Time series modelling for wastewater-based epidemiology of COVID-19 : a nationwide study in 40 wastewater treatment plants of Belgium, February 2021 to June 2022

TITLE PAGE

Title: Time series modelling for wastewater-based epidemiology of COVID-19: A nationwide study in 40 wastewater treatment plants of Belgium, February 2021 to June 2022

Authors: Xander Bertels[1] (0000-0002-4815-9067), Sven Hanoteaux[2], Raphael Janssens[2] (0000-0002-4383-1053), Hadrien Maloux[2], Bavo Verhaegen[3] (0000-0002-1709-5259), Peter Delputte[4] (0000-0003-3972-616X), Tim Boogaerts[5] (0000-0002-3802-3174), Alexander L.N. van Nuijs[5] (0000-0002-9377-6160), Delphine Brogna[6] (0000-0002-4354-4177), Catherine Linard[6] (0000-0002-0819-7755), Jonathan Marescaux[6,7], Christian Didy[8], Rosalie Pype[8] (0000-0002-8785-5946), Nancy H.C. Roosens[9] (0000-0001-9218-078X), Koenraad Van Hoorde[3], Marie Lesenfants[2], Lies Lahousse[1] (0000-0002-3494-4363)

Affiliations

1. Department of Bioanalysis, Ghent University, 9000 Ghent, Belgium
2. Epidemiology and Public Health, Epidemiology of Infectious Diseases, Sciensano, 1050 Brussels, Belgium
3. Infectious Diseases in Humans, Foodborne Pathogens, Sciensano, 1050 Brussels, Belgium
4. Laboratory for Microbiology, Parasitology and Hygiene, University of Antwerp, 2610 Wilrijk, Belgium
5. Toxicological Centre, University of Antwerp, 2610 Antwerp, Belgium
6. Institute of Life, Earth and Environment, University of Namur, 5000 Namur, Belgium
7. E-BIOM SA, 5000 Namur, Belgium
8. Société Publique de Gestion de l'Eau, 4800 Verviers, Belgium
9. Biological Health Risks, Transversal Activities in Applied Genomics, Sciensano, 1050 Brussels, Belgium

Corresponding author: Lies Lahousse (lies.lahousse@ugent.be): Ottergemsesteenweg 460, 9000 Gent, Belgium

Authors' contributions:

Study concept and design: XB, LL, SH, and CL. Sample collection, sample analyses, protocols, and data management: RJ, HM, BV, PD, TB, AvN, JM, CD, RP, NR, KVH, SH, and ML. Statistical methodology: XB, LL, and SH. Formal analysis: XB and SH. Drafting of the

manuscript: XB. Critical review of the manuscript: SH, RJ, HM, BV, PD, TB, AvN, DB, CL, JM, CD, RP, NR, KVH, ML, and LL. Supervision and management: LL and ML. All authors contributed to and approved the final version of the Article.

Funding: This work was supported by the Belgian Federal Government (grants COVID-19_SC048, COVID-19_SC063, and COVID-19_SC093). It was also supported by Société Publique de Gestion de l'Eau (SPGE), E-BIOM, and the University of Namur (CR-280) through two public procurements (MP20.051, MP20.097). SPGE was involved in wastewater sampling and communication of data regarding the Walloon WWTPs. E-BIOM was involved in wastewater analysis. Both SPGE and E-BIOM contributed to the co-authoring review process of the paper. Yet the funders had no role in the design of the study, in the interpretation of data, in drafting the manuscript, or in the decision to publish the results.

Conflicts of interest: None declared

Ethical statement: Ethical approval was not needed for this study. Detection of SARS-CoV-2 RNA in wastewater provides anonymised data. Aggregated clinical data was used and is publicly available (https://epistat.sciensano.be/covid/).

Data availability: Clinical datasets are publicly available (https://epistat.sciensano.be/covid/). Wastewater data is partially available online (https://epistat.wiv-isp.be/covid/covid-19.html). R script are available upon reasonable request.

Abbreviations:

- AICc: corrected Akaike Information Criterion
- IE: inhabitant equivalent
- IQR: interquartile range
- LOQ: limit of quantification
- NSD: normalised standard deviation
- PMMoV: pepper mild mottle virus
- RMSE: root-mean-square error
- RNA: ribonucleic acid
- SD: standard deviation
- WBE: wastewater-based epidemiology
- WWTPs: wastewater-treatment plants

ABSTRACT

Background: Wastewater-based epidemiology (WBE) has been implemented to monitor surges of COVID-19. Yet, multiple factors impede the usefulness of WBE and quantitative adjustment may be required.

Aim: We aimed to model the relationship between WBE data and incident COVID-19 cases, while adjusting for confounders and autocorrelation.

Methods: This nationwide WBE study includes data from 40 wastewater treatment plants (WWTPs) in Belgium (02/2021-06/2022). We applied ARIMA-based modelling to assess the effect of daily flow rate, pepper mild mottle virus (PMMoV) concentration, a measure of human faeces in wastewater, and variants (alpha, delta, and omicron strains) on SARS-CoV-2 RNA levels in wastewater. Secondly, adjusted WBE metrics at different lag times were used to predict incident COVID-19 cases. Model selection was based on AICc minimization.

Results: In 33/40 WWTPs, RNA levels were best explained by incident cases, flow rate, and PMMoV. Flow rate and PMMoV were associated with -13.0% (95% prediction interval: -26.1 to +0.2%) and +13.0% (95% prediction interval: +5.1 to +21.0 %) change in RNA levels per SD increase, respectively. In 38/40 WWTPs, variants did not explain variability in RNA levels independent of cases. Furthermore, our study shows that RNA levels can lead incident cases by at least one week in 15/40 WWTPs. The median population size of leading WWTPs was 85.1% larger than that of non-leading WWTPs. In 17/40 WWTPs, however, RNA levels did not lead or explain incident cases in addition to autocorrelation.

