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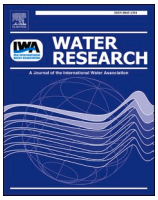

Urban wastewater-based epidemiology for multi-viral pathogen surveillance in the Valencian region, Spain

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ABSTRACT

Wastewater-based epidemiology (WBE) has lately arised as a promising tool for monitoring and tracking viral pathogens in communities. In this study, we analysed WBE's role as a multi-pathogen surveillance strategy to detect the presence of several viral illness causative agents. Thus, an epidemiological study was conducted from October 2021 to February 2023 to estimate the weekly levels of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), Respiratory Syncytial virus (RSV), and Influenza A virus (IAV) in influent wastewater samples (n = 69). In parallel, a one-year study (October 2021 to October 2022) was performed to assess the presence of pathogenic human enteric viruses. Besides, monitoring of proposed viral fecal contamination indicators crAssphage and Pepper mild mottle virus (PMMoV) was also assessed, along with plaque counting of somatic coliphages. Genetic material of rotavirus (RV), human astrovirus (HAStV), and norovirus genogroup I (GI) and GII was found in almost all samples, while hepatitis A and E viruses (HAV and HEV) only tested positive in 3.77 % and 22.64 % of the samples, respectively. No seasonal patterns were overall found for enteric viruses, although RVs had a peak prevalence in the winter months. All samples tested positive for SARS-CoV-2 RNA, with a mean concentration of 5.43 log genome copies per liter (log GC/L). The tracking of the circulating SARS-CoV-2 variants of concern (VOCs) was performed by both duplex RT-qPCR and next generation sequencing (NGS). Both techniques reliably showed how the dominant VOC transitioned from Delta to Omicron during two weeks in Spain in December 2021. RSV and IAV viruses peaked in winter months with mean concentrations 6.40 and 4.10 log GC/L, respectively. Moreover, the three selected respiratory viruses strongly correlated with reported clinical data when normalised by wastewater physico-chemical parameters and presented weaker correlations when normalising sewage concentration levels with crAssphage or somatic coliphages titers. Finally, predictive models were generated for each respiratory virus, confirming high reliability on WBE data as an early-warning system and communities illness monitoring system. Overall, this study presents WBE as an optimal tool for multi-pathogen tracking reflecting viral circulation and diseases trends within a selected area, its value as a multi-pathogen early-warning tool stands out due to its public health interest.

1. Introduction

Advances in molecular and next-generation sequencing techniques have revolutionised the analysis of viral genomes from wastewater samples, offering real-time insights into the transmission of infectious diseases among populations (Guo et al., 2022; Gonçalves et al., 2022). In this context, Wastewater-based Epidemiology (WBE) has become a powerful tool for multi-pathogen surveillance, most notably for detecting and tracking COVID-19 and other emerging infectious diseases (Mandal et al., 2020).

Traditionally, WBE has been used to detect and track enteric viruses such as poliovirus (PV), noroviruses genogroup I (HuNoV GI) and GII

Abbreviation list: Wastewater-based Epidemiology, (WBE); Norovirus genogroup I, (HuNoV GI); Norovirus genogroup II, (HuNoV GII); Rotavirus, (RV); Astrovirus, (HAstV); Hepatitis A virus, (HAV); Hepatitis E virus, (HEV); Influenza virus, (IAV); Respiratory syncytial virus, (RSV); Porcine epidemic diarrhea virus, (PEDV); Pepper Mild Mottle Virus, (PMMoV); Valencian Region, (CV); Variant of Concern, (VOC); Mean standard error, (% MSE).

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(HuNoV GII), rotaviruses (RVs), human astroviruses (HAstVs), and hepatitis A (HAV) and E viruses (HEV), which are major causes of infectious diseases, such as poliomyelitis, gastroenteritis and hepatitis, and can have a significant impact on public health, particularly among vulnerable populations like young children and the elderly (Prevost et al., 2015; Hirose et al., 2016; Cuevas-Ferrando et al., 2022). As clinical surveillance for most human enteric viruses, influenced by epidemiological, administrative, and financial constraints is very restricted, WBE testing is for instance a well-established tool for poliovirus surveillance and outbreak response (WHO, 2003; Hovi et al., 2005; Ndiaye et al., 2014; Kim et al., 2016).

