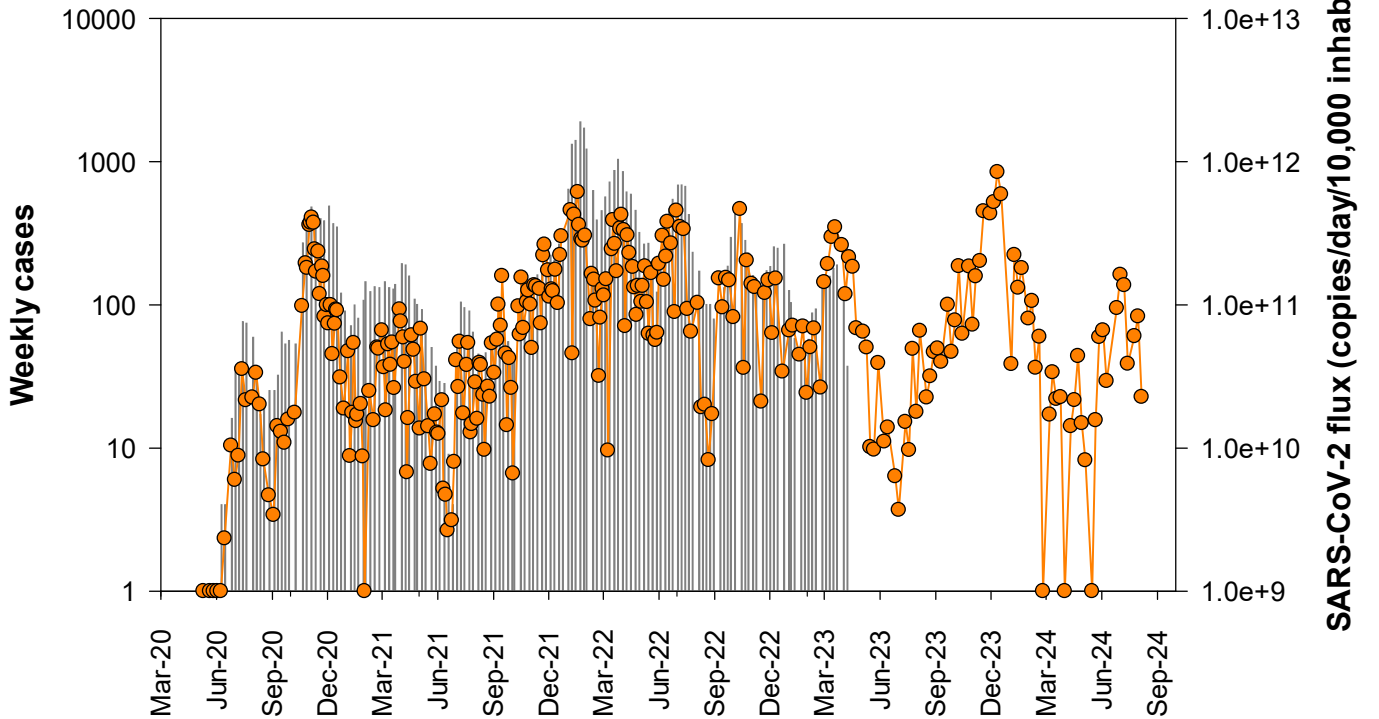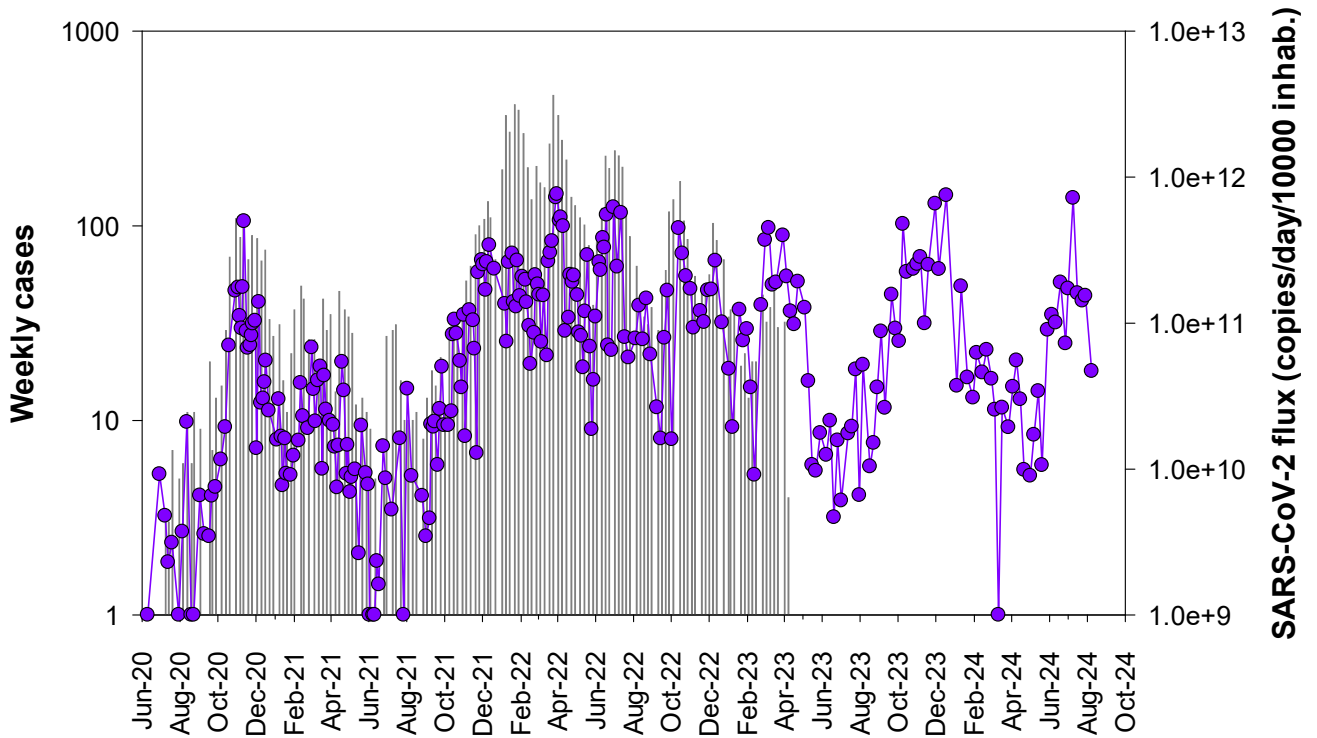