Conclusion: This study provides quantitative insights into key determinants of WBE, including the effects of wastewater flow rate, PMMoV, and variants. Substantial inter-WWTP variability was observed in terms of explaining incident cases. These findings are of practical importance to WBE practitioners and show that the early-warning potential of WBE is WWTP-specific and needs validation.

Keywords: wastewater surveillance; COVID-19; ARIMA; PMMoV; flow rate

Abstract word count: 288

MAIN TEXT

1 Introduction

Accurate monitoring of community-wide severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread is vital to estimate and reduce the societal impact of coronavirus disease (COVID-19). To this end, individual clinical testing has been used extensively to diagnose COVID-19 infections and impose quarantine measures (Vandenberg et al., 2021). Yet it is costly and has a tendency to be biased towards symptomatic infections due to ineffective detection of asymptomatic cases (Girum et al., 2020). Hence, for epidemiological monitoring, wastewater-based epidemiology (WBE) of SARS-CoV-2 has been implemented as a complementary surveillance tool (Agrawal et al., 2021; Janssens et al., 2022; Rainey et al., 2022; Rector et al., 2023). WBE is a method that enables detection of faecally and urinary excreted SARS-CoV-2 genes in influent wastewater to monitor viral surges (Anand et al., 2021; Anand et al., 2022; Cevik et al., 2021; Park et al., 2021) and has an early-warning potential (Mao et al., 2020; Shah et al., 2022). Additional advantages of WBE to clinical testing are its capability to detect both symptomatic and asymptomatic infections (Parasa et al., 2020), to provide more inclusive, privacy-friendly, and population-wide estimates, and allow more targeted clinical testing (Amman et al., 2022).

Nonetheless, the potential of WBE remains limited due to important variability in WBE estimates caused by the complexity of influent wastewater samples, external factors such rainfall and chlorination, and heterogeneity of wastewater treatment plants (WWTPs) and sewer networks. Hence, a myriad of factors affect the measured viral gene concentrations, including wastewater dilution, wastewater composition, and population factors such as variability in viral shedding and uncertainty in the size of the underlying population represented in a given wastewater sample (Bertels et al., 2022; Li et al., 2023). Therefore, the true number of viral RNA copies per resident remains unknown.

Adjusting for those key determinants, including flow rate, wastewater faecal strength, and population size, have been proposed to improve the utility of WBE estimates (Bertels et al., 2022). Yet, there is little research assessing the quantitative effects of these phenomena on viral concentrations in wastewater (Vallejo et al., 2022), which is critical to decide how to adjust for these factors. Although WBE estimates can be highly correlated to clinical cases of COVID-19 (D'Aoust et al., 2021; Vallejo et al., 2022; Westhaus et al., 2021), to the best of our knowledge there has been no study to date which optimizes the wastewater metric by adjusting for those factors to quantitatively model COVID-19 cases.

In this nationwide WBE study, wastewater was sampled twice weekly over more than one year (02/2021-06/2022) at 40 WWTPs in Belgium covering more than five million inhabitants. We aimed (i) to model the effect of flow rate, human faecal loads, and variants (alpha, delta, and omicron strains) on wastewater SARS-CoV-2 RNA levels and (ii) to optimize wastewater metrics to explain incident COVID-19 cases. Our study shows that wastewater flow rate and population dynamics, but not variants,

consistently explain RNA levels independent of cases. We provide meta-analysed effect sizes and prediction intervals, allowing other researchers to adjust RNA levels independent from incident cases. Furthermore, we show that WBE can lead clinical epidemiology by one week, but only in a minority of WWTPs due to substantial inter-WWTP variability. Lastly, we found that in some WWTPs RNA levels were not informative for incident cases in addition to autocorrelation of cases.

2 Material and methods

2.1 Data description

2.1.1 Wastewater data

Influent wastewater samples were collected at 40 Belgian wastewater treatment plants (WWTPs) covering approximately 5 million inhabitants, which represents 43% of the Belgian population (Figure 1 and Table S1). In the context of national wastewater surveillance, 24h samples are collected twice a week on Monday and Wednesday. Results from 15 February 2021 to 8 June 2022 were used in this study. During this period, quantitative SARS-CoV-2 RNA concentrations were obtained using a consistent protocol. Nucleocapsid 1 (N1), nucleocapsid 2 (N2) and envelope (E) RNA copies of SARS-CoV-2 were used as markers of viral presence in wastewater. Wastewater analyses were performed by Sciensano (Belgian public health institution), by the University of Antwerp and by E-BIOM (spin-off from the University of Namur) (Table S1), using the methods from Boogaerts et al. (2021 and Coupeau et al. (2020. A detailed overview of the sample collection, concentration, extraction, and PCR-based quantification is presented in the Supplementary File.

In each of the WWTPs, the covered population size was defined as the census-based domestic inhabitant equivalent, normalised by the geographical catchment area (Table S1) and the flow rate was measured by flowmeter as the daily incoming flow rate divided by 24h (m^3/h). Pepper mild mottle virus (PMMoV) RNA copy concentration, as indicator of human faeces in wastewater, was measured during the same period and used as a proxy for the number of people present in a catchment area. PMMoV is an extremely stable plant virus that infects plants from the *Capsicum* genus (pepper-containing food products) and shows widespread abundance in human stool and wastewater, without strong seasonal fluctuation (Rosario et al., 2009; Zhang et al., 2006).

2.1.2 Epidemiological data

The number of incident COVID-19 cases for a given WWTP was defined as the total daily number of positive COVID-19 PCR tests at the corresponding municipality normalised by the fraction of the covered municipality inhabitant equivalent by the WWTP catchment area.