The virus responsible for COVID-19, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has profoundly impacted global health and economy and its early detection and tracking is crucial to control its spread and mitigate its effects. WBE has been proven effective in detecting the presence of SARS-CoV-2 in wastewater, with results often preceding clinical cases by several days to weeks (Randazzo et al., 2020; Ahmed et al., 2020; La Rosa et al., 2020; Medema et al., 2020; Wurtzer et al., 2020; Wu et al., 2020). Additionally, the ongoing spread and evolution of the virus has led to the appearance of several variants of interest and concern, including Alpha, Beta, Gamma, Kappa/Delta, and Omicron, which can impact the transmissibility, disease severity, and the effectiveness of treatments and vaccines to varying degrees (Choi et al., 2021; Tao et al., 2021). Studies have shown that the prevalence of these variants in wastewater is correlated with clinical data, making early detection and monitoring of local variant spread in wastewater a complementary approach to genomic epidemiology based on individual patient samples (Pérez-Cataluña et al., 2021; Bar-Or et al., 2021; Crits-Christoph et al., 2021; Izquierdo-Lara et al., 2021; La Rosa et al., 2021; Rios et al., 2021; Carcereny et al., 2021; Lee et al., 2022; Haver et al., 2023).

During the COVID-19 pandemic, containment measures significantly reduced the impact of other respiratory viruses, such as influenza virus (IAV) and respiratory syncytial virus (RSV) (Chow et al., 2023). However, after the easing of COVID-19 lockdown restrictions, an increase in influenza and RSV infections has been observed, earlier than in pre-COVID years (Toribio-Avedillo et al., 2023; Hughes et al., 2022; Wolfe et al., 2022). RSV is estimated to cause 33 million cases and 66,000–199,000 deaths in children under five years old worldwide (Hall et al., 2009; Borchers et al., 2013; Shi et al., 2017; Langedijk et al., 2022), while influenza is estimated to result in 3 to 5 million severe illnesses and 290,000 to 650,000 respiratory deaths each year (Iuliano et al., 2018). The shedding of respiratory viruses such as RSV and influenza A in faeces has not been well documented yet, leading to limited use of WBE estimates for these viruses. However, it has been found that these respiratory viruses can be present and detectable in wastewater as stool is not the only source of human contribution (sputum, body secretions, etc.) to wastewater (Ahmed et al., 2023; Koureas et al., 2023; Hellmer et al., 2014).

The primary objective of this study was to gain insights into the epidemiology of respiratory and enteric viruses, particularly in the context of the COVID-19 pandemic, in the Valencian region (Spain). We monitored the levels of viruses, fecal indicators and physico-chemical parameters in wastewater samples and results were confronted with available clinical data to establish accurate and robust estimates of prevalence from wastewater, with the final aim of demonstrating WBE to be a valuable tool for multi-pathogen surveillance and support public health authorities to gain a comprehensive understanding of the spread of infectious diseases.

2. Materials and methods

2.1. Sampling site and sample collection

Grab influent wastewater samples were collected weekly from October 2021 to February 2023 from a wastewater treatment plant (WWTP) located in the Valencian region of Spain serving a total population of 321,622 individuals according to the Government of the autonomous community of Valencia (GVA) (data from 2022). Prevalence of enteric viruses in influent wastewater samples was assessed over one year (October 2021 to October 2022, $n = 53$) whilst respiratory viruses presence was analysed over a longer period (October 2021 to February 2023, $n = 69$) to better evaluate the impact of COVID-19 lockdown restrictions on the selected respiratory diseases. Samples were grabbed early in the morning (7–12 a.m.) by collecting ~500 mL of wastewater in sterile HDPE plastic containers (Labbox Labware, Spain). Collected samples were transferred on ice to the laboratory, kept refrigerated at 4 °C, and concentrated within 24 h.

