Predictive potential of SARS-CoV-2 RNA concentration in wastewater to assess the dynamics of COVID-19 clinical outcomes and infections

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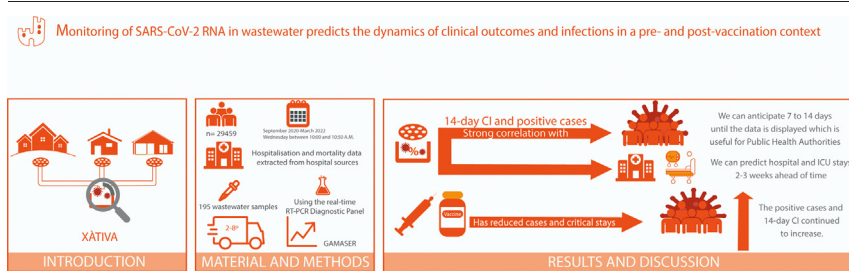[e] Faculty of Health Sciences, La Rioja University, 26006 Logroño, Spain
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HIGHLIGHTS

- Wastewater SARS-CoV-2 concentration allows to anticipate the behavior of clinical variables
- Wastewater viral concentration correlates to weekly hospitalization and critical care data and provides early warning on one week PCR+ subjects
- Wastewater viral concentrations data allows to anticipate 14-day CI 1-2 weeks in advance
- Correlations between wastewater SARS-CoV-2 [C] and 14-day CI or PCR+ are not affected by vaccination
- This methodology may aid decision-making by anticipating new outbreaks seven to 15 days in advance

GRAPHICAL ABSTRACT

Monitoring of SARS-CoV-2 RNA in wastewater predicts the dynamics of clinical outcomes and infections in a pre- and post-vaccination context

ARTICLE INFO

ABSTRACT

Coronavirus disease 2019 - caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) -, has triggered a worldwide pandemic resulting in 665 million infections and over 6.5 million deaths as of December 15, 2022. The development of different epidemiological tools have helped predict new outbreaks and assess the behavior of clinical variables in different health contexts. In this study, we aimed to monitor concentrations of SARS-CoV-2 in wastewater as a tool to predict the progression of clinical variables during Waves 3, 5, and 6 of the pandemic in the Spanish city of Xátiva from September 2020 to March 2022.

We estimated SARS-CoV-2 RNA concentrations in 195 wastewater samples using the RT-PCR Diagnostic Panel validated by the Center for Disease Control and Prevention. We also compared the trends of several clinical variables (14-day cumulative incidence, positive cases, hospital cases and stays, critical cases and stays, primary care visits, and deaths) for each study wave against wastewater SARS-CoV-2 RNA concentrations using Pearson's product-moment correlations, a two-sided Mann-Whitney U test, and a cross-correlation analysis.

We found strong correlations between SARS-CoV-2 concentrations with 14-day cumulative incidence and positive cases over time. Wastewater RNA concentrations showed strong correlations with these variables one and two weeks in advance. There were significant correlations with hospitalizations and critical care during Wave 3 and Wave 6; cross-correlations were stronger for hospitalization stays one week before during Wave 6. No association between vaccination percentages and wastewater viral concentrations was observed.

Our findings support wastewater SARS-CoV-2 concentrations as a potential surveillance tool to anticipate infection and epidemiological data such as 14-day cumulative incidence, hospitalizations, and critical care stays. Public health authorities could use this epidemiological tool on a similar population as an aid for health care decision-making during an epidemic outbreak.

1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic became a public health emergency of international concern affecting a large number of people and killing millions all over the world (Cucinotta and Vanelli, 2020). COVID-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), usually detected by testing symptomatic individuals and those suspected of being positive after a close contact with a person with confirmed COVID-19 (Landi et al., 2020).

Health professionals have tried to contain the spread of the virus and reduce the most severe symptoms of the disease by developing vaccines and drugs (Awadasseid et al., 2020; Gilbert et al., 2022; Goldblatt et al., 2022; Samy et al., 2022). Daily counts of positive cases allows assessing the progression and spread of COVID-19. However, the pathology of a particular viral load and its relation with the virulence and severity of SARS-CoV-2 are still under study (Cao et al., 2022; Walsh et al., 2020; WHO, 2023). Because of the emergence of novel variants, continuous changes of the antigen screening tests have to be made to detect the presence of the associated SARS-CoV-2 mutations e.g., Omicron and its subvariants BA.2, BA.3, BA.4, and BA.5, which delays the detection of a new COVID-19 wave (Hajissa et al., 2022). Health and political authorities are having difficulties making decisions on the restrictive measures to be implemented in their communities in the face of new outbreaks associated to SARS-CoV-2 variants (WHO, 2023).

Information on SARS-CoV-2 detection and viral burden at different stages of an outbreak in symptomatic and asymptomatic individuals is crucial for a reliable interpretation of the phase of the viral spread and its virulence in a given population beyond COVID-19 detection tests and would be of great help for healthcare and governmental decision-making (Walsh et al., 2020).

Monitoring wastewater collection systems has previously been used for early surveillance of disease prevalence, e.g., hepatitis E, hepatitis A, enterovirus, or poliovirus (Beyer et al., 2020; Bisseux et al., 2018; Gholipour et al., 2022; Hellmér et al., 2014; Hovi et al., 2001). SARS-CoV-2 RNA is present in urine and stool samples of symptomatic or asymptomatic COVID-19 patients (Davó et al., 2021; Haramoto et al., 2020), and its detection can be done from raw wastewater (Peccia et al., 2020; Vallejo et al., 2022). Increased RNA concentration in raw wastewater has been linked with a larger number of confirmed COVID-19 cases (Randazzo et al., 2019; Vallejo et al., 2022), but it also provides information on unconfirmed asymptomatic infected individuals.

Thus, SARS-CoV-2 monitoring via *water fingerprinting* may be an emerging public health tool to track the spread of the virus and assess the impact sanitary and movement restriction measures may have on its infectious capacity. Wastewater analyses studies in Japan and Italy have reported results in this direction (Haramoto et al., 2020; La Rosa et al., 2020; Mousazadeh et al., 2021). Wastewater surveillance may provide an objective and comprehensive assessment of near real-time public and environmental health status (Chavarria-Miró et al., 2021; Mousazadeh et al., 2021), and be a lagging indicator of the pandemic's progression (Haramoto et al., 2020; Randazzo et al., 2019). To the best of our knowledge, to date no wastewater analyses to evaluate incidence and other health data such as the number of cases in a population, hospital stays, intensive care unit (ICU) stays, and positive polymerase chain reactions (PCRs) have been performed in

conjunction with wastewater virus concentrations. The main purpose of this study was to correlate SARS-CoV-2 viral RNA concentrations in wastewater with compiled testing and hospitalization data in a Spanish metropolitan area from September 2020 to April 2022, which corresponds to the previous five waves of SARS-CoV-2 infection. We also aimed to analyze the effect of vaccination and mobility restrictions on all these clinical and biochemical parameters.

2. Materials and methods

2.1. Wastewater sampling

We collected wastewater samples in the town of Xàtiva (Valencia, Spain); monitored three hydraulic sectors covering the entire city (population 29.459 by January 1, 2021) once a week (on Wednesdays) and within the same time range (between 10 am and 10:50 am). To establish the limits between each hydraulic sector, the locations of hospitals, primary health attention, and health and social care centers were taken into consideration, as well as the security to access the sampling point. No industrial disposals were collected in any of the sectors. For this study, 195 wastewater samples were collected from September 7, 2020 to February 28, 2022. For the collection, neutral glass bottles with screw caps of at least 250 mL capacity and resistant to sterilization, or aseptic plastic bottles of at least 500 mL capacity were used. Samples were collected from sewers, immediately refrigerated (2 °C - 8 °C) and transported to GAMASER laboratory for analysis.

Sampling was interrupted due to bureaucratic issues for some specific weeks between May 10, 2021 and July 11, 2021 (Table 1).

2.2. Viral concentration, extraction, and quantification

To determine viral concentration we followed the protocol described by Randazzo et al. (2020), which has been adopted since the beginning of the pandemic for quantitative determination of SARS-CoV-2 in wastewater by the Spanish Ministry for the Ecological Transition and the Demographic Challenge (Ministerio de Transición Ecológica y Reto Demográfico, 2021). This protocol ensures that the methodology used is adequate for determining SARS-CoV-2 concentrations in wastewater using the control virus described and the ISO 15216-1:2017 on which it is based.

Each assay was associated with internal quality controls, including: process blank control (evaluates the absence of contamination from the concentration stage), extraction negative control (assesses the absence of contamination during the extraction stage), and PCR negative control (assesses the absence of contamination in the PCR stage). A recovery control (mengovirus) for each sample was used, to ensure the compliance of a 1 % minimum recovery criterion.

Table 1
COVID-19 ascent and descent dates; 14-day cumulative incidence.

	Ascent	Descent
Wave 3	2020-11-16 to 2021-01-18	2021-01-18 to 2021-05-03
Wave 5	2021-06-21 to 2021-07-26	2021-07-26 to 2021-09-27
Wave 6	2021-11-15 to 2022-01-03	2022-01-03 to 2022-02-28

The aluminum hydroxide adsorption-precipitation method was performed as follows: 200 mL of the water sample were adjusted to pH 6.0 and an Al(OH)3 precipitate was formed by adding 1:100 v:v of a 0.9 N ALCl3 solution. After readjusting the pH, the sample was slowly agitated for 15 min at room temperature. The precipitate was collected by centrifugation at 1700 $\times g$ for 20 min. The pellet was resuspended using 10 mL of 3 % beef extract (pH 7.4) and the sample shaken for 10 min at 150 rpm (Randazzo et al., 2019). A concentrate was next formed by centrifugation at 1900 $\times g$ for 30 min and the pellet resuspended in 1 mL of phosphate buffered saline solution ("9510 Detection of Enteric Viruses", 2018; Lodder et al., 2010). For the process control, samples were spiked with 105 PCR units of mengovirus vMCO (CECT 1000009) following a protocol similar to ISO 15216-2:2017 used in food products. This laboratory procedure was first validated for non-enveloped viruses (Randazzo et al., 2019, 2020) and recently for an enveloped virus member of the *Coronaviridae* family, which causes porcine epidemic diarrhea virus and is used as a SARS-CoV-2 surrogate.

