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Building-Scale Wastewater-Based Epidemiology for SARS-CoV-2 Surveillance at Nursing Homes in A Coruña, Spain

Noelia Trigo-Tasende [1,†], Juan A. Vallejo [1,†], Soraya Rumbo-Feal [1], Kelly Conde-Pérez [1], Mohammed Nasser-Ali [1], Javier Tarrío-Saavedra [2], Inés Barbeito [2], Fernando Lamelo [3], Ricardo Cao [2], Susana Ladra [4], Germán Bou [1] and Margarita Poza [1,*]

[1] University of A Coruña (UDC), Microbiome and Health Group (meiGAbiome), Institute of Biomedical Research (INIBIC), University Hospital of A Coruña (HUAC), Interdisciplinary Center for Chemistry and Biology (CICA), Spanish Network for Infectious Diseases (CIBERINFEC-ISCIII), Campus da Zapateira, 15008 A Coruña, Spain; noeliatrigo@gmail.com (N.T.-T.); juanvallejo@gmail.com (J.A.V.); sorayarumbofeal@gmail.com (S.R.-F.); kellyxunq@gmail.com (K.C.-P.); mohammednasserma87@gmail.com (M.N.-A.); german.bou.arevalo@sergas.es (G.B.)
[2] Research Group MODES, Research Center for Information and Communication Technologies (CITIC), University of A Coruña (UDC), Campus de Elviña, 15071 A Coruña, Spain; javier.tarrio@udc.es (J.T.-S.); barbeitocal@gmail.com (I.B.); ricardo.cao@udc.es (R.C.)
[3] Nursing Homes Support and Coordination Unit, University Hospital of A Coruña, As Xubias, 15006 A Coruña, Spain; fernando.lamelo.alfonsin@sergas.es
[4] University of A Coruña (UDC), Research Center for Information and Communication Technologies (CITIC), Database Laboratory, Campus de Elviña, 15071 A Coruña, Spain; susana.ladra@udc.es
* Correspondence: margarita.poza.dominguez@sergas.es
† These authors contributed equally to this work.

Abstract: Wastewater-based epidemiology (WBE) has become an effective tool in the surveillance of infectious diseases such as COVID-19. In this work, we performed a brief study of monitoring the SARS-CoV-2 viral load in wastewater from six nursing homes located in the metropolitan area of A Coruña (Spain) between December 2020 and March 2021. The main objective was to detect SARS-CoV-2 outbreaks among residents and study the efficacy of the vaccination campaign. SARS-CoV-2 viral load (RNA copies per L of wastewater) was determined by reverse-transcription quantitative PCR (RT-qPCR) using the quantification cycle (Cq) values for the nucleocapsid (N) gene. Our results showed that the increase in viral load preceded the increase in clinical cases, favoring an early warning system that detects COVID-19 outbreaks in advance, making it possible to contain and stop the transmission of the virus among residents. In addition, the efficacy of the new COVID-19 vaccines was evidenced, since after the vaccination campaign in nursing homes in A Coruña, it was observed that many residents did not present any symptoms of the disease, although they excreted high amounts of virus in their feces. WBE is a cost-effective strategy that should be implemented in all cities to prevent new emerging diseases or future pandemic threats.

Keywords: COVID-19; epidemiology; nursing home; early warning; environmental surveillance; public health; wastewater; SARS-CoV-2; WBE

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1. Introduction

COVID-19, a potentially deadly respiratory disease caused by the single-stranded SARS-CoV-2 RNA virus, was declared as a pandemic in March 2020 [1] causing millions of deaths all over the world. The most common symptoms of COVID-19 are fever, cough, shortness of breath, and headache, among others, which can induce an atypical pneumonia [2]. The transmission of SARS-CoV-2 occurs mainly through droplets and aerosols generated by sneezing, coughing, exhaling, speaking, or even through the ocular and nasal mucosa [3]. Airborne transmission and environmental contamination have been demonstrated by the detection of SARS-CoV-2 in the air and on surfaces around infected

patients [4]. However, a significant number of COVID-19 cases develop gastrointestinal (GI) symptoms such as nausea, anorexia, vomiting, diarrhea, or abdominal pain. This is due to the SARS-CoV-2 replication in the GI tract, confirmed by the detection of viral RNA in stool samples from infected people [5,6]. The expression of the ACE2 receptor in enterocytes mediates the virus entry into the intestine through the interaction with the spike glycoprotein [7]. The replication of SARS-CoV-2 in the GI tract has been evidenced [8], although it was suggested that it is very low or even null in stool samples [9]. Its persistence in the GI tract is longer than in the respiratory tract [10], being detected in fecal samples of infected patients even 7 months after the onset of the disease, as reported by Natarajan et al. [11], demonstrating that infection in the GI tract can continue even after finishing in the respiratory tract. For this reason, patients infected by SARS-CoV-2 still excrete viral RNA to feces despite being negative for SARS-CoV-2 by nasopharyngeal swab testing [12]. Consequently, viral RNA enters wastewater treatment plants (WWTP).