2.2. Viral concentration method and nucleic acid extraction

Wastewater samples were inoculated with porcine epidemic diarrhea virus (PEDV) strain CV777 as a coronavirus model process control. Two hundred milliliters of wastewater samples were concentrated with the aluminium-based adsorption-precipitation method (Randazzo et al., 2020; AAVV, 2018). In brief, 200 mL of sample were transferred into 250 mL PPCO centrifuge bottles (Thermo Fisher Scientific, Rochester, US) and artificially inoculated with PEDV. Then, pH was adjusted to 6.0 and $Al(OH)_3$ precipitate formed by adding 1 part 0.9 N $AlCl_3$ (Acros organics, Geel, Belgium) solution to 100 parts of sample. The pH was readjusted to 6.0 and sample mixed using an orbital shaker at 150 rpm for 15 min at room temperature. Then, viruses were concentrated by centrifugation at 1700 × *g* for 20 min and the pellet was resuspended in 10 mL of 3 % beef extract pH 7.4, transferred in 50 mL PPCO centrifuge tubes and shaken for 10 min at 150 rpm. Concentrate was recovered by centrifugation at 1900 × *g* for 30 min and pellet resuspended in 1 mL of PBS.

Nucleic acid extraction from wastewater concentrates was carried out using the Maxwell® RSC Instrument (Promega, Spain) with the Maxwell RSC Pure Food GMO and authentication kit (Promega) and the "Maxwell RSC Viral total Nucleic Acid" running program (Pérez-Cataluña et al., 2021).

2.3. SARS-CoV-2 detection and quantification

SARS-CoV-2 quantification was achieved by targeting the N1 region of the nucleocapsid gene. The One Step PrimeScript™ RT-PCR Kit (Perfect Real Time Takara Bio, USA) was used with N1 primers and conditions described by the Centers for Disease Control and Prevention (CDC, 2019).

The prevalence of SARS-CoV-2 Alpha, Delta, and Omicron variants (BA.1 and BA.2) was assessed using five different duplex TaqMan RT-qPCR procedures, with primers targeting the S and N genes - UK/Alpha (S:69/70del), Delta (S:157/158del), Omicron BA.1 (S:214insEPE, S:69/70del), and Omicron BA.2 (S:25/27del) - and a mastermix prepared using the One-Step PrimeScript RT-PCR Kit as previously (Carcereny et al., 2022, 2023).

Each RT-qPCR analysis included duplicate wells with undiluted RNA and a 10-fold dilution to check for inhibition, as well as corresponding negative controls (amplification and extraction) on the LightCycler 480 instrument (Roche Diagnostics, Germany). Standard curve used for SARS-CoV-2 genome quantitation was prepared using commercially available Twist Synthetic SARS-CoV-2 RNA Control (Control 2, MN908947.3) targeting N1. For variant-characteristic specific mutations quantification, Twist Synthetic SARS-CoV-2 RNA Controls 14 (B.1.1.7 lineage), 48 (B.1.1.529, BA.1 lineage), and 50 (B.1.1.529, BA.2 lineage) (Twist Bioscience HQ, USA) were used for Alpha and Omicron variants quantification, respectively. SARS-CoV-2 B1.617.2 (Delta) complete genome was used as Delta variant control (EPI_ISL_2,967,855, Vircell, Spain).

To estimate SARS-CoV-2 gene viral titers, Cq values ≤ 40 were converted into genomic copies per liter using the standard curve and

volumes tested. Inhibition was assessed by comparing average viral titers from duplicate wells of undiluted RNA and 10-fold diluted RNA. Inhibition was confirmed when the difference in average viral titers was $> 0.5 \log_{10}$, and viral titers were inferred from the 10-fold RNA dilution. The relative presence of each SARS-CoV-2 genomic variant was calculated according to Carcereny et al., 2023. Primers and probes, amplification conditions, and limits of quantification and detection for each genomic target detected in this work is available in the Table S3.