Viral RNA was extracted from the above-obtained concentrates with the Nucleocapsin RNA virus kit (Macherey-Nagel, Düren, Germany) following manufacturer's instructions. Briefly, 25 μL of Plant RNA Isolation Aid (Thermo Fisher Scientific, Vilnius, Lithuania) and 600 μL of lysis buffer from the nucleocapside virus were added to 150 μL of the concentrated sample and pulse-vortex mixed for 1 min. The resultant homogenate was centrifuged for five minutes at 10,000 g to remove debris and the supernatant was processed according to the manufacturer's instructions and eluted in 100 μL of RNAse-free distilled water.

Viral RNA was detected by RT-qPCR using the One-Step PrimeScript™ RT-PCR kit (Perfect Real Time) (Takara Bio, Mountain View, CA, USA), targeting the nucleoprotein (N), N1, and N2 fragments (Center for Disease Control and Prevention, 2021) and the envelope protein (E) gene (Corman et al., 2020). Primers, probes, and sequences for the genes and mengovirus are shown in Supplementary Table 1; concentrations, mastermix volumes, and final volume of analysis are listed in Supplementary Table 2. As abovementioned, all RT-qPCR runs included negative (nuclease-free water) and positive controls. Supplementary Table 3 specifies the RT-qPCR running conditions. RT-qPCR targets were quantified by plotting the quantification cycles (CT) to an external standard curve built using a ten-fold serial dilution of the 2019-nCoV_N_Positive Control and 2019-nCoV_E_Positive Control (IDT). Mengovirus RNA recovery rates were calculated and used as quality assurance parameters according to ISO 15216-1:2017. For quantification, previously built robust calibration lines were used, making sure that the criteria established was the same ISO standard of R2 > 0.98 and slope between −3.1 and − 3.6. The LoD was 670 GC/L and the LoQ 17,000 GC/L. For accurate viral RNA gene/copies/L quantification, the most unfavorable of the three target's Ct (cycle threshold) value was used.

2.3. Data interpretation: SARS-CoV-2 concentration and standardization

The local Health Department communicated new cases and deceases twice a week during the study period. Thus, to facilitate data interpretation when correlating epidemiological indicators, SARS-CoV-2 concentrations were treated as weekly aggregates (sum of the concentration values per sector for each sample collection day) for the whole city that week and expressed as GC/L. Thus, keeping in mind the delays of public health official reports, the results could be compared to our epidemiological indicators, as they could both be adjusted to the same natural week.

2.4. Epidemiological indicators

To calculate the new confirmed cases (every week) shown in the graphs - published twice a week by the Valencian Health Department (Conselleria de Sanitat Universal y Salud Pública, 2023), we subtracted from the total number of confirmed cases since the beginning of the pandemic the results available from the previous Monday. Hospitalization, admission to the critical care unit, average hospital stays, average stay in critical care, weekly mortality, and PCR + in both primary care and the hospital data were

obtained from the directorate of Health Management of the *Conselleria de Sanitat de la Generalitat Valenciana* and the town hall of Xátiva. The percentage of vaccinated individuals included the total population of the Autonomous Community of Valencia, and not only the inhabitants from Xátiva; this specific information was not available in the reports provided by the health authorities (Conselleria de Sanitat Universal y Salud Pública, 2023). Of note, in 2022, the Health Authorities provided the hospitalization data on a monthly basis; thus, data had to be scaled down every week.

2.5. Statistical analyses

Statistical data analyses were performed using the R software and graphical representations were made with ggplot2 (Wickham, 2016).

The Shapiro-Wilk Test was used on all study variables to determine normal distributions, represented as Quartile-Quartile (QQ) plots. Different factors may affect SARS-CoV-2 RNA concentrations in wastewater (temperature, precipitation, sampling site, water level, and flow) adding noise to the data (Arabzadeh et al., 2021). A local regression (LOESS) was performed to overcome this problem and obtain a better adjusted trend of this variable. The span for this regression was set at 0.15.

To determine variable relationships and their changes throughout the various study waves, multiple Pearson's product-moment correlations were performed between pairs of variables. The result is shown as a heatmap, with and without hierarchical clustering. Table 1 specifies the beginning and end of each study wave. Note COVID-19 Wave 4 could not be observed in this region.

We estimated the trends and correlations of SARS-CoV-2 RNA wastewater concentration and 14-day cumulative incidence (CI) to assess its use as a marker for epidemiological surveillance. For this, the time series data were divided into ascent and descent for every COVID-19 wave (Table 1) and data were normalized with a max-min transformation. Next, the trends were represented with a linear fit and the correlation computed a Pearson's product-moment correlation.

To assess the differences in our variables before and after the completion of the SARS-CoV-2 vaccination program, we performed a two-sided Mann-Whitney *U* test between the data of the three waves. Statistical significance (Alpha) was set at 0.05. The date for this was set for July 5, 2021, when the population vaccinated for SARS-CoV-2 with the complete schedule reached a maximum. Therefore, the pre-vaccination period includes Wave 3 and the post-vaccination period Wave 5 and Wave 6.

Cross-correlation analyses were performed to test any possible relationship between SARS-CoV-2 RNA concentrations in wastewater with epidemiological indicators for all study waves. Another cross-correlation test was performed with wastewater SARS-CoV-2 RNA concentrations against 14-day CI and positive PCR cases from September 2020 to March 2022.

3. Results

3.1. Epidemiological indicators versus SARS-CoV-2 wastewater concentrations

The epidemiological indicators for 14-day CI, positive cases (PCR +), hospital cases, hospital stays (defined as the total number of COVID-19 patients confined in the hospital at the same time for a week), critical cases, critical stays, primary care visits, and deaths were analyzed each week from September 2020 to March 2022. These clinical indicators were compared against the values of SARS-CoV-2 genome copies per liter (GC/L) in wastewater for the same period (Fig. 1). Normality tests were all significant (Supplementary Fig. 1), i.e., none of the study variables showed a normal distribution.

The overall trend of the indicators was similar to that of SARS-CoV-2 concentrations. However, each of variable fitted viral wastewater concentration differently; 14-day CI was the variable that better overlapped with viral concentration over time (Fig. 1A), followed by PCR + cases (Fig. 1B). Better correspondence with viral concentrations was found for hospitalization, critical cases, and stays during Wave 3 and Wave 5 in comparison to Wave 6. The larger number of vaccinated people with at least one
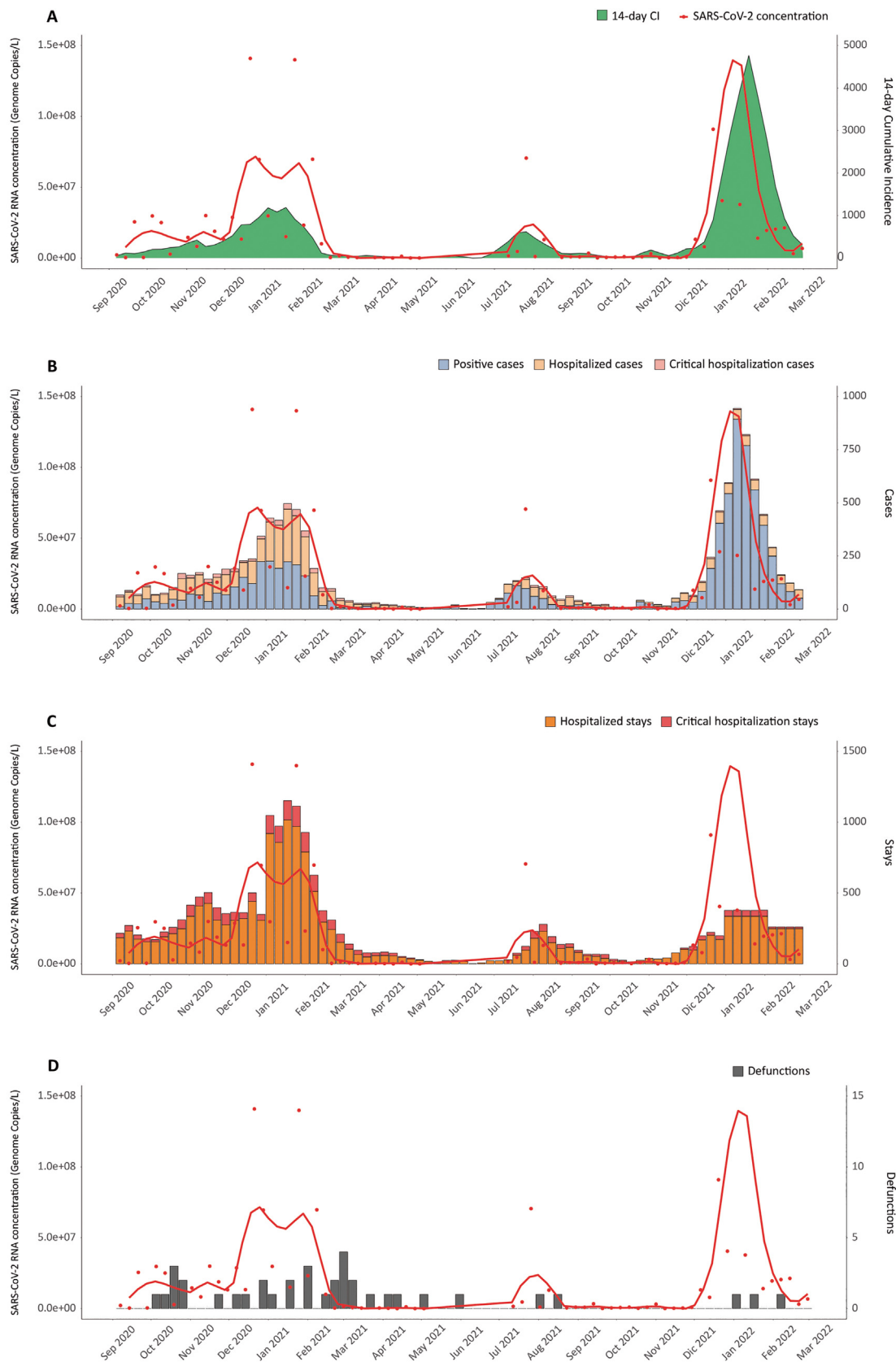
Fig. 1. Relationship between 14-day cumulative incidences (A), COVID-19 cases (B), and COVID-19 hospital stays (C) with RNA concentrations in wastewater in the region of Xátiva (Spain). Cases and stays are represented as bars, divided in critical hospitalization, hospitalization, or positive diagnosis via PCR. RNA concentrations, sampled and smoothened using a LOESS model (span = 0.15), are represented as red dots and a line, respectively. SARS-CoV-2 concentration values above $1.5 \cdot 10^8$ genome copies/L are omitted in the figure.

dose explains this: from 0 % in Wave 3 to 55 % in Wave 5, and 80 % in Wave 6 (Fig. 1B and C). Deaths per week showed a decreasing trend at the end of Wave 3; however, these data are limited due to the size of the population inoculated with the first vaccine at the beginning of Wave 6 (Fig. 1D).