Given that at least one third of COVID-19 infections do not exhibit any clinical symptoms and consequently go unnoticed in clinical testing [13], although they excrete high amounts of virus in their feces, wastewater-based epidemiology (WBE) has been widely considered in the last years as a useful tool to monitor the real magnitude of the COVID-19 pandemic evolution through the detection of SARS-CoV-2 RNA in wastewater. The total infected population excretes its viral load in feces, including symptomatic and asymptomatic persons, detected in wastewater. Although WBE made a greater impact during the COVID-19 global pandemic, it had already been implemented for tracking poliovirus, hepatitis A, and norovirus outbreaks [14,15]. Since 2020, many studies have focused on SARS-CoV-2 surveillance in wastewater to track SARS-CoV-2 infections in the community. In addition, a clear relation between COVID-19 clinical cases and the SARS-CoV-2 viral load (RNA copies per L) measured in wastewater has been reported [16–18]. This fact made it possible to predict the increase in clinical cases and anticipate future outbreaks using WBE, which served as an early warning system and as an effective surveillance tool for SARS-CoV-2 [19–22], as well as for other potentially dangerous pathogens [23–25].

Most studies have used WBE to monitor the real evolution of the COVID-19 pandemic at the community level, but it can also be used at the building level, that is, in closed facilities such as nursing homes, schools, prisons, or universities [26–31]. In this case, future outbreaks can be identified before the first case is reported, offering a lead time useful for decision making, which implies preventive measures such as clinical testing, isolation, or treatment of the positive case, avoiding the spread of the virus in the building. Since late 2020, new SARS-CoV-2 variants have emerged replacing the original variant (B.1.177), each of them with higher transmissibility than the previous one. For this reason, the European Center for Disease Prevention and Control (ECDC) classified them as variants of concern (VOC), variants of interest (VOI), or variants under monitoring (VUM), depending on the impact on their transmissibility, on their ability to escape the host's immune system, or on the severity of the disease [32]. Consequently, wastewater surveillance studies started to sequence the SARS-CoV-2 genome to identify which variants are circulating in the community [33–37]. In this work, we applied the WBE strategy at the building level to monitor the SARS-CoV-2 viral load in six nursing homes located in the metropolitan area of A Coruña (NW Spain) for the period December 2020–March 2021, before the emergence of the Alpha variant (B.1.1.7) and during the beginning of the COVID-19 vaccination programs in Spain. This work was part of a larger project called COVIDBENS, which monitored the SARS-CoV-2 viral load in wastewater at the community level in A Coruña [19].

2. Materials and Methods

2.1. Sample Collection

A total of 137 wastewater samples were collected twice a week from 6 nursing homes located in each of the five municipalities of the metropolitan area of A Coruña (Spain), Oleiros, Arteixo, Culleredo, Cambre, and A Coruña (Table 1) from 22 December 2020 to 26 March 2021. These nursing homes were Santa Teresa de Jornet, Orpea, Oleiros, Arteixo,

Brives, and Ballesol. Automatic samplers installed in the sewer of each sampling site were programed to generate a 600 mL bottle of wastewater per hour and to mix and integrate the resulting 24 bottles into a larger one, from which only 100 mL of a sample was taken. Wastewater sampling was performed respecting the rules of sterility. Finally, the 24 h composite samples were transported at 4 °C to the laboratory and processed on the same day. Dataset 1 contains the sample collection schedule for each location.

Table 1. Characteristics and surveillance periods of the nursing homes analyzed from December 2020 to March 2021 in A Coruña (Spain).

Name of the Nursing Home	No. of Residents	No. of Staff	Municipality	Surveillance Period
Santa Teresa de Jornet	157	112	A Coruña	22 December 2020–26 March 2021
Orpea	114	99	Culleredo	22 December 2020–26 March 2021
Oleiros	246	346	Oleiros	22 December 2020–26 March 2021
Arteixo	63	40	Arteixo	22 December 2020–26 March 2021 [1]
Bribes	39	27	Cambre	22 December 2020–26 March 2021 [1]
Ballesol	96	71	Oleiros	22 December 2020–26 March 2021

[1] Sample collection interrupted from 24 February 2021 to 12 March 2021.

2.2. Sample Processing

The 100 mL wastewater samples were kept at 4 °C throughout the process and concentrated by ultrafiltration in a final volume of 500 µL following the protocol previously described by Trigo-Tasende et al. [19]. Finally, 500 µL of RNAlater reagent (Thermo Fisher Scientific, Vilnius, Lithuania) was added to preserve the samples at −80 °C for further analysis.