2.4. Detection and quantification of other respiratory, enteric, and faecal indicator viruses

Levels of HuNoV GI and GII, HAstV, RV, HAV and HEV were determined using the RNA UltraSense One-Step kit (Invitrogen, USA) as previously described by Cuevas-Ferrando et al. (2022). Detection of crAssphage was established using the qPCR Premix Ex Taq™ kit (Takara Bio Inc, USA) using primers and conditions described by Stachler et al. (2017). Viral detection of PEDV was performed by RT-qPCR using the One Step PrimeScript™ RT-PCR Kit (Perfect Real Time) (Takara Bio Inc., USA) as described by Puente et al. (2020). Amplification of Pepper Mild Mottle Virus (PMMoV) was determined by using the PMMoV Fecal Indicator RT-qPCR Kit (Promega) following manufacturer's instructions. Viral detection of Influenza A virus was determined using primers from CDC (Research Use Only CDC Influenza SARS-CoV-2 "Flu SC2" Multiplex Assay Primers and Probes) and as described by CDC (protocol of realtime RTPCR for influenza A(H1N1)) by using PrimeScript RT-PCR kit (Takara Bio Inc.) (Sanghavi et al., 2012). RSV detection was performed as previously described (Sanghavi et al., 2012).

Different controls were used in all assays: negative process control consisting of PBS; whole process control to monitor the process efficiency of each sample (spiked with PEDV); and positive (reference material) and negative (RNase-free water) RT-qPCR controls.

Synthetic gBlock gene fragments (Integrated DNA Technologies, Inc., USA) comprising the PCR targeted regions of crAssphage, HuNoV GI and GII, HAstV, RV, HAV, HEV were used to prepare standard curves for quantification. For IAV and RSV quantification, Twist Synthetic InfluenzaV H1N1 RNA control (part number: 103,001) and purified RNA of RSV (Vircell, S.L) were used. PMMoV Fecal Indicator RT-qPCR Kit (Promega) provided PMMoV RNA for generating a standard curve.

2.5. SARS-CoV-2 sequencing

A monthly sample, selected for presenting RT-qPCR results for N1 with Ct<32, was used for SARS-CoV-2 genomic sequencing. Genomic amplification was performed using the primer scheme of the Artic protocol v3 for samples between October 2021 and January 2022, and v4 for the other months (https://github.com/artic-network/artic-ncov2019/tree/master/primer_schemes/nCoV-2019). Amplicon libraries were built following the Classic PCR tilling protocol and sequenced using the MinION Mk1C system (Oxford Nanopore Technologies, Oxford, UK) (Quick et al., 2020). After sequencing, basecalling was performed with guppy software (Oxford Nanopore) with the high accuracy algorithm using a cut-off value of 8 for base quality (Quick et al., 2020). Obtained reads were analysed with Freyja software (https://github.com/andersen-lab/Freyja) using default parameters.

2.6. Coliphage quantification

Five mL of sewage sample were filtered through sterile 0.45 μm pore syringe filters (Labbox Labware, S.L., Spain) to remove bacteria and fungus (Toribio-Avedillo et al., 2021; Nappier et al., 2006). Quantification was performed by using a commercial Bluephage Easy Kit for Enumeration of Somatic Coliphages (BLUEPHAGE S.L., Spain), following the manufacturer's instructions.

2.7. Data collection, normalisation, and statistical analyses

Clinical data for COVID-19, IAV, and RSV cases was obtained from the "Sentinel Surveillance of Acute Respiratory Infection in Primary Care and Hospitals" (SiVIRA) yearly reports, provided by the Microbiological surveillance network of the Valencian Region (MIVA network). SiVIRA-CV is the Acute Respiratory Infection (ARI) Surveillance System in primary care and hospitals in the Valencian Region (CV). In primary care, it is based on syndromic surveillance that provides information on the weekly incidence of ARI through the weekly cases of ARI diagnosed in primary care consultations, through the Ambulatory Information System (SIA) and the Epidemiological Surveillance Analysis (AVE) system. It also includes sentinel surveillance of a selection of patients with ARI, in which primary care family doctors and pediatricians record clinical-epidemiological data and collect a sample for virologic analysis of influenza, SARS-CoV-2 and RSV in the microbiology laboratories. In hospitals, severe ARI clinical processes correspond to hospitalised patients obtained through the ALUMBRA platform (Corporate Analysis Platform of the Regional Ministry of Health).