Bleesbruck
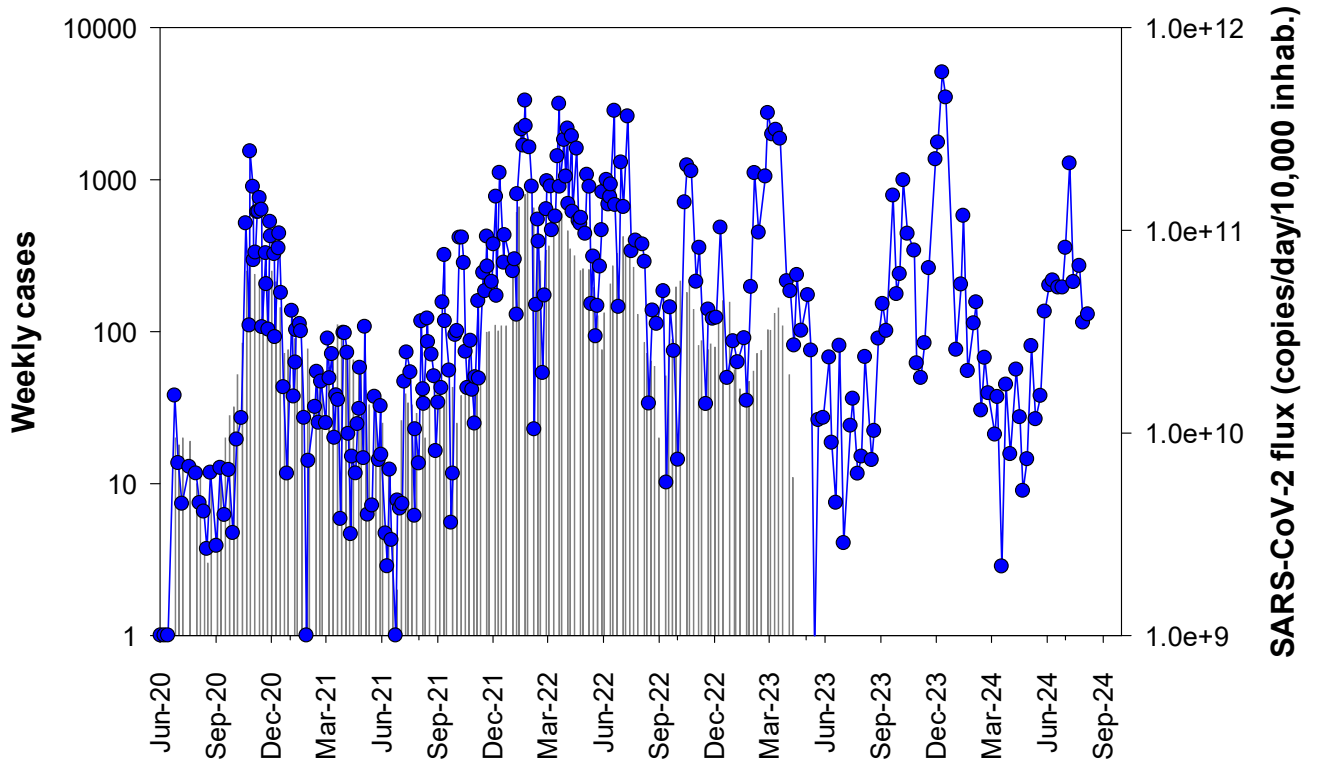