Moreover, a strong correlation was observed for 14-day CI and SARS-CoV-2 in wastewater, with Pearson correlation coefficients (r) varying between 0.79 and 0.95 (Fig. 2A, B, C). Similar correlations were found between PCR + cases and viral RNA wastewater concentrations, showing r values between 0.85 and 0.88. Correlations between 14-day CI and SARS-CoV-2 concentrations were also assessed for the ascent and descent of each wave; values between 0.65 and 0.99 were obtained, all with statistically significant p values (Supplementary Fig. 2). Wave 5 showed the strongest correlation (r values of 0.95 during the ascent of the wave and 0.99 in the descent) and Wave 3 showed the worst correlation (r values between 0.65 and 0.86).

Interestingly, r coefficients for hospitalizations and critical hospitalization cases (0.76 and 0.65, respectively) and for hospitalizations and critical hospitalization stays (0.74 and 0.66, respectively) showed high correlations

with wastewater SARS-CoV-2 RNA concentrations in Wave 3 and Wave 6. r values for these clinical variables decreased in Wave 5 and only maintained a moderate correlation with hospitalization cases (0.64). Surprisingly, no clear correlation between deaths and viral concentrations in wastewater was observed for Wave 3 (0.04), although a slight increase in the correlation was found for Wave 5 and Wave 6 (0.29 and 0.38, respectively).

r values obtained in the three heatmaps highlighted the presence of different clusters based on the percentage of vaccination (Supplementary Fig. 3) with a big cluster combining all variables except for deaths during Wave 3 (Supplementary Fig. 2A).

In Wave 5, a cluster formed by positive cases, 14-day CI, and SARS-CoV-2 concentrations showed very strong correlations (range from 0.88 to 0.97). Another cluster was formed between all other epidemiological variables.

In Wave 6, two clusters were formed. One by critical hospitalization stays, SARS-CoV-2 concentration, 14-day CI, and positive cases, and the other by critical hospitalization cases, primary care visits, and hospitalization cases and stays.

These results show that 14-day CI and positive cases are always clustered together with SARS-CoV-2 concentrations in wastewater.
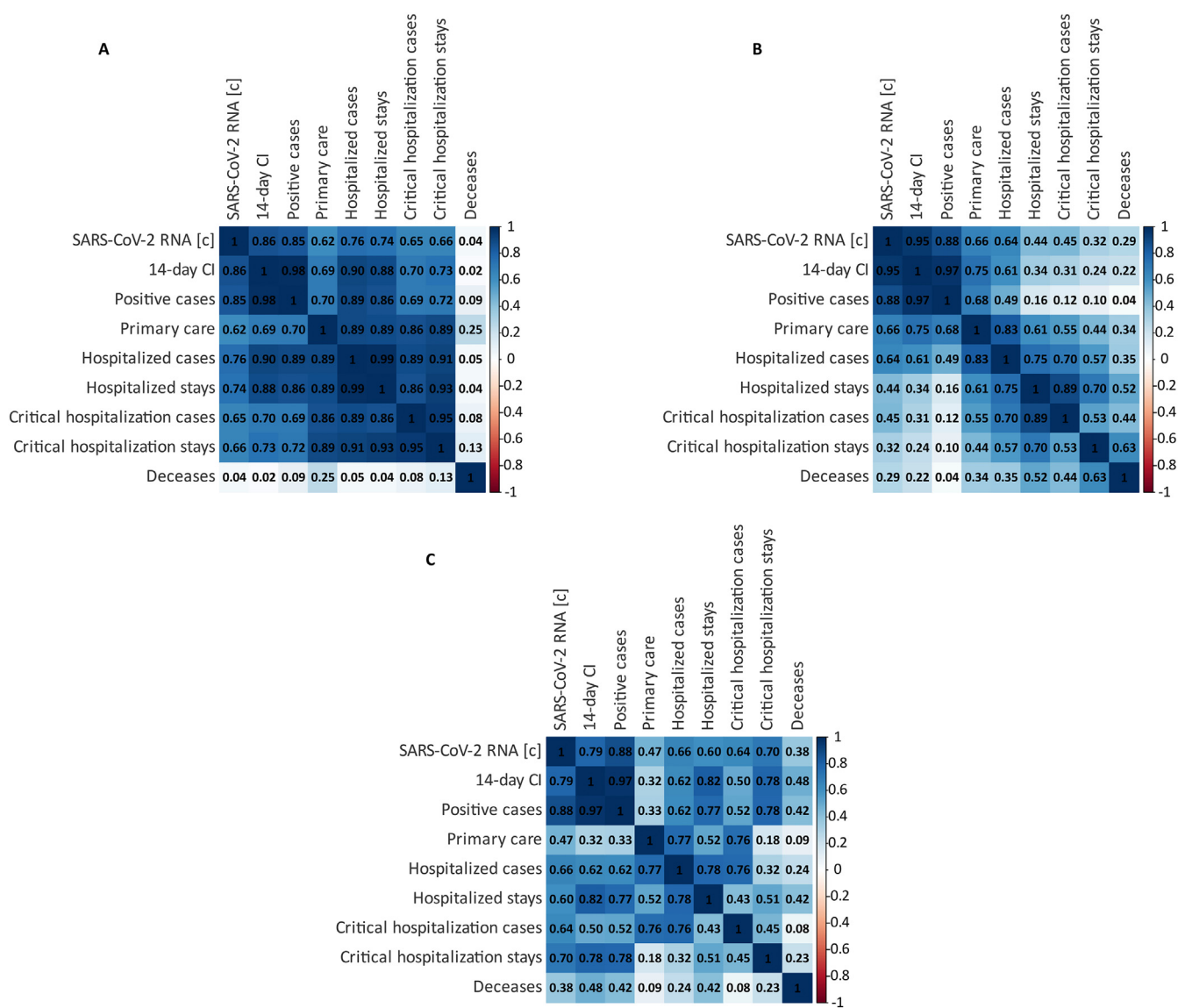

Fig. 2. Correlation heatmap matrix between wastewater SARS-CoV-2 RNA concentrations and the clinical variables 14-day cumulative incidence, positive cases (PCR +), primary care visits, hospital cases, hospital stays, critical hospitalization cases, critical hospitalization stays, and deaths per week for Wave 3 (A), Wave 5 (B), and Wave 6 (C). Highest Pearson's correlation coefficients (r) are represented in blue and the lowest in white. Red indicates maximum inverse correlation on −1.

3.2. Effect of vaccination on epidemiological variables and wastewater SARS-CoV-2 concentrations

Vaccination lowered the values of all epidemiological indicators over time, except 14-day CI and PCR + cases (Fig. 3), with clear differences between Wave 3 (no vaccination) and Wave 5 (55 % of the individuals had at least one dose) or Wave 6 (80 % of the individuals had at least one dose). No statistical differences were detected for wastewater SARS-CoV-2 concentrations between Wave 3 and Wave 6 (Mann-Whitney U test) in spite of the slight trend towards an increase in Wave 6; contrarily, a statistical difference was found between Wave 5 and Wave 6 ($p = 0.024$). Moreover, there were clear increases of 14-day CI ($p = 0.0058$) and PCR + cases ($p = 0.0098$) between Wave 3 (0 % vaccination) and Wave 6 (80 % vaccination with at least one dose). Similar differences were observed between Wave 5 (55 % vaccination with at least one dose) and Wave 6 ($p = 0.004$ and 0.0016, respectively). As for visits to primary care, no differences were seen between Wave 3 and Wave 6; statistically significant differences were only found between Wave 5 versus Wave 3 and Wave 6 ($p = 0.025$ and 0.001, respectively). Regarding hospitalization cases and stays, statistical differences were observed between Wave 5 and Wave 3 ($p = 0.011$
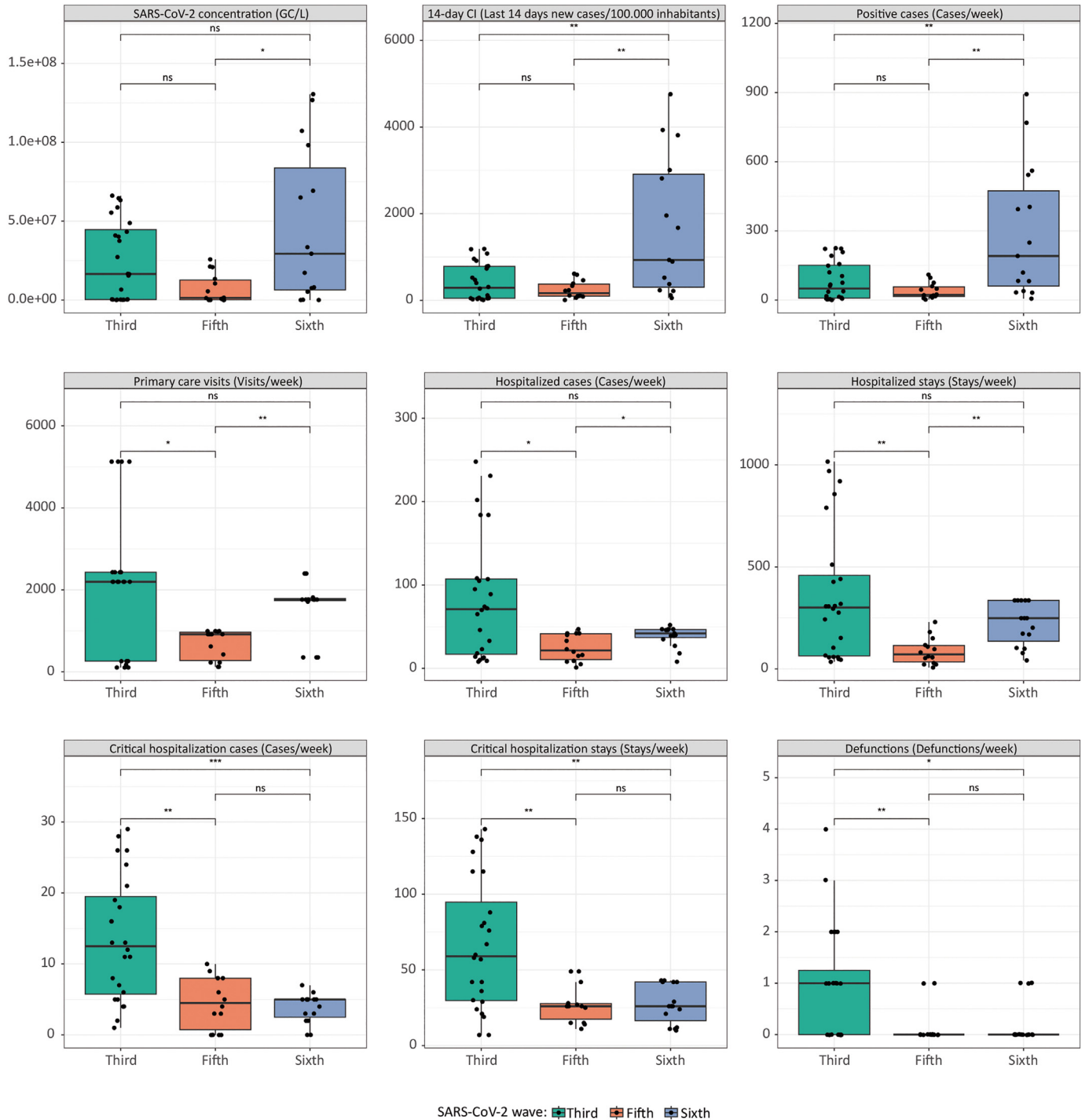

Fig. 3. Box plots comparing epidemiologic variables and wastewater SARS-CoV-2 RNA concentrations before and after vaccination. p values for the Mann-Whitney U test are shown in the figure. Statistical significance: ns: >0.05; *: <0.05; **: <0.01; ***: <0.001.