For Spearman's correlation performance, respiratory viruses levels (GC/L) were normalised by both fecal viral indicators' quantification data (crAssphage, PMMoV, and somatic coliphages) and physicochemical water samples properties (water inflow, chemical oxygen demand, biochemical oxygen demand, phosphorus, total nitrogen, solids in suspension, and electric conductivity). Physico-chemical parameters for each sample were provided by the WWTP. Spearman correlations were carried out between the unshifted time series (each clinic data with its time corresponding virus levels in wastewater) and shifting the data in time by up to 5 weeks before and after wastewater sampling dates.

Predictive models were implemented with the Random Forest algorithm using the R package *randomForest*, known for its intrinsic explainability, robustness to outliers, stability with new data, and adeptness in handling non-linear correlations (Liaw and Wiener, 2002; Keyel et al., 2019; Koureas et al., 2021; Singh et al., 2023; Marin-Ramirez et al., 2024). For each respiratory virus, the data were separated into two sets, "training" ($n = 40$) and "test" ($n = 29$), to fit the models and to have a "test" measure to compare results with. Ideally, the two sets should have exactly the same distribution of the data, so we found this separation to be sufficient. Thus, the same procedure was repeated five times so that the model metrics were the mean of the 5 training runs as a cross-validation method to avoid possible induced biases due to the random splits of the training and test data sets and to assess the performance of the method in a more robust way (Fig. S2).

3. Results

3.1. Occurrence and evolution of viruses detected in wastewater

Weekly influent wastewater samples from a WWTP located in the Valencian region (Spain) were processed by (RT)-qPCR to determine the occurrence and levels of respiratory viruses (SARS-CoV-2, RSV, and IAV), enteric viruses (HuNoVs GI and GII, HAstVs, RVs, HAV, and HEV), and proposed viral fecal indicators crAssphage and PMMoV (Fig. 1) (Table S1). Somatic coliphage quantification were assessed by plaque counting. The recoveries of PEDV, spiked as viral process control, ranged between 6.78 and 49.63 % (data not shown); thus, results of targeted viruses were validated according to Haramoto et al. (2018) and the criteria included in the ISO 15216–1:2017 (recovery of control virus ≥1 %).

Enteric viruses prevalence in wastewater was analysed over one year (October 2021 to October 2022, $n = 53$) (Fig. 1). The detection rates of HuNoV GI, HuNoV GII, HAstVs, and RVs were 98.11, 100, 100, and 92.45 %, respectively. In contrast, HAV and HEV were only detected in 3.77 % and 22.64 % of the samples, respectively. As for the mean concentrations from positive samples for each virus, the highest values were those of RV (7.95 log GC/L), HAstV (7.00 log GC/L), and HuNoV GII

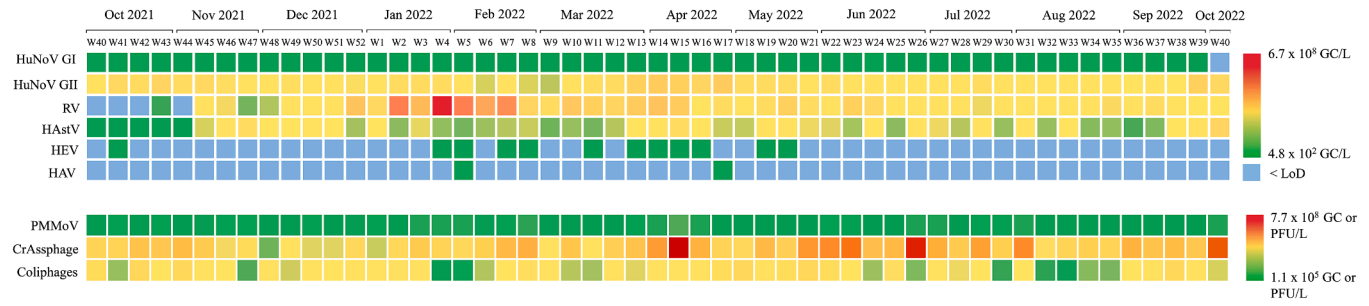


Fig. 1. Levels of enteric viruses and faecal viral indicators (GC/L or PFU/L) in wastewater samples from a single WWTP in the Valencian Region from October 2021 to October 2022. < LoD: Below limit of detection.