Mersch
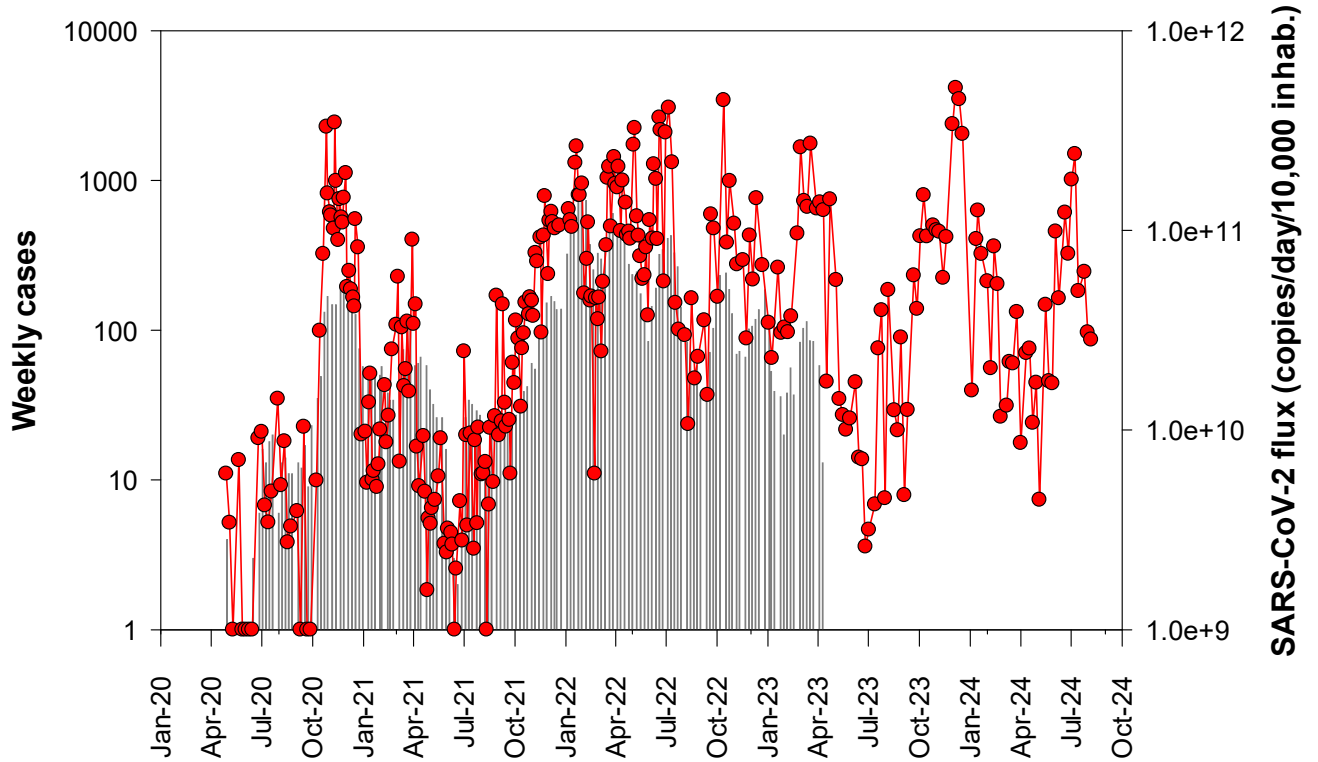
Pétange