The spread of SARS-CoV-2 has been characterised by several variants. As these variants could have an impact on the link between the epidemiological situation and the evolution of the viral concentrations in wastewater, they need to be accounted for. Data on variants circulating in the Belgian population were provided by the COVID-19 Genomics Belgium Consortium (Cuypers et al., 2022). During the period considered in this study, a variant was defined as dominant when its proportion was equal to or higher than 50%. Hence, the period under study has been divided into three subcategories depending on which variant was dominant: from 15 February 2021 to 14 June 2021, Alpha was dominant; from 21 June 2021

to 12 December 2021, Delta was dominant and from 27 December 2021 to 8 June 2022, Omicron was dominant.

2.1.3 Data sources, missing data and data transformation

All data sources are reported in the Supplementary File. For both the SARS-CoV-2 and the PMMoV RNA concentrations, concentration replicates below the limit of quantification (LOQ) of 20 copies/mL were coded as half of the LOQ (10 copies/mL) and negative replicates were coded as 1 copy/mL to allow for logarithmic transformation (Ma, 2020). To explore the link between the epidemiological situation and the viral concentrations in wastewater, additional wastewater metrics have been defined: (i) the PMMoV mass load (copies/day) is defined as the PMMoV concentration (copies/mL) x flow rate (mL/day); (ii) the viral mass load (copies/day) is defined as the SARS-CoV-2 RNA concentration (copies/mL) x flow rate (mL/day) and the viral to PMMoV ratio (-) is defined as the SARS-CoV-2 RNA concentration (copies/mL) / PMMoV concentration (copies/mL). Finally, both the viral concentration and the viral mass load were logged as +1. Missing wastewater data (1.3%) was replaced by an estimate obtained through time-dependent linear interpolation. Missing data and negative results for each treatment plant were listed in Table S2.

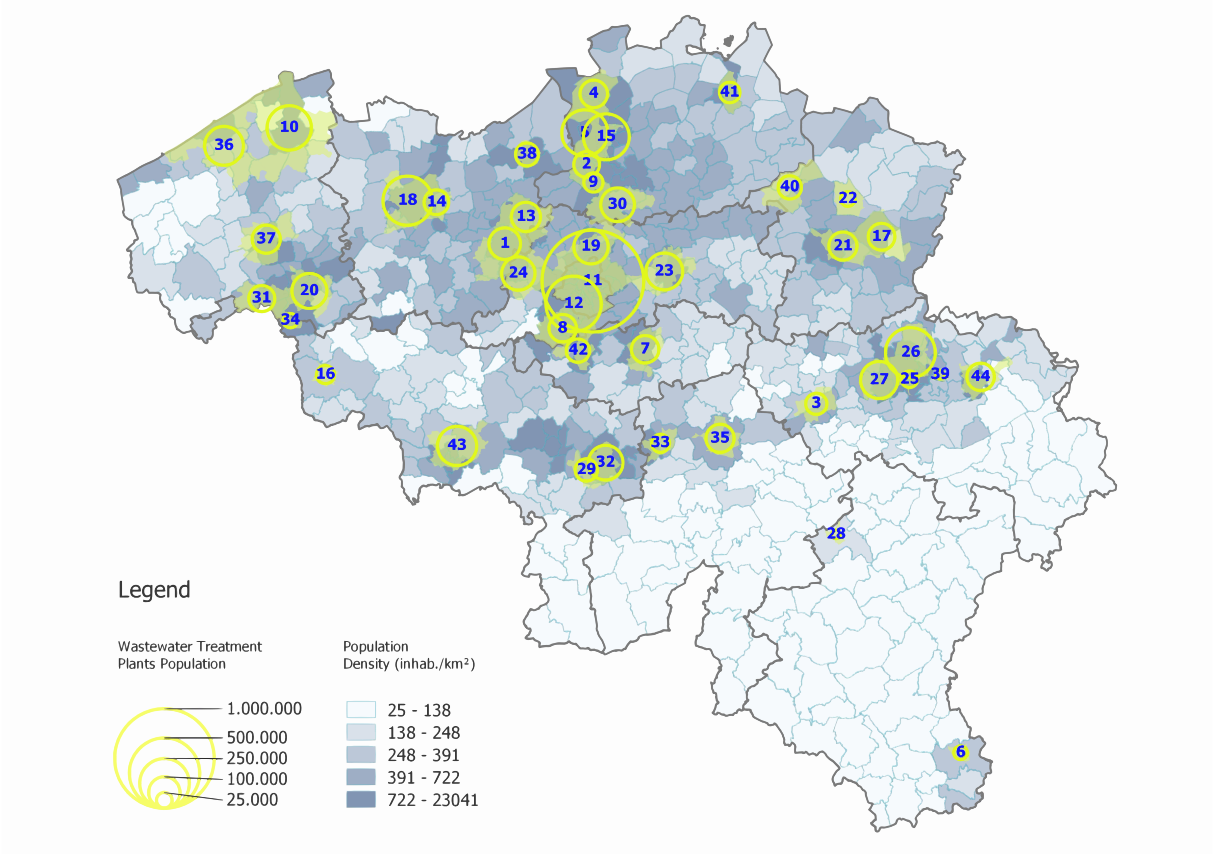

Figure 1. Map of Belgium with the location and coverage of 44 WWTPs used in the national wastewater surveillance program (Janssens et al., 2022) and municipality population density. Catchment areas are indicated by yellow surface colour. Four WWTPs were excluded from this analysis due to no available data (WWTP of Boom (nr. 9)) or shorter data coverage (WWTP of Liège Grosses Battes (nr. 25),

Soumagne (nr. 39), and Wegnez (nr. 44)) due to flooding events in July 2021. The numerical population size coverage of each WWTP is shown in Table S1. (Color print.)

2.2 Statistical analysis

2.2.1 Modelling wastewater SARS-CoV-2 RNA levels

Non-seasonal autoregressive integrated moving average (ARIMA) models and dynamic regression were applied to model SARS-CoV-2 RNA concentrations (Hyndman and Athanasopoulos, 2021). Briefly, ARIMA models are a type of time series models which describe autocorrelation. Dynamic regression models are (multiple) regression models extended with ARIMA. Dynamic regression models used in this study have a similar coefficient interpretation as standard regression but allow integrating the autocorrelation structure of the data. A comprehensive discussion of these model types is presented in the Supplementary File.