(7.58 log GC/L), while the lowest values were for HuNoV GI (4.41 log GC/L), HAV (3.05 log GC/L) and HEV (3.64 log GC/L). Notably, the mean concentration of HuNoV GII was three logarithms higher than that of HuNoV s GI. In terms of distribution, a higher presence of rotavirus was observed in winter (Fig. 1).

As regards the viruses proposed as indicators of fecal contamination, crAssphage and PMMoV were detected in all samples from October 2021 to February 2023 ($n = 69$), while somatic coliphages were also present in all analysed samples from October 2021 to October 2022 ($n = 53$) (Fig. 1). Among the three proposed indicators, mean crAssphage concentration levels were the highest (8.09 log GC/L), being almost three logarithms higher than those of PMMoV (5.71 log GC/L). The plaque count of somatic coliphages yielded a mean concentration value of 7.28 log PFU/L.

In parallel to enteric viruses and viral fecal indicators monitoring, wastewater samples were tested for respiratory viruses over a longer period covering from October 2021 to February 2023 (Table S2). SARS-CoV-2 RNA was detected in all samples tested ($n = 69$) (Fig. S1and Fig. 4A. In contrast, RSV and IAV genetic material was only found in 39.13 % (27/69) and 31.88 % (20/69) of the wastewater samples analysed, respectively (Fig. S1, Figs. 5A, 6A). Regarding the monthly distribution of these viruses, the detection of SARS-CoV-2 in wastewater was fairly constant throughout the entire period analysed, while RSV was detected more in winter and IAV presented a very pronounced peak between March and April 2022. Quantification of SARS-CoV-2 targeted RNA yielded mean concentration levels of 5.43 log GC/L. When testing positive, IAV and RSV mean concentration values in wastewater samples were 6.42 log GC/L and 3.88 log GC/L, respectively.

3.2. *Detection of SARS-CoV-2 variants of concern by molecular and sequencing techniques*

All wastewater samples collected weekly from October 2021 to February 2023 were screened using duplex RT-qPCR to detect the prevalence of specific variants of concern (VOC), namely UK/Alpha (S:69/70del), Delta (S:157/158del), and Omicron (S:69/710del,