2.3. RNA Extraction and RT-qPCR Analyses

Viral RNA was extracted from 100 µL of the concentrated samples and eluted in 70 µL of RNase-free water (Thermo Fisher Scientific, Waltham, MA, USA) using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The purity and quantity of the viral RNA was assessed by spectrophotometry (BioDrop uLite, Cambridge, UK), and the extracted RNA was stored at −80 °C until use. The detection of SARS-CoV-2 RNA was performed by reverse-transcription quantitative PCR (RT-qPCR) assay using the TaqPath COVID-19 RT-PCR Kit (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's instructions. RT-qPCR assays were conducted in sextuplicate using a CFX96 Thermal cycler (Bio-Rad, Hercules, CA, USA). Negative and positive PCR controls were used for each run using RNase-free water (Thermo Fisher Scientific, Waltham, MA, USA) and the reagents supplied in the kit, respectively.

2.4. Viral Load Determination

The viral load was determined by the construction of a calibration curve from serial dilutions of the stock solution (10,000 SARS-CoV-2 RNA copies/µL, European Virus Archive Global (EVAg, Germany), ranked from 5 to 500 copies/µL. The resulting calibration curve between the log10 SARS-CoV-2 copy number and the (quantification cycle) Cq values for the nucleocapsid (N) gene obtained for every RT-qPCR run generated a linear equation ($y = mx + b$), where y represents the SARS-CoV-2 RNA copies per L, m is the slope, x is the Cq value, and b is the y-intercept. In addition, the limit of detection (LoD) and the limit of quantification (LoQ), described as the lowest concentration at which 95% of the positive samples were detected, and the lowest concentration at which the relative standard devia-

tion was less than 25%, respectively, were calculated by randomly selecting 15 replicates of the calibration curves. The amplification efficiency (E) was also calculated following the recommendations by the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines [38].

2.5. COVID-19 Clinical Cases at Nursing Homes

The number of COVID-19 clinical cases reported at nursing homes analyzed in this study was collected between December 2020 and March 2021 (Table 2). In addition, the vaccination campaign in nursing homes in A Coruña started with the first dose in early January 2021 and the second dose at the end of February (Table 2). Dataset 2 contains the evolution of the COVID-19 cases over time at each sampling point during the surveillance period analyzed.

Table 2. Number of COVID-19 clinical cases and the vaccination progress at six nursing homes located in the metropolitan area of A Coruña (Spain) from December 2020 to March 2021.

Nursing Home Name	COVID-19 Cases (Reported by the Health System)	Number of Hospitalized Patients	Infection Rate (%) [1]	Vaccination First Dose	Vaccination Second Dose
Santa Teresa de Jornet	39	2	25	14 January 2021	4 December 2021
Orpea	1	0	1	7 January 2021	28 January 2021
Oleiros	0	0	0	30 December 2020	20 January 2021
Arteixo	0	0	0	8 January 2021	29 January 2021
Bribes	0	0	0	9 January 2021	30 January 2021
Ballesol	3	0	3	7 January 2021	28 January 2021

[1] percentage of infected patients with respect to the number of residents.

2.6. Statistical Analysis

A correlation analysis was carried out considering the Santa Teresa de Jornet nursing home, first, to determine approximately how many days were needed to obtain an increase in the clinical COVID-19 cases curve after an increase in the viral load curve and, second, to approximately determine how much time was required for the first dose of the vaccine to achieve its effect. In this latter study, two different subsamples were considered. One was set up until 14 January 2021, when the first dose of the vaccine had been administered, and the second one was set up from that date on. Correlation was obtained for the first subsample considering the viral load curve at day "t" and active cases curve at day "t+k", with "k" meaning days of delay. After that, by choosing the optimal "k", namely "k0", as the one in which the correlation attained its maximum, the correlation of the second subsample considering "k0" was calculated. Then, the subtraction, namely DCC(d), between these two correlations calculated in the previous stages was obtained. Additionally, smoothing methods were used to obtain some smoothed version of the viral load and active cases curves in each nursing home. Specifically, the Loess method was considered with a smoothing parameter varying within the set {0.25, 0.3, 0.35, 0,4, 0,45}, depending on the sample considered.

3. Results and Discussion

3.1. Standard Curve Parameters

The LoD and LoQ were 100 and 50 SARS-CoV-2 RNA copies per PCR reaction, respectively. The supplementary file includes the standard curve for the N gene obtained from the RT-qPCR assays, which was $y = -3.5251x + 43.132$, where y represents the SARS-CoV-2 RNA copies per L of wastewater, and x represents the Cq value for the N gene (Figure S1). The y-intercept was 43.132, the slope was -3.5251, the R^2 value was 0.9964, and the amplification efficiency was 92.17%. The SARS-CoV-2 detection rates, the percentage of positive wastewater samples, and the Cq values are included in Table S1.