(cases) and 0.0027 (stays)) or Wave 6 ($p = 0.022$ (cases) and 0.0015 (stays)), although values were lower in Wave 6 in comparison to Wave 3, an indication towards decrease after vaccination.

Critical hospitalization cases and stays, as well as deaths, showed a significant decrease after vaccination, as observed when Wave 3 values were compared with those of Wave 5 ($p = 0.0014$; $p = 0.0038$; $p = 0.0067$) and Wave 6 ($p = 0.00027$; $p = 0.0055$; $p = 0.012$).

3.3. Wastewater SARS-CoV-2 quantification as a tool for an early surveillance system

Wastewater as a public health surveillance tool can predict and anticipate the spread of a new epidemic outbreak. We carried out a cross-correlation study between wastewater SARS-CoV-2 concentrations and a series of epidemiologic variables (Fig. 4) to determine any correlation between study variables over time. The statistical methodology was tested for all study parameters in Wave 3 (0 % vaccination) and Wave 6 (maximum percentage of vaccination). Relevant cross-correlation r values and 95 % confidence intervals are available in Supplementary Table 4.

The highest correlations were found for 14-day CI and positive cases at lag 0 (no anticipation) for Wave 3. As for Wave 6, RNA concentrations showed stronger correlations with study variables 1 and 2 weeks in advance

(r values of 0.90 and 0.91, respectively, for −1 week lag and 0.82 and 0.74, respectively, for −2 week lag). For hospitalization cases and stays, cross-correlations showed stronger correlations for stays one week before in Wave 6 (0.69), showing maximum correlation with no advance in Wave 3 (cases = 0.76; stay = 0.74). Critical hospitalization cases and stays showed a slight correlation increase three weeks before only for Wave 3 (0.70 and 0.74, respectively), while the advance in correlation for Wave 6 was only one week before for critical cases and stays (0.67 and 0.8 for −1 week delay in critical hospitalization cases and stays, respectively). Primary case visits showed stronger correlation with a lag of three weeks in Wave 3 (0.75) and a lag of two weeks in Wave 6 (0.6).

The cross-correlation for the variable *deceased* did not reach the confidence interval threshold, probably due to the limited number of deaths registered in the small region were the study was carried out. However, in the absence of vaccination, there was a trend towards an increased correlation three to five weeks in advance and of one week in the population with at least one dose.

Cross-correlations for wastewater SARS-CoV-2 RNA concentrations and 14-day CI or PCR + cases included all data from September 2020 to March 2022 and the three waves were analyzed (Fig. 5). The trend for these variables was the same, revealing strong correlations ($r = 0.82$ and $r = 0.87$, respectively). Moreover, the highest correlations between SARS-CoV-2
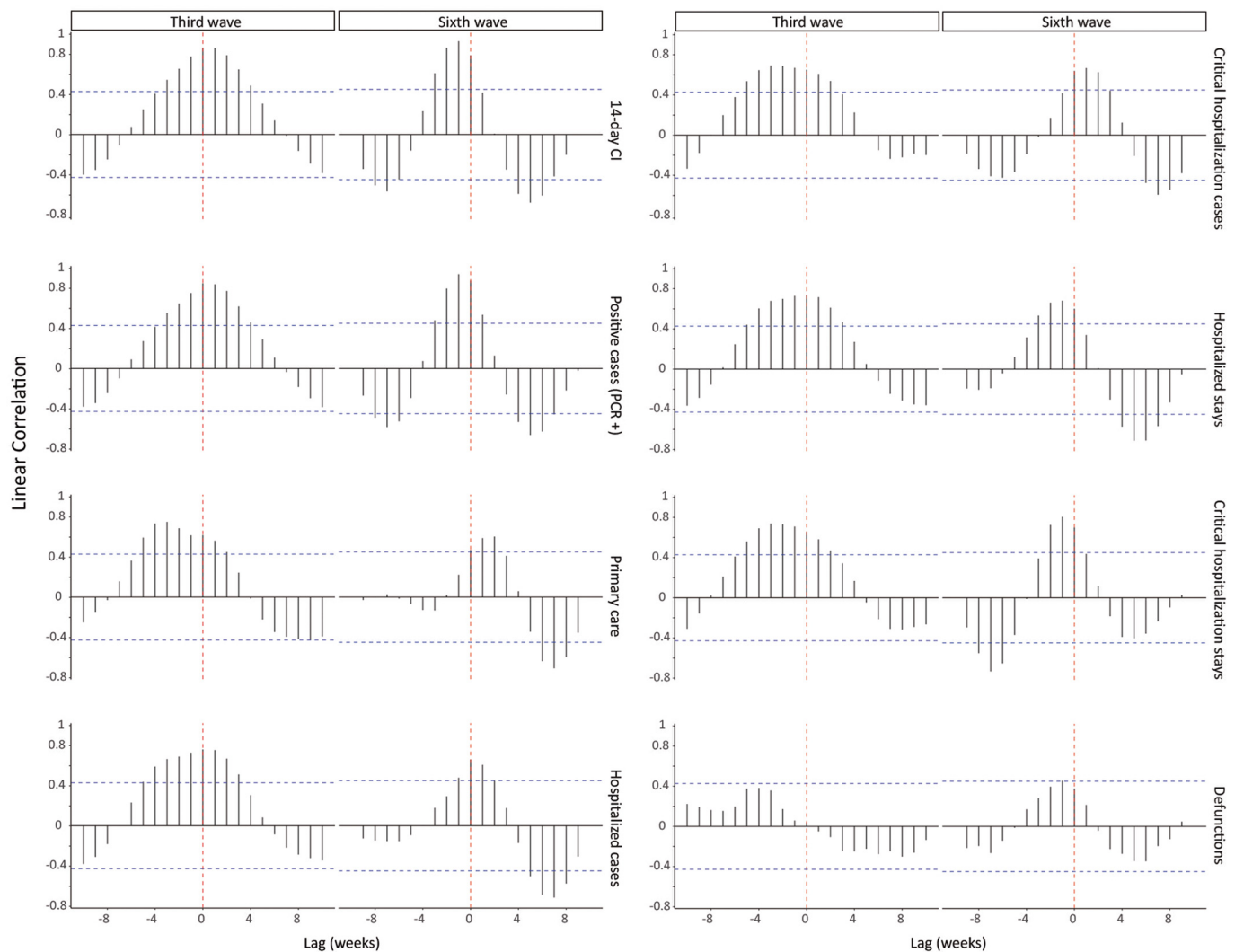

Fig. 4. Cross-correlation between wastewater SARS-CoV-2 RNA concentrations and epidemiological variables for each wave analyzed. Lag represents the number of time intervals (i.e., weeks) lapsed to perform the correlation; 95 % confidence intervals for the correlations are indicated as horizontal blue dashed lines and the breakpoint at 0 (no lag) is indicated as a vertical red dashed line.

Fig. 5. Cross-correlation between wastewater SARS-CoV-2 RNA concentrations and 14-day cumulative incidence (above) and PCR + cases (below) from September 2020 to March 2022. Lag represents the number of time intervals (i.e., weeks) lapsed to perform the correlation; 95 % confidence intervals for the correlations are indicated as horizontal blue dashed lines and the breakpoint at 0 (no lag) is indicated as a vertical red dashed line.
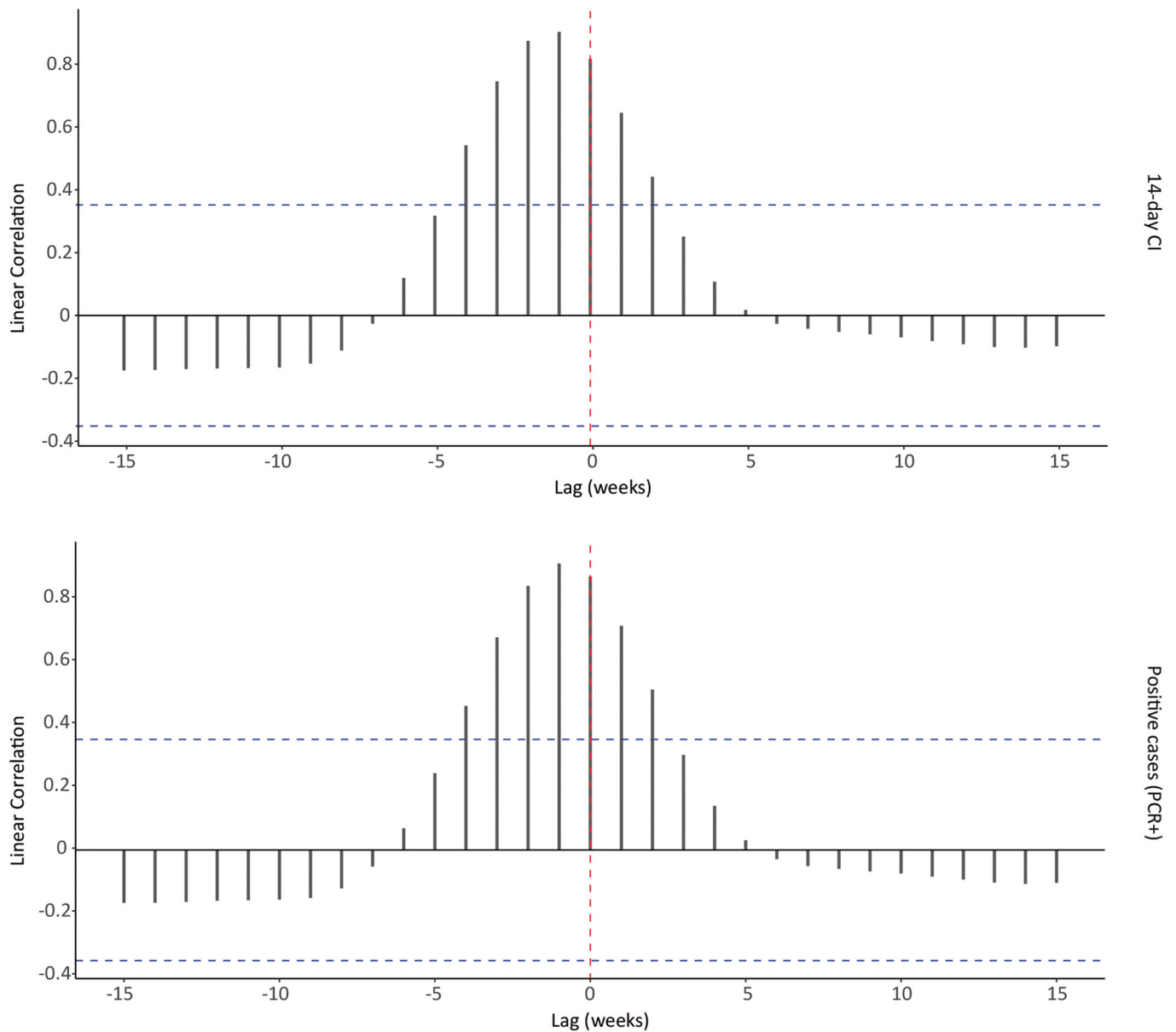
concentrations and 14-day CI or PCR + cases were seen 1 and 2 weeks in advance in comparison to correlations without lag. The strongest correlation was 0.90 in 14-day CI and 0.91 in PCR + cases one week in advance.