The logarithm ($\log_{10}$) of wastewater SARS-CoV-2 RNA concentration, defined as the average concentration of N1-, N2-, and E-gene RNA copies, was modelled. For every WWTP, an ARIMA model and 8 dynamic regression models were fitted. Dynamic regression models were adjusted for $\log_{10}$(COVID-19 cases) and with combinations of the following predictors (Table S3): (i) daily flow rate (m^3/h), (ii) PMMoV concentration (copies/L) or PMMoV mass load (copies/day), and (iii) dichotomous predictors of SARS-CoV-2 variants (alpha, delta, and omicron strains), based on 50% or higher prevalence of sequenced clinical samples.

2.2.2 Modelling incident COVID-19 cases

Incident $\log_{10}$(cases) were modelled by an ARIMA model and dynamic regression models, which included one of the following wastewater metric combinations: viral concentrations (copies/mL), viral mass load (copies/day), viral to PMMoV ratio (-), or viral mass load and viral to PMMoV ratio. Each of the four combinations was tested with the wastewater metrics lagged up to 2 weeks (i.e., up to 4 distinct sampling dates). This resulted in 16 dynamic regression models. All the dynamic regression models used the dichotomous predictors of the variants as a covariate. An exhaustive list of the considered models is presented in Table S4.

2.2.3 Model selection and meta-analysis

The optimal ARIMA specification of the models was set in a data-driven way by non-stepwise corrected Akaike Information Criterion (AICc) minimization, and with a first order of differencing ($d = 1$) to account for non-stationarity (Hyndman and Athanasopoulos, 2021; Kwiatkowski et al., 1992). Once the optimal ARIMA specification was obtained for each of the proposed model structures, the best model for a given WWTP was selected based on AICc scores, as a measure of predictive accuracy. The most selected RNA model was fitted on all WWTPs, and effect sizes were meta-analysed by a random-effects

model using inverse-variance weighting. The root-mean-square error (RMSE), a goodness-of-fit indicator, of the selected model was compared to a standard multiple regression model based on backward stepwise selection. All analyses were performed in R 4.0.5 (Vienna, Austria) using the forecast package for the ARIMA models and all data visualization was done with the ggplot2 package (Hyndman et al., 2022; Hyndman and Khandakar, 2008; R Core Team, 2022; Wickham, 2016).

3 Results

3.1 Determinants of SARS-CoV-2 RNA levels in wastewater

In 33/40 WWTPs a dynamic regression model of $\log_{10}$(cases), wastewater flow rate and PMMoV concentration was selected as the most accurate model to explain wastewater SARS-CoV-2 RNA levels (Table S5). This model was applied on all WWTPs and effect sizes were meta-analysed. One standard deviation (SD) increase in flow rate was associated with 13.0% (95% prediction interval (95%PI): -26.2 to +0.2%) decrease in RNA levels, independent of cases and PMMoV (Figure 2). Reversely, one SD increase in PMMoV levels was associated with 13.0% (95%PI: +5.1 to +21.0%) increase in RNA levels, independent of cases and flow rate (Figure 2). The removal of flow rate, PMMoV, or both variables from this model significantly reduces the predictive accuracy (median ΔAICc: +10.9, +12.8, and +27.5, respectively, Table S6). Independent of flow rate and PMMoV, a 10.0% increase in incident cases was associated with 4.5% (95%PI: +1.0 to 8.0%) increase in RNA levels. Overall, the best models explained on average 64.7% (R^2, SD = 10.4%) of the variation in RNA levels. Detailed meta-analyses for flow rate and PMMoV are presented in the Supplementary File (Figures S1-2).

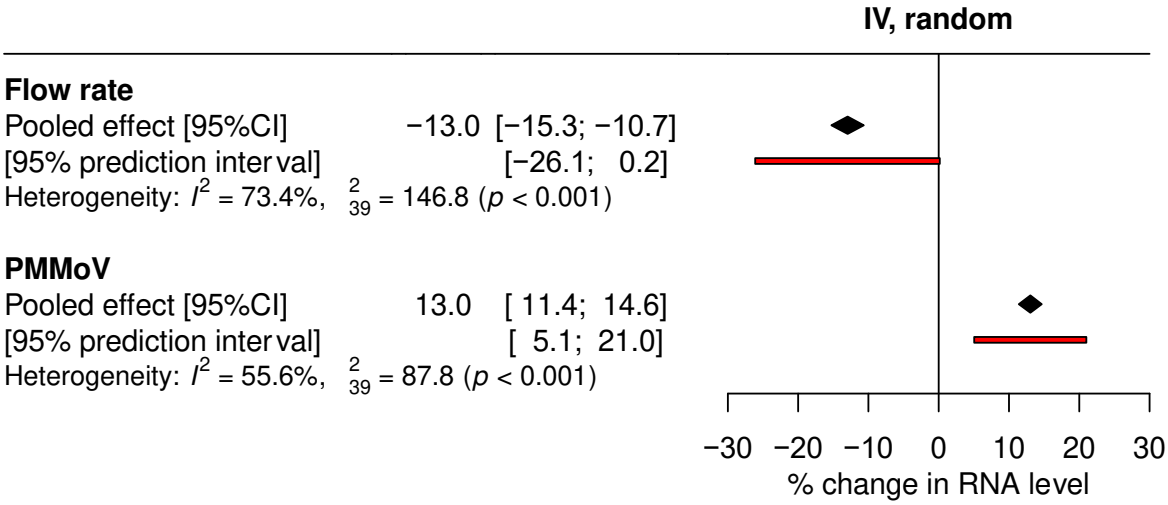

Figure 2. Meta-analysis of the independent effect of flow rate and pepper mild mottle virus (PMMoV) on SARS-CoV-2 RNA levels in wastewater, adjusted for incident cases. Effect sizes are expressed as percentage change in RNA level per one standard deviation increase in flow rate and PMMoV, respectively.