Bettembourg

Uebersyren

Echternach
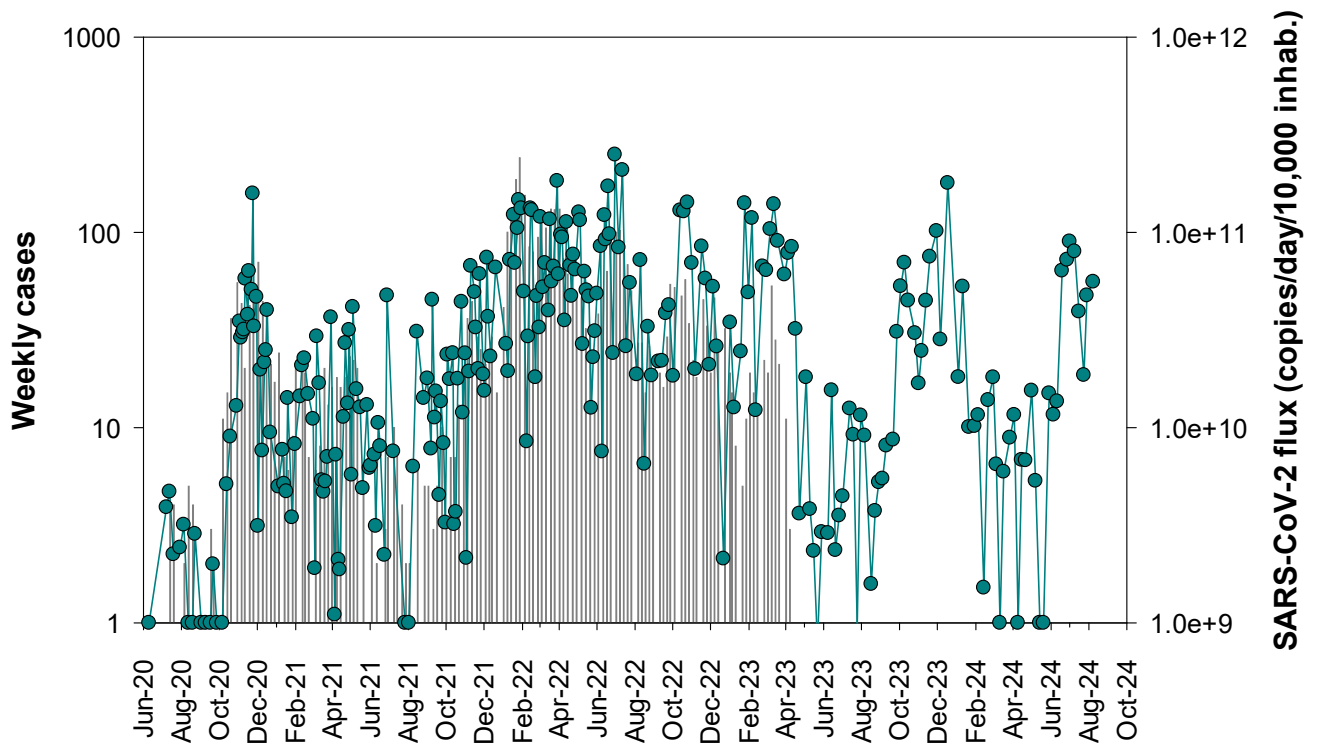
Table 3- Sewage sampling since the beginning of the CORONASTEP study