4. Discussion

Previous works have analyzed the progression of SARS-CoV-2 infections measuring wastewater concentrations of the virus (Chavarria-Miró et al., 2021; Haramoto et al., 2020). Here, we aimed to correlate wastewater SARS-CoV-2 RNA concentrations with the following variables: CI of SARS-CoV-2, positive cases (PCR +), primary care visits, deaths, hospitalization, and critical cases and stays. For this, we collected data on the study variables for a period of 18 months (September 2020 to March 2022) in Xativa (Spain). Our study shows that the best overlap over time with wastewater SARS-CoV-2 concentrations is 14-day CI, followed by positive cases.

Our results show that 14-day CI and positive cases have strong correlations with SARS-CoV-2 concentration in wastewater ($R = 0.79–0.95$ and

$R = 0.85–0.88$ respectively) with an anticipation period that ranges between seven and 14 days. These correlations are in line with previous findings (Li et al., 2023; Barrios et al., 2021; Kitamura et al., 2021; Róka et al., 2021; Weidhaas et al., 2021) which demonstrated correlations ranging from 0.71 to 0.96 between the SARS-CoV-2 concentration and new cases per week. Moreover, Barrios et al. and Rusiñol et al. found correlations from 0.53 to 0.88 between SARS-CoV-2 concentration and 7–14 days future cases, indicating that the concentration of the virus is a good anticipating variable for new cases (Barrios et al., 2021; Rusiñol et al., 2021).Thus, wastewater SARS-CoV-2 concentrations are a good predictor of viral behavior and spread. The anticipation period is sufficiently long and precise as to alert public health services and political authorities on a new outbreak and take the necessary measures to protect citizens and alert the health system. The lack of information on the behavior of the SARS-CoV-2 virus was one of the main reasons certain drastic decisions were taken, which would probably had been different should there had been more information in advance on viral spread and its clinical impact (Stephens et al., 2022) (particularly in periods of low incidence). The decisions taken during the pandemic were

based on risk management techniques - such as the Monte Carlo simulation (with little information or uncertainty) - that incorporated not only health, but also economic and even ethical criteria (Daumann et al., 2021). Some social studies have called it the management of ignorance (Parviainen et al., 2021). Thus, the results of the present study is to provide reliable, predictive, and anticipatory information to reduce uncertainty with valuable data that will allow informed decision-making adjusted to real clinical practice. Clinical implications and detection of wastewater RNA have been key when planning a public health response. Several studies report that determining the presence of wastewater SARS-CoV-2 RNA from sites with a population of >100,000 inhabitants allows early detection of outbreaks (Weidhaas et al., 2021). A potential limitation of these claims (which do not affect our results), is that wastewater RNA analysis may not be a reliable source of data in different geographical contexts, depending on whether the water has not been treated or disinfected and may be contaminated. The concentration of viral RNA in wastewater depends on different factors such as persistence of the virus in the organism, its stability in the sewer, sampling methodology, RNA extraction, detection protocols, and mode of quantification in relation to water flow. These factors generate variations in the results (20 % to 40 %) and thus affect correlation values (Li et al., 2021b). Presence of the virus may decrease significantly on Day 5 of infection or be detectable for weeks in stool samples (Wu et al., 2020). It is important to mention that in populations where the wastewater sample represents >10 COVID-19 positive cases, the influence of the variable "presence of the virus in the organism" is significantly reduced (Li et al., 2021b). Moreover, temperature as well as the season at which samples are collected, may affect RNA degradation in wastewater (Li et al., 2021b). Similarly, the presence of chemicals (e.g., bleach) and/or biological compounds may alter RNA degradation (Li et al., 2021b). In this sense, the presence of hospitals or health centers can affect the concentration of viral RNA detected, since the wastewater from these institutions is chemically treated. Therefore, it is important to mention that in our study the hospital of Xátiva was outside the area analyzed, not affecting the concentration of the virus in the wastewater. The time of the day samples were collected should also be taken into account, in this study at 10 am the same day of the week, coinciding with the highest water consumption peak. And the type of sampling (simple or composed), which can significantly affect the concentration (Li et al., 2021b), being the in-sewer decay the major contributor to uncertainty of SARS-CoV-2 estimation in wastewater. All these issues should address in future studies (Wang et al., 2021; Zhou et al., 2021) and not underestimated in line with World Health Organization recommendations regarding the use of ultraviolet radiation, disinfection, and filtration for SARS-CoV-2 positive wastewater, not only because of this pandemic, but also for potential new outbreaks (WHO, 2020).

The results of our study show that vaccination schemes have been particularly effective in reducing cases, critical hospital stays, and deaths, while positive cases and 14-day CI continued to increase. The high morbidity and mortality rates associated to the pandemic, resulted in hospital overcrowding and very high economic costs in the health sector (Wang et al., 2021). If having data on wastewater virus concentrations contributes to reduce morbidity, mortality, health system stress, and costs, it should be incorporated as a surveillance tool in health systems to help anticipate restrictive and protective decision-making.

We also examined the influence of vaccination on the predictive potential of wastewater SARS-CoV-2 concentrations after a novel outbreak. PCR+ cases and 14-day CI data (Fig. 3) indicate that the vaccination in the second half of 2021 had no effect on viral spread. On the contrary, there is significant increase in 14-day CI and positive cases between Wave 3 (0 % of the population had been vaccinated) and Wave 6 (80 % of the population had been vaccinated). This can be explained by the combination of the vaccination/relaxation of restriction measures, the subsequent increase in mobility and contacts (Google COVID-19 Community Mobility Reports, 2022), and the irruption of new SARS-CoV-2 variants such as Omicron with an important and higher transmission potential (Araf et al., 2022).

Wastewater SARS-CoV-2 RNA shows high correlation with the anticipation (lag $-1/2$ weeks) of positive cases ($r_{-1\ week} = 0.91$; $r_{-2\ weeks} = 0.74$) and 14-day CI ($r_{-1\ week} = 0.90$; $r_{-2\ weeks} = 0.82$) in the study period in which 80 % of the population had been vaccinated with at least one dose (Fig. 4).

When using wastewater concentrations and epidemiological indicators lag patterns, it is important to consider how health authorities manage and collect the epidemiological data. Thus, in our study, at least seven days had to be added to a specific date for each clinical data when the calculations were made, because data were known and officially made public seven days after the wastewater samples were collected, processed, and the SARS-CoV-2 concentration determined. For that, correlation values can be shifted until 7 days and show better correlations when no lag was considered; this is for Wave 3 in absence of vaccination (December 2020), for example. For this period, there were many undetected asymptomatic cases, and the reported PCR+ cases and 14-day CI were inaccurate. Conversely, the results indicate that for Wave 6, when case detection methods were standardized and widespread, the increase in observed cases (symptomatic and asymptomatic) correlates with the measurement of wastewater virus 1–2 weeks in advance. Fig. 5 shows that wastewater viral concentrations anticipate 14-day CI and PCR+ data one or two weeks in advance, a very useful surveillance tool for health authorities in the event of a new outbreak, since it can predict the behavior and spread of the virus even if the vaccination status of the analyzed population is unknown.

Wastewater SARS-CoV-2 concentrations follow hospitalizations and critical care COVID-19 cases in real time during post-vaccination (lag 0). In Wave 6, decrease of cases and hospital stays were observed in numerous studies and different settings (Barchuk et al., 2022; Iacobucci, 2021; Marcellusi et al., 2022). For Wave 3, wastewater viral concentrations anticipate critical care hospitalizations and primary care visits in three weeks ($r = 0.69$ and $r = 0.78$ respectively). This is due to the high virulence of the virus before vaccination, which affects primary care visits, hospitalizations, and critical care cases. Moreover, wastewater SARS-CoV-2 RNA concentrations anticipates hospital stays (lag -1 week; $r = 0.73$) and intensive care stays (lag -3 weeks; $r = 0.74$) (Fig. 4) in the pre-vaccination period (Wave 3). Post-vaccination (Wave 6), one week anticipation is shown for both variables ($r = 0.68$ and $r = 0.81$, respectively). These results are in line with other studies (Bivins et al., 2020; Hegazy et al., 2022; Stephens et al., 2022). Wastewater viral concentrations can be helpful for planning hospital and ICU bed requirements and as a tool to predict hospitalizations during future seasonal vaccination campaigns. Other studies (Choi et al., 2022; Puhach et al., 2022) reported a decrease on the timeframe for viral shedding in the context of the Omicron variant and vaccinated individuals, which may have affected the lags reported in this study. However, with the high increase of cases in Wave 6, higher wastewater viral concentrations were still determined and the shift in lag for the cross-correlations seems to achieve a better linear correlation with 1–2 weeks of anticipation using our epidemiological variables and sampling conditions.