In 35/40 WWTPs, increasing flow rate was associated with a statistically significant drop in wastewater RNA levels, independent of cases and PMMoV. Exceptions were the WWTPs of Houthalen Centrum, Marchienne-au-Pont, Vallée du Hain (l'Orchis), Montignies-sur-Sambre and Wasmuel (Figure S1). In the latter WWTP, a nominal positive trend was observed (+4.7% (95%CI: -2.2 to +11.5%)). The optimal model for this WWTP did not include additive flow rate adjustment, although implicitly included

through correction for PMMoV mass load (copies/day). The WWTP of Wasmuel was the 8th largest catchment area in terms of covered population size in this study and showed the lowest normalised standard deviation (NSD) of flow rate (0.21) and the second smallest NSD of PMMoV (0.55).

In 36/40 WWTPs, increasing PMMoV was associated with a statistically significant increase in RNA levels, adjusted for cases and flow. Exceptions were the WWTPs of Tessenderlo, Turnhout, Hasselt, and Mouscron-versant-Espierres (Figure S2). In the former three WWTPs, no SARS-CoV-2 RNA was detected (i.e., RNA concentration below the limit of detection) for a substantial number of dates (Table S2). In the latter WWTP (Mouscron-versant-Espierres), wastewater was collected from both the Belgian (~21,200 IE) and France (~120,000 IE) population. Collection of French wastewater represented a substantial flow which was not covered in the clinical testing surveillance. Three out of five of the most impacted treatment plants included large student campuses (UC Louvain (Basse-Wavre), University of Liège (Liège Oupeye), and KU Leuven (Leuven)).

Lastly, an intercept for dominant variants improved the model accuracy only in 2/40 WWTPs (Destelbergen and Marchienne-au-Pont). In those two WWTPs, RNA levels of SARS-CoV-2 were 71% lower during the delta wave (B.1.617.2 strain) and 69% lower during the omicron waves (BA.1, BA.2, BA.2.75, BA.2+L452X, and BA.4 strains) compared to the period when the alpha variant (B.1.1.7) was dominant for a given number of cases, and adjusted for flow rate and PMMoV levels.

In 38/40 WWTPs, the selected dynamic regression model showed a lower RMSE value than the optimal standard multiple regression model. Overall, the average RMSE difference of dynamic regression models was 3.9 times lower than those of standard multiple regression models (Table S9).

3.2 Wastewater-based surveillance data to model incident COVID-19 cases

3.2.1 Optimal wastewater metric to link incident COVID-19 cases

In 28/40 WWTPs, the optimal model for incident COVID-19 cases included wastewater-based surveillance data. In the remaining 12/40 WWTPs, a standard ARIMA model, which does not include wastewater information, outperformed dynamic regression models in terms of predictive accuracy (Table 1a).

Of the 28 models that included a WBE metric, a flow-adjusted viral mass load was included in 15/28 WWTPs (Table 1a), while a viral-to-PMMoV gene ratio was included in 8/28 WWTPs. Overall, the flow-adjusted mass load was selected in larger WWTPs (87,633 (IQR = 102,225) vs 78,290 (IQR = 68,030) IE) while viral-to-PMMoV gene ratio was selected in smaller WWTPs (67,077 (IQR = 63,443) vs 82,082 (IQR = 92,296) IE) in terms of population coverage.

An unadjusted and unlagged viral concentration was selected in 2 of the 28 WWTPs (WWTPs of Aartselaar and Tessenderlo). These WWTPs were modestly sized WWTPs (68,031 and 55,546 vs 82,156 (IQR = 85,479) IE) and showed large variability in log(RNA) levels (0.46 and 0.82 vs 0.29 (IQR = 0.12) NSD) and in PMMoV mass load (1.31 and 1.10 vs 0.76 (IQR = 0.29) NSD).

Table 1. Optimal models to link wastewater data with incident COVID-19 cases.

A) Optimal wastewater metric (n = 40)	
WWTPs (n)	**Metric**
12/40	No wastewater metric*
7/40	Unadjusted viral concentration
13/40	Viral mass load
6/40	Viral-to-PMMoV ratio
2/40	Viral mass load + viral-to-PMMoV ratio
B) Optimal lag time of wastewater metric (n = 28)	
WWTPs (n)	**Lag time**
5/28	Unlagged wastewater metric
8/28	1-sample leading wastewater metric
10/28	2-sample leading wastewater metric (1 week)
3/28	3-sample leading wastewater metric
2/28	4-sample leading wastewater metric (2 weeks)

Viral concentration = SARS-CoV-2 RNA copies/mL; viral mass load = flow-adjusted SARS-CoV-2 RNA copies per day; viral-to-PMMoV ratio = SARS-CoV-2 gene copies per PMMoV gene copy. *Dynamic regression models with ARIMA-modelled errors were applied, except when no wastewater metric was included (standard ARIMA). Dynamic regression models adjusted for dominance of alpha, delta, or omicron variants (dichotomously coded (0/1) depending on dominant prevalence (≥ 50% of samples)).

3.2.2 Early-warning potential of wastewater metric

Among the 28 catchment areas, a leading wastewater indicator of at least one week showed the best predictive accuracy in 15/28 WWTPs, with the one-week leading indicator being selected in most (10/28) treatment plants (Table 1b, Table S7). The median covered population size in leading WWTPs was 85.1% larger than in those where a non-leading wastewater indicator was selected (102,800 (IQR 66,735) vs 55,546 (IQR = 33,891) IE). The coefficients of all 28 models are presented in Table S8. A sensitivity analysis using correlation coefficients (Table S7) showed similar results in 12/28 WWTPs, a more pronounced lead time in 8/28 WWTPs, and a less pronounced lead time in 8/28 WWTPs. Figure 3 illustrates the optimal model (1-week leading viral mass load) at the largest WWTP (Brussels-North, 1,045,900 IE) to explain incident cases of COVID-19.