3.2. Statistical Analysis

Correlation analysis for the Santa Teresa de Jornet nursing home considering clinical cases and viral load data revealed that 6 days were needed to observe an increase in the clinical cases after the increase in the viral load. In addition, we also obtained that the maximum effect of the vaccine occurred 9 days after receiving the first dose, specifically on 23 January (Table 3).

Table 3. Correlation analysis for the Santa Teresa de Jornet nursing home.

d [1]	k0 [2]	Maximum Correlation before Vaccination	Maximum Correlation after Vaccination	DCC (d) [3]
14 January 2021	6	0.7399414	−0.1424553	0.8823967
15 January 2021	7	0.7399414	−0.1641223	0.9040637
16 January 2021	8	0.7399414	−0.3012689	1.04121
17 January 2021	8	0.7399414	−0.3012689	1.04121
18 January 2021	8	0.7399414	−0.1641223	0.9040637
19 January 2021	10	0.7399414	−0.3098003	1.04121
20 January 2021	9	0.7399414	−0.2092073	0.9491487
21 January 2021	8	0.7399414	−0.2277364	0.9676778
22 January 2021	9	0.7399414	−0.2690311	1.008972
23 January 2021	6	0.7399414	−0.3103899	1.050331
24 January 2021	6	0.7399414	−0.3103899	1.050331
25 January 2021	7	0.7399414	−0.3103899	1.050331
26 January 2021	7	0.7399414	−0.3103899	1.050331
27 January 2021	6	0.7399414	−0.3103899	1.050331
28 January 2021	6	0.7399414	−0.3103899	1.050331

[1] Date corresponding to the cutoff point between the first and second subsample. [2] Days of delay whose correlation reaches its maximum. [3] Subtraction between the two correlations.

3.3. SARS-CoV-2 Infections at Nursing Homes in A Coruña

Only three of the nursing homes analyzed in this study reported COVID-19 clinical cases (Table 2). Specifically, the Santa Teresa de Jornet nursing home reported the highest infection rate (percentage of infected patients with respect to number of residents), which was around 25% (Table 2). From the beginning of the COVID-19 pandemic, monitoring of SARS-CoV-2 in nursing homes has been a fundamental prevention measure to control possible outbreaks among residents, since older people have a higher risk and vulnerability to infectious diseases. Patients older than 70 years are more likely to have more severe symptoms of the disease, be admitted to the intensive care unit (ICU), or even have a higher risk of mortality [39]. The RT-PCR of nasopharyngeal swabs was the main screening procedure for COVID-19 detection, but due to the high number of SARS-CoV-2 infections and the pressure on the health care system at the beginning of the pandemic, nursing homes started to use the antigen tests to control the transmission of the virus among residents. However, its lower sensitivity increased the risk of false negatives [40], which incremented the spread of the virus in the population. Consequently, the real number of patients infected with SARS-CoV-2 was much higher than those reported by the health system. Additionally, it should be noted that clinical testing for COVID-19 in nursing homes was not routinely performed but was only carried out in the event that the patient manifested any symptoms or an outbreak was suspected in the building. It should be noted that almost a third of the population infected with SARS-CoV-2 comprises asymptomatic people [14], whose cases are usually not detected by the health system. On the other hand, the workers were not required to undergo clinical tests, which means that the viral load in the wastewater of the nursing homes might not fully correspond to the clinical cases of the residents but could also come from the staff or visitors.

3.4. SARS-CoV-2 in Wastewater Samples

The RNA copies of the virus per L in wastewater from the six nursing homes analyzed in this study were determined by RT-qPCR procedures (Dataset 3). SARS-CoV-2 RNA was detected in 61% of the total wastewater samples, and generally, the detection rates exceeded 50%, except in the case of Brives (42%) (Figure 1).

