WEEKLY REPORT – WASTEWATER-BASED EPIDEMIOLOGICAL SURVEILLANCE OF THE SARS-COV-2

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RESULTS OF 05/01/2022

Authors: R. Janssens[1], H. Maloux[1], S. Hanoteaux[1], B. Verhaegen[2], K. Van Hoorde[2], K. Dierick[2], S. Quoilin[1], K. Blot[1], M. Lesenfants[1]*

[1] Sciensano, Service Epidemiology of infectious diseases
[2] Sciensano, Service Foodborne pathogens
*Project responsible and contact: marie.lesenfants@sciensano.be

In collaboration with:

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1. Summary

The circulation of the SARS-CoV-2 virus in the environment is assessed in the present work based on three indicators. The analysis of wastewater samples collected in 42 wastewater treatment plants covers 45% of the Belgian population, with increased accuracy in the urban areas. The results of the wastewater surveillance is a source of complementary information to the infection cases number as the populations represented are different. Indeed, the wastewater results do notably include all asymptomatic persons, and are independent of the testing strategy.
Here are the conclusions based on the latest results of January 05th 2022:

- At the national level: The number of "High circulation" areas remains stable at a high level while the fast increase and increasing trend have both recently risen. It is important to note that the numbers of areas in which any indicator is fulfilled are underestimated as the concentrations measured in the wastewaters were diluted last week by rainy events (from the 27th of December onwards). Based on the dilution data, it was estimated that the double of areas should have been highlighted in both the fast increase and increasing trend indicators.
- The impact of the Omicron Variant of Concern on the wastewater surveillance was assessed to be moderate (see 4.2 Variants). A source of uncertainty stands in the possible modification of the excretion load of the Omicron variant compared to the Delta variant. A weekly investigation of the possible impact of Omicron is conducted to screen its evolution in time.

The wastewater situation can be followed on a weekly basis on:

- The graphics available on the public COVID-19 dashboard
- The Risk Assessment Group (RAG) updating the weekly epidemiological situation through a report validated by the Risk Management Group (RMG) and published every Friday in French and Dutch.
- Further details on the methodology applied for the wastewater surveillance can be found in the Appendix Methodology document (access online).

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2. Introduction

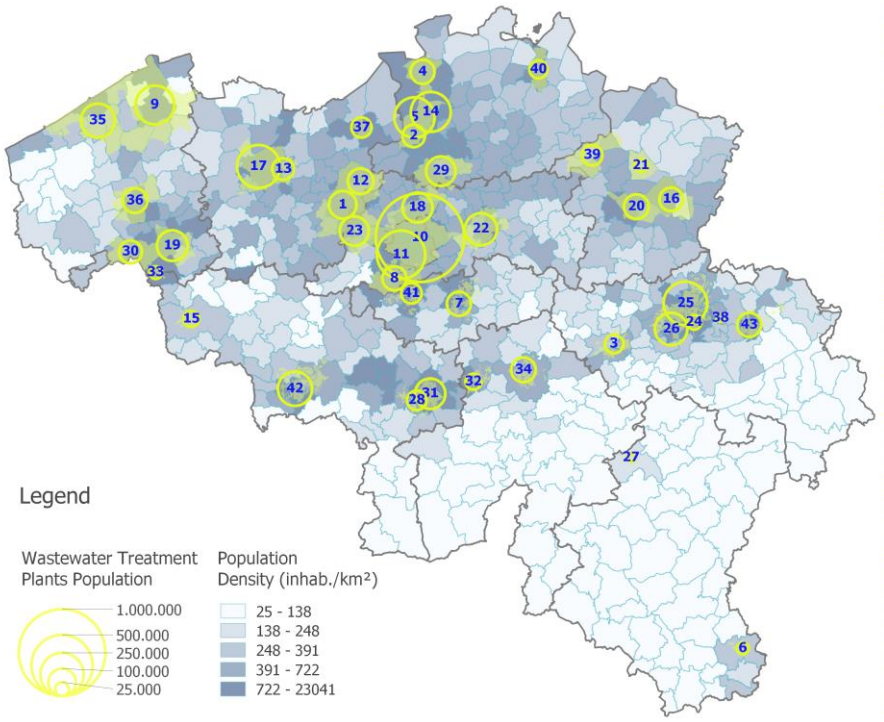
In mid-September 2020 started the SARS-CoV-2 national wastewater surveillance project. The present report is an outcome of this project aiming to assess weekly the wastewater-based epidemiological situation of Belgium.
The surveillance is based on the analysis of water samples collected twice per week from the influent of 42 WasteWater Treatment Plants (WWTPs) spread over Belgium. The evolution of the SARS-CoV-2 viral concentrations measured over time in the different treatment plants is analyzed at different levels: regional, provincial, and the catchment area covered by the individual treatment plants. Also, alerting indicators were developed to highlight areas of concern regarding the high circulation, the fast evolution, and the increasing trend of the observed viral concentrations.