The study and evaluation of wastewater SARS-CoV-2 RNA has high applicability in areas of high vaccination coverage, providing benefit to mass vaccination plans with coverage of ≥80 %. In the absence of vaccination, SARS-Cov-2 concentrations have higher correlation with critical hospitalization stays and cases, which decrease with vaccination coverage, a clear immunization indicator of the reduction of critical illness. Based on what has been learnt in dealing with new pandemics or emerging epidemics (such as SARS-COV-2), population-based immunization should be a strategic priority, although in the initial stages this may not be sufficient and restrictive measures may still be necessary (Cot et al., 2021). Wastewater SARS-Cov-2 analysis can become a key tool for epidemiological surveillance, as it would facilitate decision making by public health authorities regarding potential geographic and temporal restrictions. This is critical given the rejection of drastic mobility restrictions by a large part of the population, who accept the use of masks or telephone monitoring, but not prolonged lockdowns, hospital closures, or restrictions of leisure travel (Blayac et al., 2021). Indirectly, it could contribute to mitigate the pandemic-related economic burden (Monika, 2021) and facilitate health care for the

population, who during restriction periods had limited face-to-face interactions and more telephone/digital encounters, with the consequent rejection by the population (Lima-Serrano et al., 2020).

The possibility to anticipate significant incidence increases through wastewater analysis may allow the implementation and deployment of health contingency plans with enough time to ensure their operability. Wastewater monitoring is useful not only for public health services, but also for primary care centers and hospital mangers (especially emergency and ICU services), who would be able to organize their human and material resources before a forecasted increase in the incidence. Wastewater analysis may also be useful for primary health care services, a direct strategic link with first care, who had to be adjusted during the pandemic, including the incorporation of telehealth when face-to-face care was interrupted. Thus, transferring our results to health managers would support public health planning decisions and clinical practice by organizing health care teams.

Acting when the incidence has risen abruptly has been shown to have negative consequences in terms of hospital admissions, mortality, and health system overload, as incidence and mortality are known to correlate strongly with the observed daily cumulative rate (Lai et al., 2020). A quick and accurate response by the healthcare system is critical. In countries where people are more confident with their system, they are often more responsive to protective measures, e.g., reduced mobility (Chan et al., 2020).

There are some limitations to our study. We had no access to viral variants during the study period. This does not affect our results and conclusions, but having this information might have contributed to a more detailed wave-based prediction. In the initial waves, we assumed important under-diagnosis, contrarily to the situation in the final waves, were COVID-19 diagnoses were closer to the real health scenario due to the incorporation of mass testing in Spanish pharmacies. Covid-19-related hospital stays and ICU admissions data do not take into account the medical history of the patients; thus, the patients´ profile could not be used given the nature of our study. Moreover, hospital admission criteria probably changed (protocolized or not) depending on the wave due to hospital overcrowding, particularly ICUs (Andrés-Esteban et al., 2021).

Several biases influence data interpretation when it comes to a wastewater sampling. Some examples are water and environmental temperatures, heavy rainfalls, catchment flow, in-sewer decay, number of citizens the sewer collects from, distance of the collection point, type of sampling, and presence of hospitals, healthcare centers etc., among others (Jiang et al., 2022), In addition, different methodologies have been used in other studies to normalize concentration values (PMMV virus in a separate PCR, the physicochemical parameters of the sample, i.e., CDO or ammonium values), and even models and formulas (Jiang et al., 2022) that approach real case number estimation. The objective in this study was not inter-municipality comparisons, but to analyze viral load trends and correlate them with certain epidemiological indicators specifically for Xàtiva. Due to some technical limitations, data are expressed as weekly raw wastewater concentrations calculated from samplings carried out the same day of the week at the same time during the whole study, coinciding with the highest water consumption peak.

Some researchers have used 14-day CI and wastewater SARS-CoV-2 concentrations to develop mathematical (Joseph-Duran et al., 2022; McMahan et al., 2021; Watson et al., 2022) or machine learning modeling (Aberi et al., 2021; Li et al., 2021a; Vallejo et al., 2020; Zhu et al., 2022). However, to the best of our knowledge, our study uses clinical parameters such as hospitalizations and ICU data for the first time and wastewater viral concentrations as a predictive tool for novel outbreaks.

5. Conclusions and future perspectives

Wastewater SARS-CoV-2 concentrations show high correlation with the epidemiological variables 14-day CI, PCR+, hospitalization, and ICU admission. Our results provide data that allows tracking and anticipating the epidemiological situation in future outbreaks, taking into account for the first time

not only 14-day CI and PCR+ but also mortality, hospitalization/ICU data, and stays. Our model may anticipate the trend of these clinical data in seven to 15 days, providing a novel tool for health authorities to support any decisions during an adverse epidemiological situation. Future studies should focus on developing machine-learning models incorporating these clinical data, as well as the effect of different SARS-CoV-2 variants, mobility, and climatological data, and test these predictive models in similar populations.

The success of this wastewater-based epidemiology could contribute to the surveillance of incipient pathogens.

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CRediT authorship contribution statement

Dr. Benavent-Cervera: study design and coordination; review and approval of the final manuscript; provided all clinical data. **Dr. Fernández-Garrido:** study design and coordination; review and approval of the final manuscript; provided all clinical data. **Dr. Gea-Caballero:** study design and coordination; review and approval of the final manuscript. **Dra. Pellín-Carcelén:** study design and coordination; review and approval of the final manuscript; writing of the initial draft. **Dr. Juárez-Vela:** study design and coordination; review and approval of the final manuscript. **Dr. Andreu-Fernández:** study design and coordination; review and approval of the final manuscript; writing of the initial draft; performed all the statistical analyses. **Dr. Seguí López-Peñalver:** writing of the initial draft; performed the biochemical analyses of wastewaters; performed the statistical analyses. **Mr. Ruben Cañas-Cañas:** writing of the initial draft; performed the biochemical analysis of wastewaters; performed all statistical analyses. **Dr. Casaña-Mohedo:** writing of the initial draft; performed all the statistical analyses.

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The data that has been used is confidential.

Declaration of competing interest

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References

Aberi, P., Arabzadeh, R., Insam, H., Markt, R., Mayr, M., Kreuzinger, N., Rauch, W., 2021. Quest for optimal regression models in SARS-CoV-2 wastewater based epidemiology. Int. J. Environ. Res. Public Health 18 (20), 10778. https://doi.org/10.3390/IJERPH182010778/S1.

Andrés-Esteban, E.M., Quintana-Diaz, M., Ramírez-Cervantes, K.L., Benayas-Peña, I., Silva-Obregón, A., Magallón-Botaya, R., Santolalla-Arnedo, I., Juárez-Vela, R., Gea-Caballero, V., 2021. Outcomes of hospitalized patients with COVID-19 according to level of frailty. PeerJ 9, e11260. https://doi.org/10.7717/PEERJ.11260/SUPP-2.

Arabzadeh, R., Grünbacher, D.M., Insam, H., Kreuzinger, N., Markt, R., Rauch, W., 2021. Data filtering methods for SARS-CoV-2 wastewater surveillance. Water Sci. Technol. 84 (6), 1324–1339. https://doi.org/10.2166/wst.2021.343.

Araf, Y., Akter, F., Tang, Y. dong, Fatemi, R., Parvez, M.S.A., Zheng, C., Hossain, M.G., 2022. Omicron variant of SARS-CoV-2: genomics, transmissibility, and responses to current COVID-19 vaccines. J. Med. Virol. 94 (5), 1825–1832. https://doi.org/10.1002/JMV.27588.

Awadasseid, A., Wu, Y., Tanaka, Y., Zhang, W., 2020. Initial success in the identification and management of the coronavirus disease 2019 (COVID-19) indicates human-to-human transmission in Wuhan, China. Int. J. Biol. Sci. 16 (11), 1846–1860. https://doi.org/10.7150/IJBS.45018.

Barchuk, A., Cherkashin, M., Bulina, A., Berezina, N., Rakova, T., Kuplevatskaya, D., Stanevich, O., Skougarevskiy, D., Okhotin, A., 2022. Vaccine effectiveness against referral to hospital after SARS-CoV-2 infection in St. Petersburg, Russia, during the Delta variant surge: a test-negative case-control study. BMC Med. 20 (1), 1–8. https://doi.org/10.1186/S12916-022-02509-8/FIGURES/1.

Barrios, M.E., Díaz, S.M., Torres, C., Costamagna, D.M., Blanco Fernández, M.D., Mbayed, V.A., 2021. Dynamics of SARS-CoV-2 in wastewater in three districts of the Buenos Aires metropolitan region, Argentina, throughout nine months of surveillance: a pilot study. Sci. Total Environ. 800, 149578. https://doi.org/10.1016/J.SCITOTENV.2021.149578.

Beyer, S., Szewzyk, R., Gnirss, R., Johne, R., Selinka, H.-C., 2020. Detection and characterization of hepatitis E virus genotype 3 in wastewater and urban surface waters in Germany. Food Environ. Virol. 12 (2), 137–147. https://doi.org/10.1007/s12560-020-09424-2.

Bisseux, M., Colombet, J., Mirand, A., Roque-Afonso, A.-M., Abravanel, F., Izopet, J., Archimbaud, C., Peigue-Lafeuille, H., Debroas, D., Bailly, J.-L., Henquell, C., 2018. Monitoring human enteric viruses in wastewater and relevance to infections encountered in the clinical setting: a one-year experiment in Central France, 2014 to 2015. Euro Surveillance 23 (7). https://doi.org/10.2807/1560-7917.ES.2018.23.7.17-00237.

Bivins, A., North, D., Ahmad, A., Ahmed, W., Alm, E., Been, F., Bhattacharya, P., Bijlsma, L., Boehm, A.B., Brown, J., Buttiglieri, G., Calabro, V., Carducci, A., Castiglioni, S., Cetecioglu Gurol, Z., Chakraborty, S., Costa, F., Curcio, S., De Los Reyes, F.L., ... Bibby, K., 2020. Wastewater-based epidemiology: global collaborative to maximize contributions in the fight against COVID-19. Environ. Sci. Technol. 54 (13), 7754–7757. https://doi.org/10.1021/acs.est.0c02388.