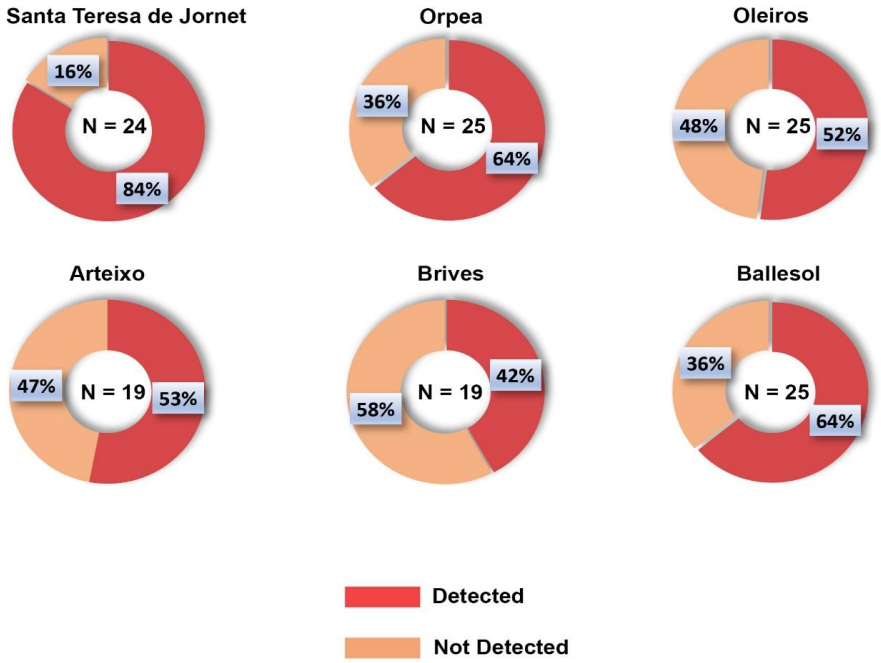

Figure 1. SARS-CoV-2 detection rates in the nursing homes' wastewater located in the metropolitan area of A Coruña (Spain) between December 2020 and March 2021.

Previous studies had already demonstrated the relationship between the viral load in wastewater and the clinical cases [41,42]. Simultaneously, our team performed a two-year WBE study for the detection of SARS-CoV-2 in wastewater samples at the Bens WWTP in A Coruña (Spain) [19], where statistical modeling was performed to determine the real number of people infected by SARS-CoV-2 from the viral load data obtained in wastewater analysis [19]. These previous works have served as an early warning system throughout the COVID-19 pandemic in A Coruña, evidencing the potential of wastewater surveillance to alert about future outbreaks. In this study, we again found a good correlation between the clinical cases of COVID-19 reported by the health system and the viral load measured in the wastewater samples of nursing homes. In all cases, the viral load in wastewater increased 6–15 days before the clinical cases were reported by the health system (Figures 2–4). This alert system was very useful to control the transmission of SARS-CoV-2 in the nursing homes of the metropolitan area of A Coruña when the vaccination period had not yet begun.

In the Santa Teresa de Jornet nursing home, 39 COVID-19 active cases were diagnosed between December 2020 and February 2021 by RT-PCR or antigen testing (Dataset 2). In this case, the SARS-CoV-2 viral load in wastewater preceded the increase in clinical cases 6 days in advance (Figure 2).

The maximum number of active clinical cases was reported on 13 January. This notable increase in both the SARS-CoV-2 viral load and the number of clinical cases reported corresponded to the emergence of the Alpha variant (B.1.1.7), detected for the first time in the wastewater of the metropolitan area on 16 December 2020 [19]. This emerging VOC totally replaced the previous variant installed in the area in January 2021 due to its greater transmissibility capacity [43,44] and its greater persistence capacity in the human respiratory tract, which leads to a higher viral load in the host. [45,46]. For this reason,

the Alpha variant caused a relevant pandemic wave in January 2021, leading to a notable and abrupt increase in SARS-CoV-2 infections. The 39 clinical cases reported in this nursing home coincided with this large viral load peak. After that, both the viral load in wastewater and the active clinical cases decreased progressively until mid-February, when the vaccination campaign started. On 4 February 2021, all residents had been vaccinated with the second dose, and consequently, the active clinical cases radically stopped.