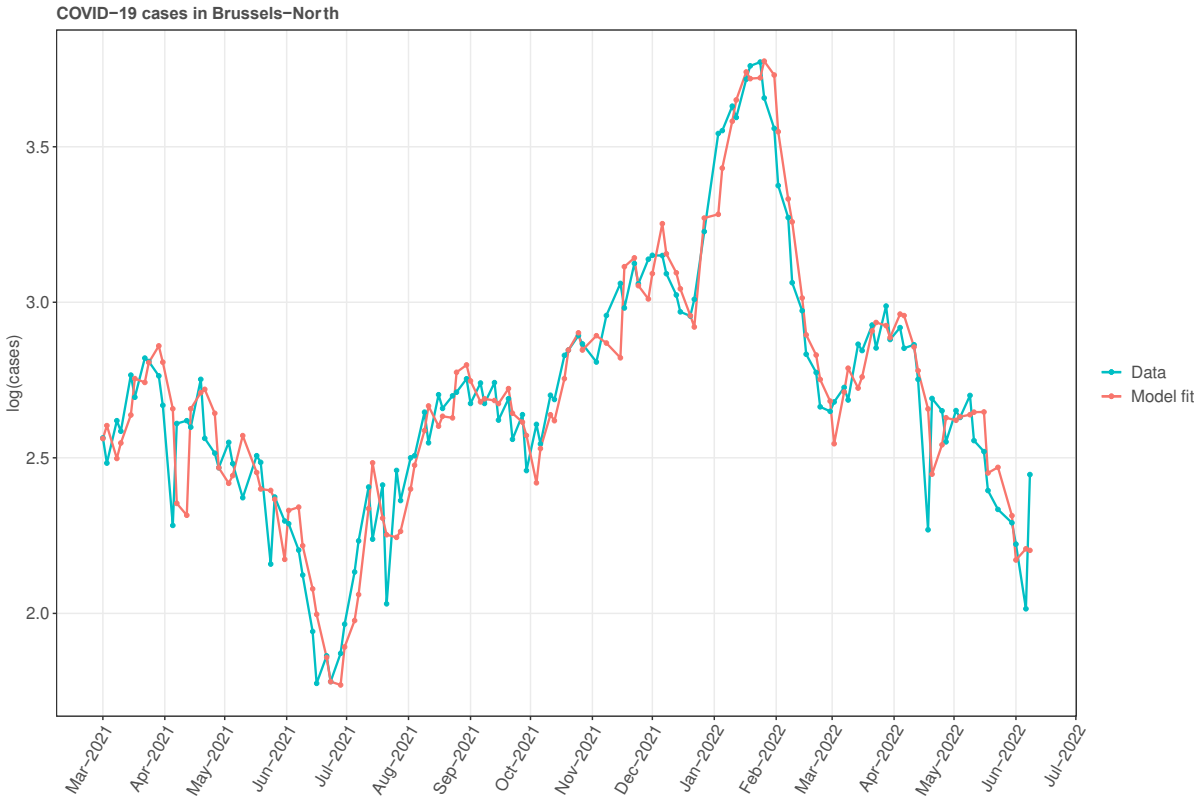

Figure 3. Logarithm of incident COVID-19 cases (blue) at the largest WWTP (Brussels-North, covering approximately one million inhabitants) and predicted incident cases based on a model including the one-week leading viral mass load (RNA copies/day) (red). Model diagnostics are presented in Figure S3. (Color print.)

4 Discussion

This nationwide study modelled the relationship between wastewater-based SARS-CoV-2 RNA levels and incident COVID-19 cases, covering approximately 5 million Belgian inhabitants for more than one year. This is the first study to show the relative effect size of wastewater flow rate and PMMoV concentrations on SARS-CoV-2 RNA levels in wastewater, while accounting for autocorrelation. Secondly, SARS-CoV-2 variants did not explain variability of RNA levels for a given number of cases in the large majority of WWTPs (38/40). Furthermore, different WBE metrics were tested at different lag times for subsequent use in monitoring COVID-19 epidemiology. This study confirms that WBE data can lead incident cases by at least one week but only in a minority of WWTPs (15/40). In 17/40 WWTPs, different wastewater metrics did not lead or explain incident cases in addition to autocorrelation. Future studies should therefore validate the early-warning potential of WWTPs and investigate whether WBE adds beyond autocorrelation to support the additional efforts/costs of determining RNA levels at these areas/WWTPs for predicting incident cases.

This analysis showed that increasing daily flow rate reduces RNA levels by on average -13.0% per SD increase, independent of incident cases and PMMoV (e.g., dilution by rainfall and other sources including industrial water and drain water). Flow-adjusted viral mass loads approach viral dynamics more accurately, which was demonstrated through its empirical support in our incident case models. Viral mass loads were mainly selected in WWTPs serving larger populations.

Secondly, our results validate that PMMoV is a key contributor to RNA variability, independent of cases and flow. Higher PMMoV levels were associated with increasing viral RNA levels and may serve as a proxy for the number of persons contributing to a wastewater sample. This is reinforced by the observation that the PMMoV was not selected in the station of Mouscron-versant-Espierres, where the cases are not truly linked with the represented population. Also, PMMoV may be used as a normalization standard for additional variability which is not explicitly defined in the models. Unmeasured phenomena such as RNA adsorption, aqueous-solid phase distribution and degradation may be implicitly modelled, partly, by normalizing for PMMoV. RNA of SARS-CoV-2 will likely be affected in similar ways as PMMoV RNA due to their common physicochemical properties of RNA including molecule size and stability, overall negative charge, and as substrates of RNases. The ratio of viral-to-PMMoV gene copies improved case models in about one in five WWTPs. A lower number of inhabitants was covered in these WWTPs, presumably increasing the relevance of relative changes in population size. In contrast, PMMoV levels did not associate with viral RNA levels in five smaller WWTPs in which the dynamic of the viral evolution was not connected with the true underlying population due to for example zero-inflation of the viral concentrations. To allow more model flexibility, one may need a normalization marker for in-sewage factors and a different marker to account for the underlying population size and dynamics of a catchment area (e.g., mobility data from telecom providers).