Blayac, T., Dubois, D., Duchêne, S., Nguyen-Van, P., Ventelou, B., Willinger, M., 2021. Population preferences for inclusive COVID-19 policy responses. Lancet Public Health 6 (1), e9. https://doi.org/10.1016/S2468-2667(20)30285-1.

Cao, L., Lou, J., Chan, S.Y., Zheng, H., Liu, C., Zhao, S., Li, Q., Mok, C.K.P., Chan, R.W.Y., Chong, M.K.C., Wu, W.K.K., Chen, Z., Wong, E.L.Y., Chan, P.K.S., Zee, B.C.Y., Yeoh, E.K., Wang, M.H., 2022. Rapid evaluation of COVID-19 vaccine effectiveness against symptomatic infection with SARS-CoV-2 variants by analysis of genetic distance. Nat. Med. 28 (8), 1715–1722. https://doi.org/10.1038/s41591-022-01877-1.

Chan, H.F., Brumpton, M., Macintyre, A., Arapoc, J., Savage, D.A., Skali, A., Stadelmann, D., Torgler, B., 2020. How confidence in health care systems affects mobility and compliance during the COVID-19 pandemic. PLoS One 15 (10), e0240644. https://doi.org/10.1371/JOURNAL.PONE.0240644.

Chavarria-Miró, G., Anfruns-Estrada, E., Martínez-Velázquez, A., Vázquez-Portero, M., Guix, S., Paraira, M., Galofré, B., Sánchez, G., Pintó, R.M., Bosch, A., 2021. Time evolution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in wastewater during the first pandemic wave of COVID-19 in the metropolitan area of Barcelona, Spain. Appl. Environ. Microbiol. 87 (7), 1–9. https://doi.org/10.1128/AEM.02750-20.

Choi, G., Lim, A.-Y., Choi, S., Park, K., Lee, S.Y., Kim, J-H., 2022. Viral shedding patterns of the symptomatic SARS-CoV-2 infection according to virus-type dominant periods and vaccination status in Gyeonggi Province, Korea. Epidemiol. Health e2023008. https://doi.org/10.4178/EPIH.E2023008.

Conselleria de Sanitat Universal y Salud Pública, 2023. Datos Coronavirus. Generalitat Valenciana Conselleria de Sanitat https://coronavirus.san.gva.es/es/estadisticas.

Corman, V.M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D.K.W., Bleicker, T., Brünink, S., Schneider, J., Schmidt, M.L., Mulders, D.G.J.C., Haagmans, B.L., Van Der Veer, B., Van Den Brink, S., Wijsman, L., Goderski, G., Romette, J.L., Ellis, J., Zambon, M., Drosten, C., 2020. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance 25 (3). https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.

Cot, C., Cacciapaglia, G., Islind, A.S., Óskarsdóttir, M., Sannino, F., 2021. Impact of US vaccination strategy on COVID-19 wave dynamics. Scientific Reports 11 (1), 1–11. https://doi.org/10.1038/s41598-021-90539-2.

Cucinotta, D., Vanelli, M., 2020. WHO declares COVID-19 a pandemic. Acta Biomed 91 (1), 157–160. https://doi.org/10.23750/abm.v91i1.9397.

Daumann, F., Follert, F., Gleißner, W., Kamarás, E., Naumann, C., 2021. Political decision making in the COVID-19 pandemic: the case of Germany from the perspective of risk management. Int. J. Environ. Res. Public Health 19 (1), 397. https://doi.org/10.3390/IJERPH19010397.

Davó, L., Seguí, R., Botija, P., Beltrán, M.J., Albert, E., Torres, I., López-Fernández, P.Á., Ortí, R., Maestre, J.F., Sánchez, G., Navarro, D., 2021. Early detection of SARS-CoV-2 infection cases or outbreaks at nursing homes by targeted wastewater tracking. Clin. Microbiol. Infect. 27 (7), 1061–1063. https://doi.org/10.1016/J.CMI.2021.02.003.

Detection of enteric viruses, 2018. Standard Methods For the Examination of Water and Wastewater. American Public Health Association https://doi.org/10.2105/SMWW.2882.202.

Gholipour, S., Ghalhari, M.R., Nikaeen, M., Rabbani, D., Pakzad, P., Miranzadeh, M.B., 2022. Occurrence of viruses in sewage sludge: A systematic review. Sci. Total Environ. 824, 153886. https://doi.org/10.1016/j.scitotenv.2022.153886.

Gilbert, P.B., Montefiori, D.C., McDermott, A.B. Fong, Y., Benkeser, D., Deng, W., Zhou, H., Houchens, C.R., Martins, K., Jayashankar, L., Castellino, F., Flach, B., Lin, B.C., O'Connell, S., McDanal, C., Eaton, A., Sarzotti-Kelsoe, M., Lu, Y., Yu, C., Koup, R.A., 2022. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. Science 375 (6576), 43–50. https://doi.org/10.1126/science.abm3425.

Goldblatt, D., Fiore-Gartland, A., Johnson, M., Hunt, A., Bengt, C., Zavadska, D., Snipe, H.D., Brown, J.S., Workman, L., Zar, H.J., Montefiori, D., Shen, X., Dull, P., Plotkin, S., Siber, G., Ambrosino, D., 2022. Towards a population-based threshold of protection for COVID-19 vaccines. Vaccine 40 (2), 306–315. https://doi.org/10.1016/j.vaccine.2021.12.006.

Google COVID-19 Community Mobility Reports, 2022. https://www.google.com/covid19/mobility/.

Hajissa, K., Mussa, A., Karobari, M.I., Abbas, M.A., Ibrahim, I.K., Assiry, A.A., Iqbal, A., Alhumaid, S., Mutair, A. Al, Rabaan, A.A., Messina, P., Scardina, G.A., 2022. The SARS-CoV-2 antibodies, their diagnostic utility, and their potential for vaccine development. Vaccines 10 (8), 1346. https://doi.org/10.3390/VACCINES10081346.

Haramoto, E., Malla, B., Thakali, O., Kitajima, M., 2020. First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan. Sci. Total Environ. 737, 140405. https://doi.org/10.1016/J.SCITOTENV.2020.140405.

Hegazy, N., Cowan, A., D'Aoust, P.M., Mercier, É., Towhid, S.T., Jia, J.J., Wan, S., Zhang, Z., Kabir, M.P., Fang, W., Graber, T.E., MacKenzie, A.E., Guilherme, S., Delatolla, R., 2022. Understanding the dynamic relation between wastewater SARS-CoV-2 signal and clinical metrics throughout the pandemic. Sci. Total Environ. 853 (September). https://doi.org/10.1016/j.scitotenv.2022.158458.

Hellmér, M., Paxéus, N., Magnius, L., Enache, L., Arnholm, B., Johansson, A., Bergström, T., Norder, H., 2014. Detection of pathogenic viruses in sewage provided early warnings of hepatitis A virus and norovirus outbreaks. Appl. Environ. Microbiol. 80 (21), 6771–6781. https://doi.org/10.1128/AEM.01981-14.

Hovi, T., Stenvik, M., Partanen, H., Kangas, A., 2001. Poliovirus surveillance by examining sewage specimens. Quantitative recovery of virus after introduction into sewerage at remote upstream location. Epidemiol. Infect. 127 (1), 101–106. https://doi.org/10.1017/S0950268801005787.

Iacobucci, G., 2021. Covid-19: two doses of Pfizer vaccine are "highly effective" against infection, hospital admission, and death, study finds. BMJ 373, n1164. https://doi.org/10.1136/BMJ.N1164.

Jiang, G., Wu, J., Weidhaas, J., Li, X., Chen, Y., Mueller, J., Li, J., Kumar, M., Zhou, X., Arora, S., Haramoto, E., Sherchan, S., Orive, G., Lertxundi, U., Honda, R., Kitajima, M., Jackson, G., 2022. Artificial neural network-based estimation of COVID-19 case numbers and effective reproduction rate using wastewater-based epidemiology. Water Res. 218 (February), 118451. https://doi.org/10.1016/j.watres.2022.118451.

Joseph-Duran, B., Serra-Compte, A., Sàrrias, M., Gonzalez, S., López, D., Prats, C., Català, M., Alvarez-Lacalle, E., Alonso, S., Arnaldos, M., 2022. Assessing wastewater-based epidemiology for the prediction of SARS-CoV-2 incidence in Catalonia. Sci. Rep. 12 (1), 1–11. https://doi.org/10.1038/s41598-022-18518-9.

Kitamura, K., Sadamasu, K., Muramatsu, M., Yoshida, H., 2021. Efficient detection of SARS-CoV-2 RNA in the solid fraction of wastewater. Sci. Total Environ. 763, 144587. https://doi.org/10.1016/J.SCITOTENV.2020.144587.

La Rosa, G., Iaconelli, M., Mancini, P., Bonanno Ferraro, G., Veneri, C., Bonadonna, L., Lucentini, L., Suffredini, E., 2020. First detection of SARS-CoV-2 in untreated wastewaters in Italy. Sci. Total Environ. 736. https://doi.org/10.1016/j.scitotenv.2020.139652.

Lai, C.C., Wang, C.Y., Wang, Y.H., Hsueh, S.C., Ko, W.C., Hsueh, P.R., 2020. Global epidemiology of coronavirus disease 2019 (COVID-19): disease incidence, daily cumulative index, mortality, and their association with country healthcare resources and economic status. Int. J. Antimicrob. Agents 55 (4), 105946. https://doi.org/10.1016/J.IJANTIMICAG.2020.105946.

Landi, F., Gremese, E., Rota, E., Carfi, A., Benvenuto, F., Ciciarello, F., Lo Monaco, M.R., Martone, A.M., Pagano, F., Paglionico, A., Petricca, L., Rocchi, S., Tritto, M., Sanguinetti, M., Bernabei, R., 2020. Positive RT-PCR nasopharyngeal swab in patients recovered from COVID-19 disease: when does quarantine really end? J. Infect. 81 (6), e1–e3. https://doi.org/10.1016/j.jinf.2020.08.034.

Li, X., Kulandaivelu, J., Zhang, S., Shi, J., Sivakumar, M., Mueller, J., Luby, S., Ahmed, W., Coin, L., Jiang, G., 2021a. Data-driven estimation of COVID-19 community prevalence through wastewater-based epidemiology. Sci. Total Environ. 789, 147947. https://doi.org/10.1016/J.SCITOTENV.2021.147947.