In this report, the weekly wastewater-based epidemiological situation is presented and discussed at the above-mentioned levels based on the three categories of alerting indicators. Moreover, the remaining sources of uncertainties are discussed together with their expected impacts on the wastewater results interpretation.

3. Methodology

3.1. SAMPLE COLLECTION AND ANALYSIS

The surveillance project, which started in mid-September 2020 covers around 45% of the Belgian population. The population covered is 40% in the Flemish region, 35% in the Walloon region, and nearly 100% in the Brussels region. Figure 1 shows the catchment areas covered by the 42 WWTPs included in the project, which are located in high population density areas. A catchment area is defined by the area delimiting the population covered by a specific wastewater sample, collected at the inlet of the WWTPs. Further coverage details can be found in Table 1 by province (see also Table A1 in the Appendix Methodology document (access online)) and on the Sciensano public dashboard.
In practice, 24-hour composite samples are collected twice a week on Mondays and Wednesdays from the influent of WWTP and are analysed for the presence of SARS-CoV-2 RNA by three different laboratories. The resulting SARS-CoV-2 concentrations (3 targeted genes) are delivered to Sciensano within 2 days for data analysis purpose. Concretely, the results which are representative of Mondays and Wednesdays are respectively available on Wednesdays and Fridays.



Treatment Plant	Id	Population
Aalst	1	102800
Aartselaar	2	68031
Amay	3	47038
Antwerpen-Noord	4	76949
Antwerpen-Zuid	5	215704
Arlon	6	19440
Basse Wavre (Dyle)	7	78290
Beersel	8	78540
Brugge	9	200401
Bruxelles Nord/Brussel Noord	10	1045863
Bruxelles Sud/Brussel Zuid	11	311866
Dendermonde	12	87633
Destelbergen	13	63771
Deurne	14	213048
Froyennes	15	34710
Genk	16	73364
Gent	17	247550
Grimbergen	18	120627
Harelbeke	19	125230
Hasselt	20	81988
Houthalen-Centrum	21	22357
Leuven	22	137365
Liedekerke	23	112933
Liège (Grosses Battes)	24	27934
Liège Oupeye	25	253780
Liège Sclessin	26	142239
Marche-en-Famenne	27	8633
Marchienne-au-Pont	28	52017
Mechelen-Noord	29	116363
Menen	30	70041
Montignies-sur-Sambre	31	123576
Mornimont	32	34140
Mouscron versant Espierres	33	21179
Namur-Brumagne	34	82175
Oostende	35	153401
Roeselare	36	82137
Sint-Niklaas	37	55038
Soumagne	38	7955
Tessenderlo	39	55546
Tumhout	40	44524
Vallée du Hain (L'Orchis)	41	57050
Wasmuel	42	157342
Wegnez	43	78244

Figure 1: The population located in the areas covered by the wastewater treatment plants (highlighted in yellow) and the population density for each municipality (indicated by the blue scale). Note that due to the recent flooding, the treatment plant of Wegnez is out of order. This area is, hence, not considered anymore in the wastewater surveillance and has been replaced by the treatment plant of Soumagne.

3.2. WASTEWATER RESULTS

The quantification of SARS-CoV-2 in RNA copies/ ml (concentration) was generalized in mid-February 2021 to all the treatment plants investigated. Before this date, the quantitative values were estimated based on the retrospective application of the quantification method (see details in Appendix) from mid-September 2020 to mid-February 2021 for Flanders and Brussels. In Wallonia, the quantitative results were available since the start.

Preliminary results allowed for estimating the limit of quantification of the analytical method at 20 copies/ml.