In 38/40 WWTPs, additively correcting for the dominant SARS-CoV-2 strain did not improve model predictive accuracy for RNA levels. Hence, faecal shedding kinetics of SARS-CoV-2 variants were likely stable over time. This suggests that increasing infectiousness of variants may be caused by increased infectiousness of viral particles and/or selective respiratory shedding but was not associated with increased faecal shedding. In the two WWTPs with an informative variant term, less RNA was detected for a given number cases in the delta and omicron waves compared to the alpha wave. Importantly, shedding kinetics may be affected by increasing immunity among the population over time (Puhach et al., 2022).

Finally, this study demonstrates that, although different WWTPs share common dynamical characteristics, every WWTP has its particular dynamic in time as demonstrated by the amplitude of the measured effect sizes within the same model structure, this for both the flow rate and the PMMoV concentrations. The diversity of dynamics unravelled in this study thus shows that care must be taken when comparing RNA levels measured at different WWTP and that aggregation of quantitative data in a fixed effect model should be avoided. Aggregation and comparison are still possible but should be paired with a normalization process and/or using indicators (3). Also, additional factors which were not accounted for in this study, including the organic load and the number of solid particles in sewage, wastewater pH, and water chlorination will contribute to the remaining unexplained variability (~ 35%) (Bertels et al., 2022; Li et al., 2021).

The main strengths of this study were the nationwide population scale, the large number and heterogeneity of WWTPs, the long duration (> 1 year), and the high resolution of the data (twice-weekly sampling). Secondly, this study was performed during a period with the highest frequency of diagnostic COVID-19 tests in Belgium (Sciensano, 2023). Lastly, through ARIMA-based modelling, we accounted for autocorrelation enabling in-depth inferences of effect sizes. The added value of dynamic regression models was corroborated by its superior accuracy compared to standard multiple regression models in this context.

Limitations

A main limitation is the potential of model misspecification due to additional factors influencing RNA levels in wastewater and the true number of incident cases. Some of these factors are challenging to quantify (RNA degradation and testing strategy bias during the study period). Another main limitation is the uncertainty of the underlying population size. Capturing population dynamics may require other more accurate ways, for example through mobile data records or other big data sources (Deville et al., 2014). However, PMMoV showed to be of added value to tackle both the issue of standardization and population dynamics. Thirdly, vaccination coverage was not included in this analysis, which may have a profound effect on viral shedding (Puhach et al., 2022). As the effect of vaccination is time-dependent, we assume that it is implicitly accounted for through ARIMA-modelling of the residuals. However, its

effects cannot be quantitatively deduced from this study. Fourthly, variant strains were based on clinical samples and not on wastewater detection of variants. Finally, we used unevenly spaced time series which complicates the interpretation of lag times.

Future work should adjust for population dynamics, consider inter-WWTP variability, and may overcome some of the limitations of this research by using additional quantitative data sources such as vaccination coverage and mobility data, and by considering other epidemiological outcomes such as hospitalizations. Additionally, future studies should investigate spatiotemporal variation in the lead time, including the effect of seasonality, variant strains, and changes in shedding kinetics.

5 Conclusions

This study provides quantitative insights into the effect of key determinants to reduce unexplained variability of wastewater-based epidemiology (WBE). Adjusting for daily flow rate and PMMoV (population dynamics), but not variants, substantially improves COVID-19 modelling by WBE. Secondly, our findings show that WBE can lead individual clinical testing by one week, yet important heterogeneity between catchment areas was observed. This shows that the early-warning potential of WBE needs to be validated on a WWTP-specific level.

6 Acknowledgement

We would like to acknowledge Aquiris, Aquafin, Hydria and the Walloon approved sanitation organisations (AIDE, IDEA, IDELUX, IGRETEC, INASEP, inBW and IPALLE) for contributing to this project.

7 References

Agrawal S, Orschler L, Lackner S. Long-term monitoring of SARS-CoV-2 RNA in wastewater of the Frankfurt metropolitan area in Southern Germany. Sci Rep 2021; 11: 5372.

Amman F, Markt R, Endler L, Hupfauf S, Agerer B, Schedl A, et al. Viral variant-resolved wastewater surveillance of SARS-CoV-2 at national scale. Nat Biotechnol 2022; 40: 1814-1822.

Anand U, Adelodun B, Pivato A, Suresh S, Indari O, Jakhmola S, et al. A review of the presence of SARS-CoV-2 RNA in wastewater and airborne particulates and its use for virus spreading surveillance. Environ Res 2021; 196: 110929.

Anand U, Li X, Sunita K, Lokhandwala S, Gautam P, Suresh S, et al. SARS-CoV-2 and other pathogens in municipal wastewater, landfill leachate, and solid waste: A review about virus surveillance, infectivity, and inactivation. Environ Res 2022; 203: 111839.

Bertels X, Demeyer P, Van den Bogaert S, Boogaerts T, van Nuijs ALN, Delputte P, et al. Factors influencing SARS-CoV-2 RNA concentrations in wastewater up to the sampling stage: A systematic review. Sci Total Environ 2022: 153290.

Boogaerts T, Jacobs L, De Roeck N, Van den Bogaert S, Aertgeerts B, Lahousse L, et al. An alternative approach for bioanalytical assay optimization for wastewater-based epidemiology of SARS-CoV-2. Sci Total Environ 2021; 789: 148043.

Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. Lancet Microbe 2021; 2: e13-e22.

Coupeau D, Burton N, Lejeune N, Loret S, Petit A, Pejakovic S, et al. SARS-CoV-2 Detection for Diagnosis Purposes in the Setting of a Molecular Biology Research Lab. Methods Protoc 2020; 3.