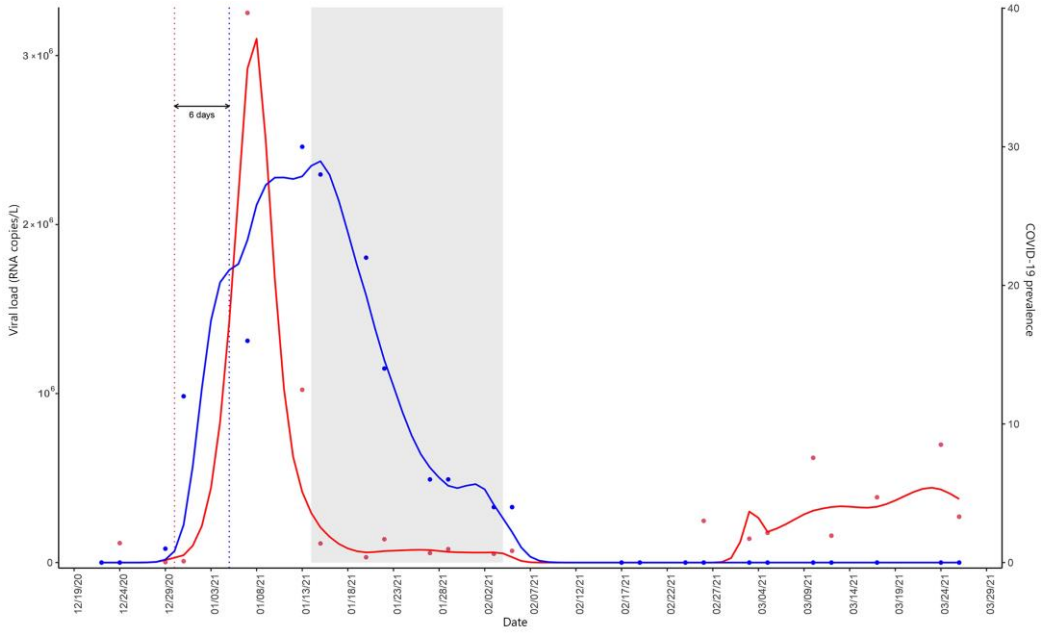

Figure 2. SARS-CoV-2 viral load measured in wastewater (red line) and COVID-19 cases reported over time (blue line) at the Santa Teresa de Jornet nursing home. Exact data corresponding to viral load (red dots) and clinical cases (blue dots). The period in which residents received the two doses of the vaccination is represented in shadow. Arrows indicate the time lag (anticipation) between the increase in SARS-CoV-2 RNA viral load in wastewater and the emergence of the clinical cases.

In the Orpea nursing home, a small increase in viral load on 5 January anticipated two weeks in advance the only COVID-19 clinical case reported at this sampling point, which was detected on 20 January (Figure 3). The maximum viral load observed in the wastewater showed an amount of 1,126,078 SARS-CoV-2 RNA copies/L.

Similarly, in the Ballesol nursing home, a small viral load peak was observed on 5 January, 15 days before the number of clinical cases increased (Figure 4). At this sampling point, the maximum viral load in wastewater reached 879,160 SARS-CoV-2 RNA copies/L.

We served as an early warning system for the nursing homes which reported clinical cases, predicting SARS-CoV-2 outbreaks 6–15 days in advance (Figures 2–4). After administration of the first dose of the vaccine against COVID-19, both the viral load and clinical cases decreased progressively in all nursing homes. However, the SARS-CoV-2 viral load increased considerably after the administration of the second dose. Although the clinical cases stopped, we detected an important viral load increase in wastewater in all nursing homes analyzed in this study from late February onward (Figures 2–5). In case of the Arteixo and Brives nursing homes, the viral load curve in wastewater started to increase on 10 March, since the sample collection was interrupted during the two previous weeks (Figure 5).

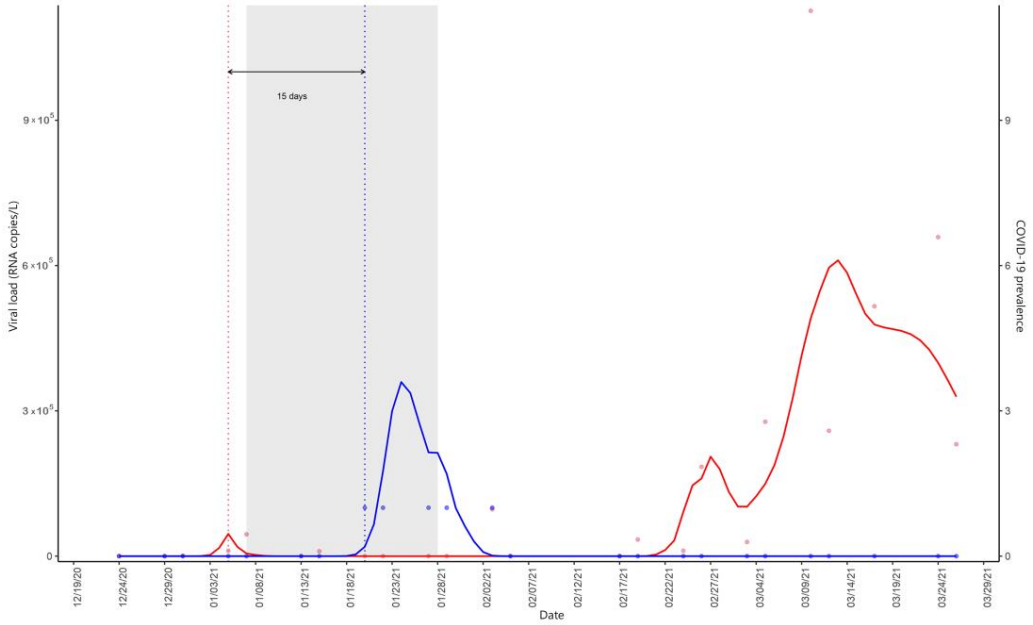

Figure 3. SARS-CoV-2 viral load measured in wastewater (red line) and COVID-19 cases reported over time (blue line) at the Orpea nursing home. Exact data corresponding to viral load (red dots) and clinical cases (blue dots). The period in which residents received the two doses of the vaccination is indicated in shadow. Arrows indicate the time lag (anticipation) between the increase in the viral load in wastewater and the emergence of clinical cases.