3.3. ALERTING INDICATORS

To highlight the areas of possible concerns, the three following types of alerting indicators are assessed twice a week, based on the viral concentration (RNA copies/ml) measured for the three targeted gene fragments (E, N1, and N2):

1. The **High circulation** indicator highlights the catchment areas where the viral concentrations are high. It corresponds to a situation where the viral concentrations exceed half of the highest value recorded during the third wave (i.e. from mid-February 2021 till begin of May).
2. The **Fast increase** indicator highlights the catchment areas where the viral concentrations have rapidly increased for the last week. It corresponds to a situation where the moving average on the past 7 days of the viral concentration has increased faster than 70% per week if being above the estimated limit of quantification. The increasing slope is normalized for each treatment plant.

3. The **Increasing trend** indicator highlights the catchment areas where the viral concentrations have been increasing for more than 6 days. The indicator is computed based on the moving average on the past two weeks of the viral concentration.

The indicators were developed in order to be able to track the different phases of an outbreak. Typically, when the viral concentrations in wastewaters start to increase in an area, the indicator Increasing trend will be the first fulfilled. If the concentrations increase quickly, the Fast increase indicator will then be fulfilled. Finally, after the initial increasing phase, the concentrations in an area will be sufficiently high to result in the High circulation to be fulfilled.

3.4. CASES RESULTS

The cases number data presented in this report come from the COVID-19 laboratory-confirmed cases database centralized by Sciensano. The cases number, used to compute the 14 days-incidence, only accounts for the physical areas covered by the wastewater project (see Figure 1).

3.5. CAUTION POINTS FOR THE RESULTS INTERPRETATION

Only the trends, observed through the alerting indicators, should be assessed for the comparison of different areas. Absolute values should not be compared as the concentration values differ from an WWTP to another, notably due to the differences in population sizes covered. Additionally, the situation comparison between the regions should be considered with caution. The degree of comparability is not yet known and depends on the comparability of the results between the different laboratories performing the analysis (see the Appendix Methodology document (access online)).
Moreover, the wastewater concentrations and the cases numbers presented in this report do not originate from the same population, even though the positive cases are selected only for the municipalities covered by the wastewater surveillance. For instance, the wastewater results account for all infected persons (whatever age or symptomatic condition) while the cases include only the persons tested clinically positive. Likewise, an infected person covered by the wastewater results could be associated with another area in the clinical surveillance as the person's postal code is used for clinical statistics (mobility bias). Therefore, the correlation between the wastewater concentrations and the cases number varies according to the area considered. The wastewater results are thus complementary and independent information to the results of the cases.

The correlation between the wastewater viral concentrations and the cases numbers could also be influenced by the vaccination campaign and the circulation of variants.

Finally, all values below the limit of quantification (< 20 RNA copies/ml) should be considered as non-quantitatively reliable values. A reported value lower than 20 copies/ml only shows that SARS-CoV-2 has been detected in the sample at an undetermined concentration.

4. Results

4.1. REGIONS

Figure 2 presents the quantitative SARS-CoV-2 RNA concentration in the wastewaters and the 14 days incidence for each region, compiling the incidence data of the area covered by the wastewater surveillance. The estimated viral concentrations can also be seen for the period before mid-February (see 2.2. in the Appendix Methodology document (access online) for more details).
The second wave peak occurring in November 2020 can be seen in the three regions in Figure 2 below. This remains true for the third wave, but to a lower extend in Wallonia. Several hypotheses could explain this: (1) the sizes of the treatment plants in Wallonia are smaller than the ones located in the two other regions, affecting the viral concentrations measured; (2) the extent to which the results are comparable between the different laboratories is unknown (see Methodology for more details)

Recently, the viral concentrations in the wastewater were increasing since the beginning of October, illustrating the fourth wave (Figure 2). The last regional trends cannot be assessed this week due to significant rainy events which resulted in dilution in the wastewater samples.
It should be noted that the last viral concentrations in wastewaters are not displayed in the figure as the regional corresponding trends are still to be validated with the next week results[1].

[1] The trends of SARS-CoV-2 circulation in wastewater, given by the dark line on the graphs, corresponds to a 14 days centred mean of the concentrations measured.