Cuypers L, Dellicour S, Hong SL, Potter BI, Verhasselt B, Vereecke N, et al. Two Years of Genomic Surveillance in Belgium during the SARS-CoV-2 Pandemic to Attain Country-Wide Coverage and Monitor the Introduction and Spread of Emerging Variants. Viruses 2022; 14.

D'Aoust PM, Mercier E, Montpetit D, Jia JJ, Alexandrov I, Neault N, et al. Quantitative analysis of SARS-CoV-2 RNA from wastewater solids in communities with low COVID-19 incidence and prevalence. Water Res 2021; 188: 116560.

Deville P, Linard C, Martin S, Gilbert M, Stevens FR, Gaughan AE, et al. Dynamic population mapping using mobile phone data. Proc Natl Acad Sci U S A 2014; 111: 15888-93.

Girum T, Lentiro K, Geremew M, Migora B, Shewamare S. Global strategies and effectiveness for COVID-19 prevention through contact tracing, screening, quarantine, and isolation: a systematic review. Trop Med Health 2020; 48: 91.

Hyndman R, Athanasopoulos G. Forecasting: Principles and Practice: OTexts, 2021.

Hyndman R, Athanasopoulos G, Bergmeir C, Caceres G, Chhay L, O'Hara-Wild M, et al. forecast: Forecasting functions for time series and linear models, 2022.

Hyndman RJ, Khandakar Y. Automatic Time Series Forecasting: The forecast Package for R. Journal of Statistical Software 2008; 27.

Janssens R, Hanoteaux S, Maloux H, Klamer S, Laisnez V, Verhaegen B, et al. SARS-CoV-2 Surveillance in Belgian Wastewaters. Viruses 2022; 14: 1950.

Kwiatkowski D, Phillips PCB, Schmidt P, Shin Y. Testing the null hypothesis of stationarity against the alternative of a unit root. Journal of Econometrics 1992; 54: 159-178.

Li X, Zhang S, Sherchan S, Orive G, Lertxundi U, Haramoto E, et al. Correlation between SARS-CoV-2 RNA concentration in wastewater and COVID-19 cases in community: A systematic review and meta-analysis. J Hazard Mater 2023; 441: 129848.

Li X, Zhang S, Shi J, Luby SP, Jiang G. Uncertainties in estimating SARS-CoV-2 prevalence by wastewater-based epidemiology. Chem Eng J 2021; 415: 129039.

Ma J. Estimating epidemic exponential growth rate and basic reproduction number. Infect Dis Model 2020; 5: 129-141.

Mao K, Zhang K, Du W, Ali W, Feng X, Zhang H. The potential of wastewater-based epidemiology as surveillance and early warning of infectious disease outbreaks. Curr Opin Environ Sci Health 2020; 17: 1-7.

Parasa S, Desai M, Thoguluva Chandrasekar V, Patel HK, Kennedy KF, Roesch T, et al. Prevalence of Gastrointestinal Symptoms and Fecal Viral Shedding in Patients With Coronavirus Disease 2019: A Systematic Review and Meta-analysis. JAMA Netw Open 2020; 3: e2011335.
Park SK, Lee CW, Park DI, Woo HY, Cheong HS, Shin HC, et al. Detection of SARS-CoV-2 in Fecal Samples From Patients With Asymptomatic and Mild COVID-19 in Korea. Clin Gastroenterol Hepatol 2021; 19: 1387-1394 e2.
Puhach O, Meyer B, Eckerle I. SARS-CoV-2 viral load and shedding kinetics. Nat Rev Microbiol 2022: 1-15.
R Core Team. R: A Language and Environment for Statistical Computing, Vienna, Austria, 2022.
Rainey AL, Loeb JC, Robinson SE, Lednicky JA, McPherson J, Colson S, et al. Wastewater surveillance for SARS-CoV-2 in a small coastal community: Effects of tourism on viral presence and variant identification among low prevalence populations. Environ Res 2022; 208: 112496.
Rector A, Bloemen M, Thijssen M, Delang L, Raymenants J, Thibaut J, et al. Monitoring of SARS-CoV-2 concentration and circulation of variants of concern in wastewater of Leuven, Belgium. J Med Virol 2023; 95: e28587.
Rosario K, Symonds EM, Sinigalliano C, Stewart J, Breitbart M. Pepper Mild Mottle Virus as an Indicator of Fecal Pollution. Applied and Environmental Microbiology 2009; 75: 7261-7267.
Sciensano. Dashboard COVID-19 Epidemiological Situation Belgium, 2023.
Shah S, Gwee SXW, Ng JQX, Lau N, Koh J, Pang J. Wastewater surveillance to infer COVID-19 transmission: A systematic review. Sci Total Environ 2022; 804: 150060.
Vallejo JA, Trigo-Tasende N, Rumbo-Feal S, Conde-Perez K, Lopez-Oriona A, Barbeito I, et al. Modeling the number of people infected with SARS-COV-2 from wastewater viral load in Northwest Spain. Sci Total Environ 2022; 811: 152334.
Vandenberg O, Martiny D, Rochas O, Van Belkum A, Kozlakidis Z. Considerations for diagnostic COVID-19 tests. Nature Reviews Microbiology 2021; 19: 171-183.
Westhaus S, Weber FA, Schiwy S, Linnemann V, Brinkmann M, Widera M, et al. Detection of SARS-CoV-2 in raw and treated wastewater in Germany - Suitability for COVID-19 surveillance and potential transmission risks. Sci Total Environ 2021; 751: 141750.
Wickham H. ggplot2: Elegant Graphics for Data Analysis. New York: Springer-Verlag, 2016.
Zhang T, Breitbart M, Lee WH, Run JQ, Wei CL, Soh SW, et al. RNA viral community in human feces: prevalence of plant pathogenic viruses. PLoS Biol 2006; 4: e3.