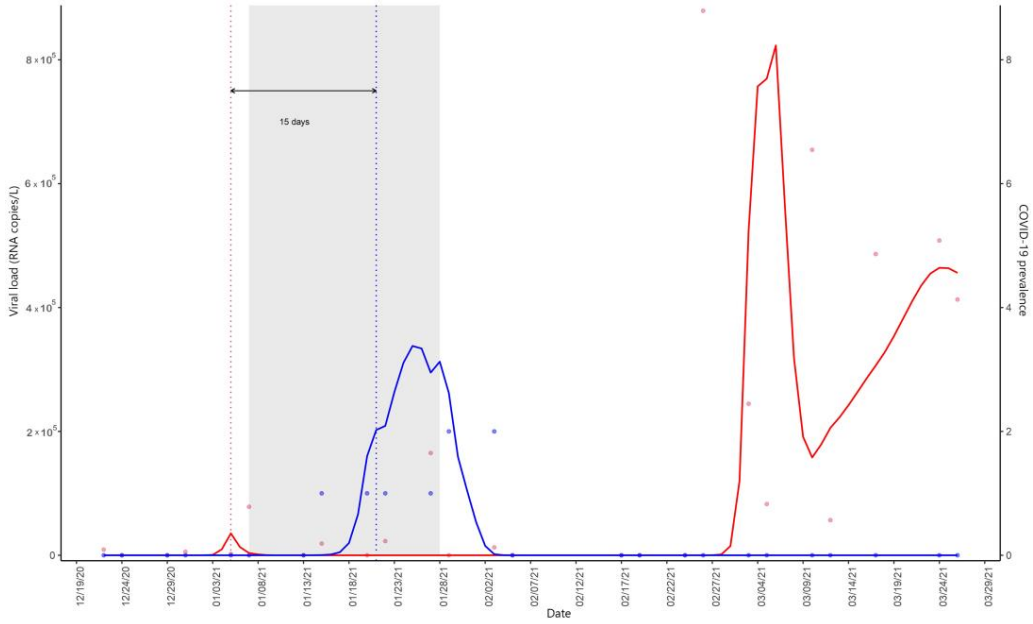

Figure 4. SARS-CoV-2 viral load measured in wastewater (red line) and COVID-19 cases reported over time (blue line) at the Ballesol nursing home. Exact data corresponding to viral load (red dots) and clinical cases (blue dots). The period in which residents received the two doses of the vaccination is indicated in shadow. Arrows indicate the time lag (anticipation) between the increase in the viral load in wastewater and the emergence of clinical cases.

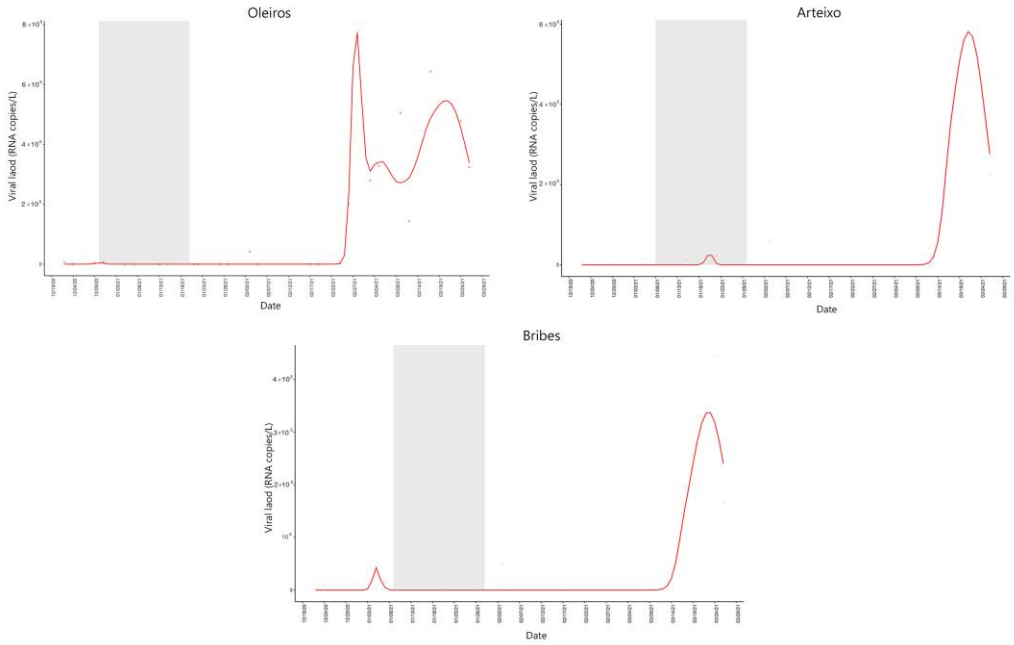

Figure 5. SARS-CoV-2 viral load trends in wastewater at the Oleiros, Arteixo, and Brives nursing homes over time. Exact data corresponding to viral load (red dots). The period in which residents received the two doses of the vaccination is indicated in shadow.