Li, X., Zhang, S., Shi, J., Luby, S.P., Jiang, G., 2021b. Uncertainties in estimating SARS-CoV-2 prevalence by wastewater-based epidemiology. Chem. Eng. J. 415 (February), 129039. https://doi.org/10.1016/j.cej.2021.129039.

Li, X., Zhang, S., Sherchan, S., Orive, G., Lertxundi, U., Haramoto, E., Honda, R., Kumar, M., Arora, S., Kitajima, M., Jiang, G., 2023. Correlation between SARS-CoV-2 RNA concentration in wastewater and COVID-19 cases in community: A systematic review and meta-analysis. J. Hazard. Mater. 441, 129848. https://doi.org/10.1016/J.JHAZMAT.2022.129848.

Lima-Serrano, M., Carrión Robles, T., Herráiz Mallebrera, A., Arrabal-Téllez, A.B., 2020. Enfermería Comunitaria y salud digital en tiempos de la COVID-19. RIdEC 13 (2), 34–42.. https://www.enfermeriacomunitaria.org/web/index.php/ridec/290-ridec-2020-volumen-13-numero-2/2538-originales-2-enfermeria-comunitaria-y-salud-digital-en-tiempos-de-la-covid-19.

Lodder, W.J., Van Den Berg, H.H.J.L., Rutjes, S.A., De Roda Husman, A.M., 2010. Presence of enteric viruses in source waters for drinking water production in the Netherlands. Appl. Environ. Microbiol. 76 (17), 5965–5971. https://doi.org/10.1128/AEM.00245-10.

Marcellusi, A., Fabiano, G., Sciattella, P., Andreoni, M., Mennini, F.S., 2022. The impact of COVID-19 vaccination on the Italian healthcare system: A scenario analysis. Clin. Drug Investig. 42 (3), 237–242. https://doi.org/10.1007/S40261-022-01127-9/FIGURES/1.

McMahan, C.S., Self, S., Rennert, L., Kalbaugh, C., Kriebel, D., Graves, D., Colby, C., Deaver, J.A., Popat, S.C., Karanfil, T., Freedman, D.L., 2021. COVID-19 wastewater epidemiology:

a model to estimate infected populations. Lancet Planet. Health 5 (12), e874–e881. https://doi.org/10.1016/S2542-5196(21)00230-8.

Ministerio de Transición Ecológica y Reto Demográfico, 2021. Protocolo de detección de SARS-CoV-2 en aguas residuales. Control microbiológico en aguas residuales como indicador epidemiológico de alerta temprana de propagación de COVID-19. https://www.miteco.gob.es/es/agua/temas/concesiones-y-autorizaciones/protocolo-deteccion-sars-cov-2-en-aguas-residuales_tcm30-528265.pdf.

Monika, A.K., 2021. The utility of 'Covid-19 mobility report' and 'Google trend' for analysing economic activities. Syntax Idea 3 (6), 1256–1268. https://doi.org/10.36418/syntax-ideav3i6.1224.

Mousazadeh, M., Ashoori, R., Paital, B., Kabdaşlı, I., Frontistis, Z., Hashemi, M., Sandoval, M.A., Sherchan, S., Das, K., Emamjomeh, M.M., 2021. Wastewater Based Epidemiology Perspective as a Faster Protocol for Detecting Coronavirus RNA in Human Populations: A Review with Specific Reference to SARS-CoV-2 Virus. Pathogens (Basel, Switzerland). vol. 10(8). https://doi.org/10.3390/pathogens10081008.

Parviainen, J., Koski, A., & Torkkola, S. (2021). "Building a Ship while Sailing It." Epistemic Humility and the Temporality of Non-knowledge in Political Decision-making on COVID-19. Soc. Epistemol., 35(3), 232–244. doi: https://doi.org/10.1080/02691728.2021.1882610.

Peccia, J., Zulli, A., Brackney, D.E., Grubaugh, N.D., Kaplan, E.H., Casanovas-Massana, A., Ko, A.I., Malik, A.A., Wang, D., Wang, M., 2020. Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics. Nat. Biotechnol. 38 (10), 1164–1167.

Puhach, O., Meyer, B., Eckerle, I., 2022. SARS-CoV-2 viral load and shedding kinetics. Nat. Rev. Microbiol. 21 (March). https://doi.org/10.1038/s41579-022-00822-w.

Randazzo, W., Piqueras, J., Evtoski, Z., Sastre, G., Sancho, R., Gonzalez, C., Sánchez, G., 2019. Interlaboratory comparative study to detect potentially infectious human enteric viruses in influent and effluent waters. Food Environ. Virol. 11 (4), 350–363. https://doi.org/10.1007/S12560-019-09392-2.

Randazzo, W., Truchado, P., Cuevas-Ferrando, E., Simón, P., Allende, A., Sánchez, G., 2020. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. Water Res. 181. https://doi.org/10.1016/J.WATRES.2020.115942.

Róka, E., Khayer, B., Kis, Z., Kovács, L.B., Schuler, E., Magyar, N., Málnási, T., Oravecz, O., Pályi, B., Pándics, T., Vargha, M., 2021. Ahead of the second wave: early warning for COVID-19 by wastewater surveillance in Hungary. Sci. Total Environ. 786, 147398. https://doi.org/10.1016/J.SCITOTENV.2021.147398.

Rusiñol, M., Zammit, I., Itarte, M., Forés, E., Martínez-Puchol, S., Girones, R., Borrego, C., Corominas, L., Bofill-Mas, S., 2021. Monitoring waves of the COVID-19 pandemic: inferences from WWTPs of different sizes. Sci. Total Environ. 787, 147463. https://doi.org/10.1016/J.SCITOTENV.2021.147463.

Samy, A., Maher, M.A., Abdelsalam, N.A., Badr, E., 2022. SARS-CoV-2 potential drugs, drug targets, and biomarkers: a viral-host interaction network-based analysis. Sci. Rep. 12 (1), 1–14. https://doi.org/10.1038/s41598-022-15898-w.

Stephens, N., Béen, F., Savic, D., 2022. An analysis of SARS-CoV-2 in wastewater to evaluate the effectiveness of nonpharmaceutical interventions against COVID-19 in the Netherlands. ACS ES and T Water 2 (11), 2158–2166. https://doi.org/10.1021/acsestwater.2c00071.

Vallejo, J., Rumbo-Feal, S., Conde, K., Tarrío, J., Reif, R., Rodiño-Janeiro, B., Cid, A., Veiga, M., Acevedo, A., Lamora, C., Bens, E., Bou, G., 2020. Highly predictive regression model of active cases of COVID-19 in a population by screening wastewater viral load. Sci. Total Environ. 811 (152334), 13. https://doi.org/10.1016/j.scitotenv.2021.152334.

Vallejo, J.A., Trigo-Tasende, N., Rumbo-Feal, S., Conde-Pérez, K., López-Oriona, Á., Barbeito, I., Vaamonde, M., Tarrío-Saavedra, J., Reif, R., Ladra, S., Rodiño-Janeiro, B.K., Nasser-Ali, M., Cid, Á., Veiga, M., Acevedo, A., Lamora, C., Bou, G., Cao, R., Poza, M., 2022. Modeling the number of people infected with SARS-COV-2 from wastewater viral load in Northwest Spain. Sci. Total Environ. 811. https://doi.org/10.1016/j.scitotenv.2021.152334.

Walsh, K.A., Jordan, K., Clyne, B., Rohde, D., Drummond, L., Byrne, P., Ahern, S., Carty, P.G., O'Brien, K.K., O'Murchu, E., O'Neill, M., Smith, S.M., Ryan, M., Harrington, P., 2020. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. J. Infect. 81 (3), 357–371. https://doi.org/10.1016/J.JINF.2020.06.067.

Wang, W.C., Fann, J.C.Y., Chang, R.E., Jeng, Y.C., Hsu, C.Y., Chen, H.H., Liu, J.T., Yen, A.M.F., 2021. Economic evaluation for mass vaccination against COVID-19. J. Formos. Med. Assoc. 120, S95–S105. https://doi.org/10.1016/J.JFMA.2021.05.020.

Watson, O.J., Barnsley, G., Toor, J., Hogan, A.B., Winskill, P., Ghani, A.C., 2022. Global impact of the first year of COVID-19 vaccination: a mathematical modelling study. Lancet Infect. Dis. 22 (9), 1293–1302. https://doi.org/10.1016/S1473-3099(22)00320-6.

Weidhaas, J., Aanderud, Z.T., Roper, D.K., VanDerslice, J., Gaddis, E.B., Ostermiller, J., Hoffman, K., Jamal, R., Heck, P., Zhang, Y., Torgersen, K., Laan, J. Vander, LaCross, N., 2021. Correlation of SARS-CoV-2 RNA in wastewater with COVID-19 disease burden in sewersheds. Sci. Total Environ. 775. https://doi.org/10.1016/J.SCITOTENV.2021.145790.

WHO, 2020. Water, sanitation, hygiene, and waste management for SARS-CoV-2, the virus that causes COVID-19. Interim Guidance. Issue 29 July .. https://www.who.int/publications/i/item/water-sanitation-hygiene-and-waste-management-for-the-covid-19-virus-interim-guidance.

WHO, 2023. Traking SARS-CoV-2 Variants. World Health Organzation Page https://www.who.int/activities/tracking-SARS-CoV-2-variants/tracking-SARS-CoV-2-variants.

Wickham, H., 2016. ggplot2 Elegant Graphics for Data Analysis. 2nd editio. Springer New York, NY https://doi.org/10.1007/978-0-387-98141-3.

Wu, Y., Guo, C., Tang, L., Hong, Z., Zhou, J., Dong, X., Yin, H., Xiao, Q., Tang, Y., Qu, X., Kuang, L., Fang, X., Mishra, N., Lu, J., Shan, H., Jiang, G., Huang, X., 2020. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. Lancet Gastroenterol. Hepatol. 5 (5), 434–435. https://doi.org/10.1016/S2468-1253(20)30083-2.

Zhou, J.B., Kong, W.H., Wang, S., Long, Y.B., Dong, L.H., He, Z.Y., Liu, M.Q., 2021. Detection of SARS-CoV-2 RNA in medical wastewater in Wuhan during the COVID-19 outbreak. Virol. Sin. 36 (5), 1077–1079. https://doi.org/10.1007/S12250-021-00373-Z/TABLES/1.

Zhu, Y., Oishi, W., Maruo, C., Bandara, S., Lin, M., Saito, M., Kitajima, M., Sano, D., 2022. COVID-19 case prediction via wastewater surveillance in a low-prevalence urban community: a modeling approach. J. Water Health 20 (2), 459–470. https://doi.org/10.2166/WH.2022.183.