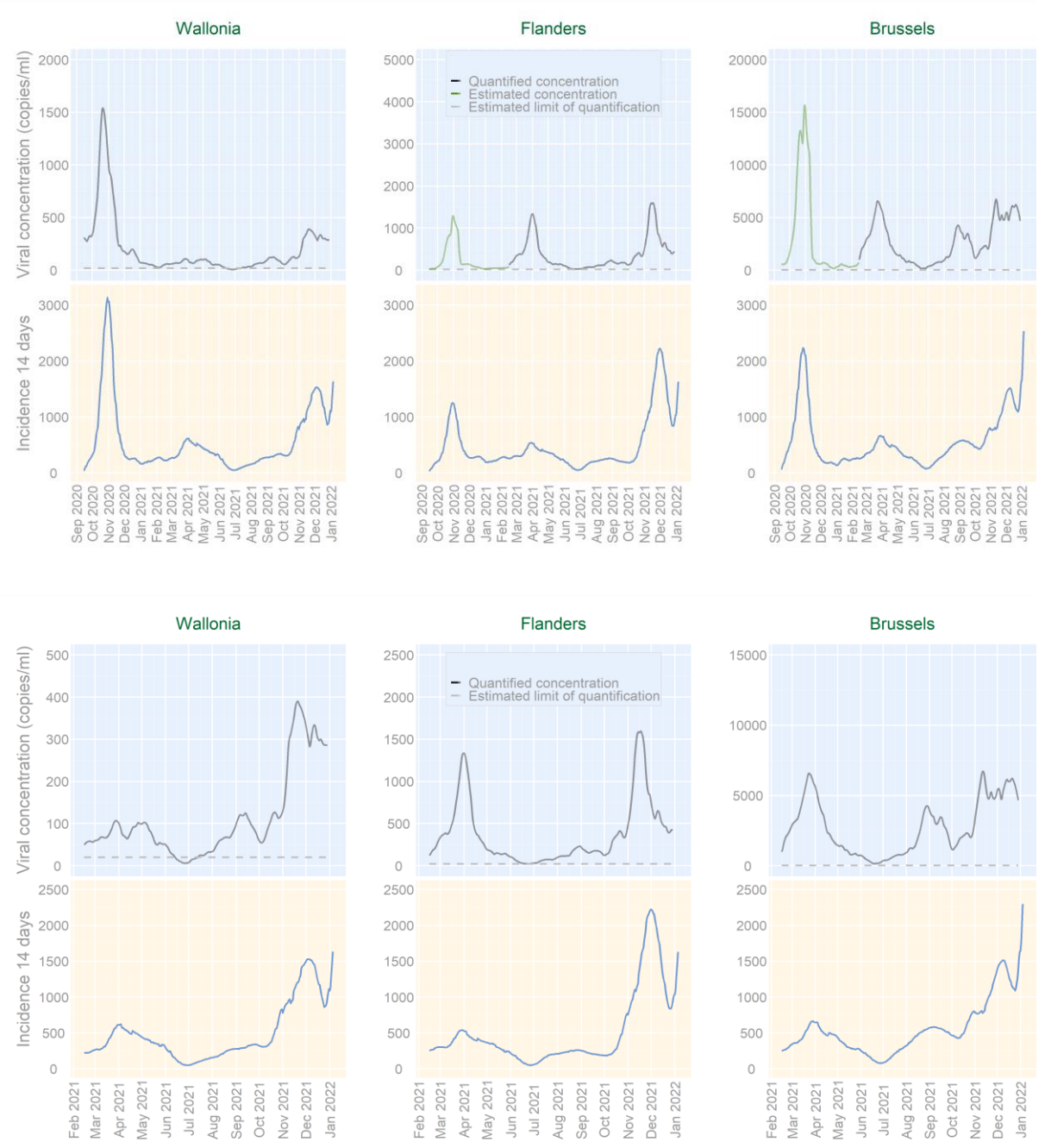


Figure 2: The SARS-CoV-2 RNA estimated and quantified concentrations expressed as copies/ml (two weeks centered moving average applied on the linear interpolation), the estimated limit of quantification of 20 SARS-CoV-2 RNA copies/ ml, and the 14 days incidence in the population covered by the wastewater surveillance since the surveillance starting date (graph set above) and mid-February 2021 (graph set below).

4.2. VARIANTS

Mutations are present in the Omicron variant on the N1 and E genes which could potentially lead to a drop out of those two genes leading to an underestimation of the viral concentrations in wastewaters. As the Omicron variant represents a substantial proportion of all sequences reported in Belgium [2], the correlations between the measured concentrations on the N1, E and N2 genes have decreased over the last two weeks. However, these correlations remain high and significant and the impact of the Omicron variant on the wastewater surveillance this week was assessed to be moderate. A source of uncertainty stands in the possible modification of the excretion load of the Omicron variant compared to the Delta variant.

A weekly investigation of the possible impact of Omicron is conducted in order to screen its evolution in time.

[2] Source :COVID-19 dashboard