WWTP	2019	2020	2021	2022	2023	2024	Total
Beggen		52	92	77	50	31	**302**
Bettembourg		45	91	75	50	32	**293**
Schifflange	4	56	96	76	51	32	**315**
Bleesbrück		44	92	77	51	32	**296**
Mersch		47	91	77	51	32	**298**
Pétange	4	51	96	77	51	32	**311**
Hesperange		35	48	50	51	3	**187**
Echternach		36	79	77	50	31	**273**
Uebersyren		38	78	77	50	31	**274**
Grevenmacher		38	81	76	51	2	**248**
Troisvierges		38	92	77	51	3	**261**
Boevange sur Attert		20	48	51	51	3	**173**
Wiltz		28	92	77	51	3	**251**
Total	**8**	**528**	**1076**	**944**	**659**	**267**	**3482**

Materials and Methods

Sewage samples

From March 2020 to August 2024, up to thirteen wastewater treatment plants (WWTPs) were sampled at their inlet according to the planning presented in Table 3. Since January 2024, the number of WWTPs followed was reduced to eight. The operators of the WWTPs collected a 24-hour composite sample according to their routine sampling procedure. Composite samples were 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 sampling day. Larger particles (debris, bacteria) were removed from the samples by centrifugation at 2,400 x g for 20 min at 4°C. A 120 mL 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 COVID-19 pandemic, it can be assumed that only SARS-CoV-2 strains will be detected by this assay given that the 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 protocol[1]. 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 Viia7 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.

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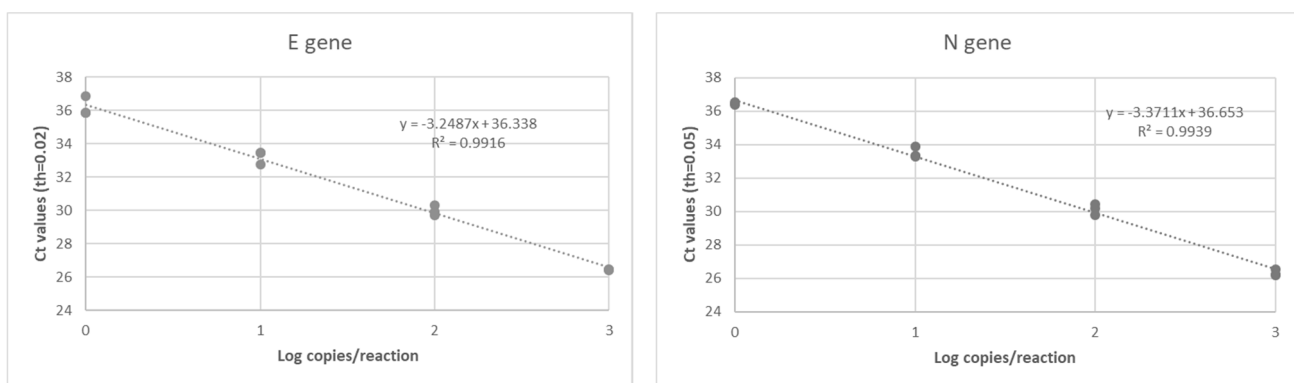
Figure 6 – RT-qPCR standard curves established for both target genes (E gene and N gene) of SARS-CoV-2 using a commercially available standard (Biorad).
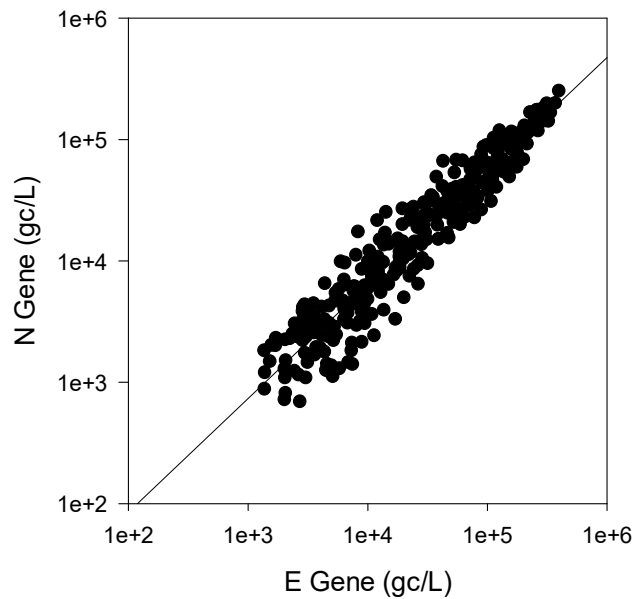
Data interpretation

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, the 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 = 5.979.10^{-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),

Acknowledgements