One of the reasons that could explain this considerable increase in SARS-CoV-2 viral load after the vaccination campaign was the relaxation of the measures imposed by the government from February 2021. In the case of nursing homes in A Coruña, ten days after the administration of the second dose of the COVID-19 vaccine, for the first time in a long time, visits were allowed three times a week, and outings outside of the residents resumed as normal. The opening of the nursing homes to the visitors produced an increase in interpersonal contacts and, consequently, a rise in the risk of viral transmission, which led to a high increase in viral load, although the residents remained healthy. Another possible reason is the relationship of the viral load with age, which is higher in elderly patients [47].

According to the correlation analysis performed for the Santa Teresa de Jornet nursing home, the maximum effect of the first dose of the COVID-19 vaccine was 9 days after its administration (14 January 2021) (Table 3). This correlated with the data observed in the graphs, given that despite the considerable increase in the viral load in wastewater, the curve of clinical cases decreased from this date to the end of the study (Figures 2–5). Thus, we demonstrated the effectiveness of vaccination against potentially dangerous pathogens such as SARS-CoV-2. No correlation analysis was carried out for the other nursing homes due to the low or null number of reported clinical cases, but we consider that this same situation can be extrapolated in all the cases. Previously, it was demonstrated that vaccination reduced the disease severity, the mortality, and the asymptomatic infection, especially after administration of the second dose [48–50]. Furthermore, booster vaccines further reduced the risk of mortality in older people [51]. Although we detected a high level of viral load in the wastewater samples, PCRs or antigen tests from nasopharyngeal samples performed by the health system were negative for SARS-CoV-2. This may be due to the short persistence of the virus in the respiratory tract of vaccinated people compared to unvaccinated people [52]. Petter et al. [53] demonstrated a reduction in the viral load in vaccinated subjects. We also demonstrated the effectiveness of the vaccination program in A Coruña in our previous work during the Alpha epidemic wave [19].

Although there is still a risk of possible COVID-19 outbreaks among fully vaccinated people [54–56], vaccines are effective in reducing the risk of transmission and the symp-

tomatic infection, but complementary measures are necessary to control the virus in the community. In addition to health strategies such as the use of a mask, social distancing, lockdowns, etc., countries must have a strategy that also integrates socioeconomic, environmental, and institutional measures. That is, measures such as contact tracing, investment in new technologies, and greater support for R&D for the development of new vaccines, among others, are also necessary to be able to face future pandemic threats [57]. The methodology used in this study can be implemented at any sampling point at the building level as well as at the community level to monitor new emerging variants of SARS-CoV-2 or other potentially dangerous pathogens. Although since mid-2023 the COVID-19 has reduced its impact on society around the world, nations should take into account the lessons from the pandemic and establish strategies typical of a post-pandemic situation. At the community level, WWTPs allow for epidemiological studies based on wastewater, but on the contrary, the presence of sampling stations is not as common at the building level. Therefore, urban planners must consider the significance of wastewater as a public health service and redesign the sewerage network in future buildings to enable the implementation of WBE systems [58].

This work had several limitations. Sequencing of the SARS-CoV-2 genome complements WBE studies improving the reliability of the results. However, on this occasion, we did not perform sequencing analysis to corroborate the predominant variants in the nursing homes. In addition, the lack of data of COVID-19 cases on workers and visitors makes it difficult to distinguish between the viral load coming from them and from the patients. However, the SARS-CoV-2 viral load released from workers or visitors is not comparable to that from patients who spend much more time in the building. Moreover, data on when and who uses the building's toilets would be difficult to obtain. So, we could not calculate the contribution of outsiders' human waste to the sewer system, which may limit the interpretation of the results. Periodic SARS-CoV-2 screening of both residents and workers or visitors, even if they have no symptoms, would improve the analysis and could be very useful to control the spread of the virus in nursing homes.

4. Conclusions

This study demonstrates the efficacy of the WBE method in monitoring infectious diseases at the building level. The correlation between viral load in wastewater and active clinical cases made it possible to predict the emergence of new SARS-CoV-2 outbreaks at nursing homes, serving as a cost-effective system to anticipate future COVID-19 outbreaks. This strategy provided valuable information to face the pandemic situation when new SARS-CoV-2 variants emerged in the community. In addition, the comparison of the clinical cases reported by the health system with the detection of the viral load in wastewater allowed evaluating in real time the great effectiveness of the vaccination campaign in A Coruña.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environments10110189/s1. Figure S1: Straight pattern performed with the Human 2019-nCoV RNA standard (EVAg) for viral load quantification through RT-qPCR assays. Linear regression analysis showed a calibration curve of $y = -3.5251x + 43.132$, $R^2 = 0.9964$; Table S1: Lower limit of detection (LoD) and limit of quantification (LoQ) determination for SARS-CoV-2 RT-qPCR.

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