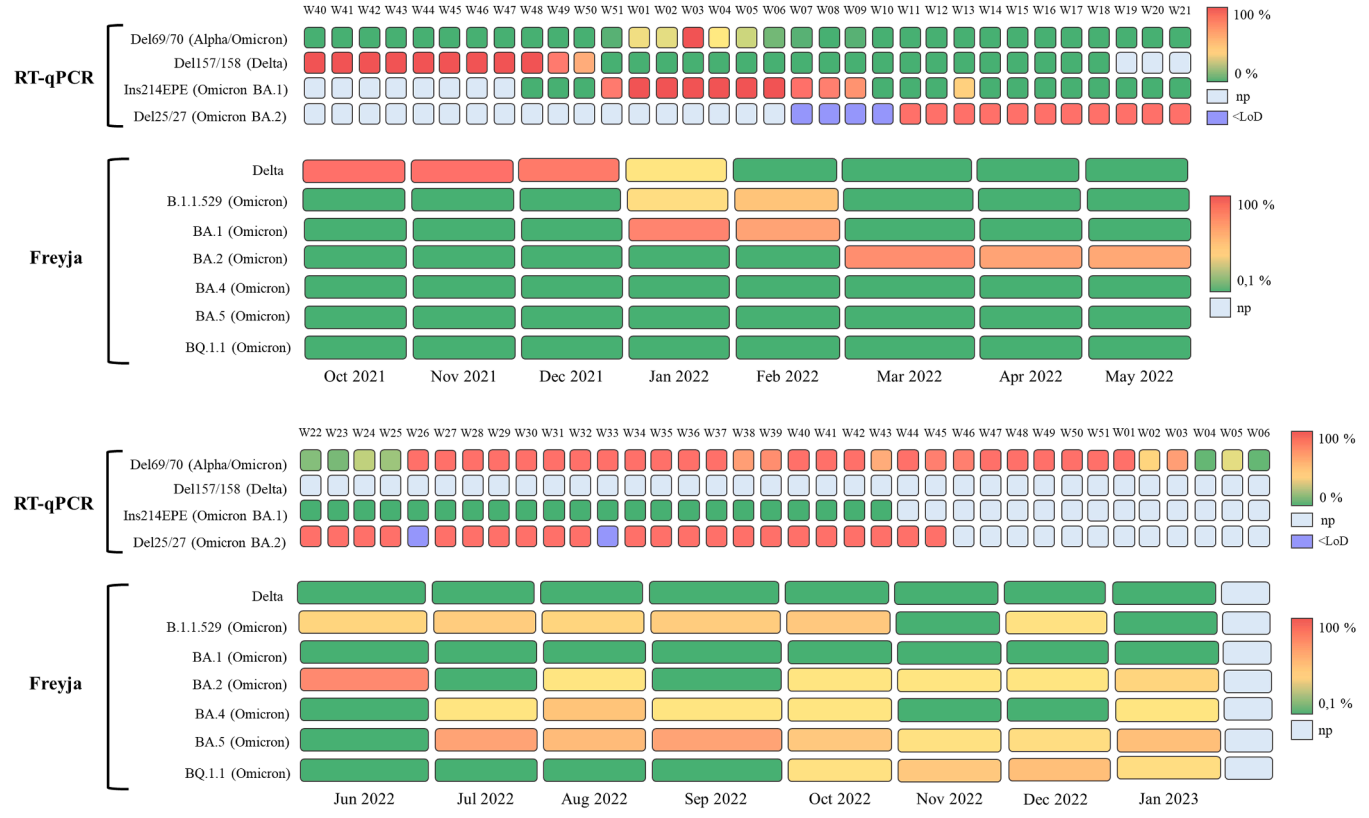


Fig. 2. Percentage of SARS-CoV-2 variants of concern in wastewater detected by duplex RT-qPCR and sequencing analysis with Freyja from October 2021 to February 2023. Np: not performed; < LoD: below the limit of detection.

S:214insEPE, and S:25/27del) (Fig. 2). In addition, these samples were sequenced with an Oxford Nanopore MinION system and analysed using Freyja software, a tool created to recover relative lineage abundances from mixed SARS-CoV-2 samples from a sequencing dataset (Fig. 2).

Duplex RT-qPCR results showed a 100 % prevalence of the Delta variant between weeks 40 and 48 of 2021. Then, between weeks 49 (W49) and 50 (W50, December) of 2021 a reduction in the prevalence of Delta was observed (W49: 90 %, W50: 50 %). Finally, in week 51, the imposition of the Omicron variant (S:214insEPE) was already observed, with a prevalence of 91 %. From week 12 of 2022, the S.25/27del characteristic of Omicron variant BA.2 was imposed. The S:69/70del, associated with Alpha VOC at that time, was not detected during the Delta VOC period but could be found again in the Omicron VOC majority period, as both Alpha and Omicron BA.1 share this mutation.

Freyja analysis included fewer samples as only one sample with sufficient genomic load for efficient sequencing was processed per month. Even so, the results were consistent with those obtained by duplex RT-qPCR. In week 48 of 2021 a slight decrease in the prevalence of the Delta variant was observed (from 100 % in previous weeks to 93.5 %) (Fig. 2) and in January 2022 a majoritarian prevalence of the Omicron variant was already observed, which extends throughout the following weeks of 2022. In line with duplex RT-qPCR results, Freyja analyses detected the emergence of Omicron BA.1 (Jan 2022 – Feb 2022), Omicron BA.2 (Mar 2022 – Jun 2022), and BA.5 (from Jul 2022) (Fig. 3). Also, the emergence of variant of concern BQ.1.1 on October 2022 could be efficiently detected by using Freyja software analyses (Fig. 3). Raw data regarding mutation prevalences can be found in supplementary table S4.

3.3. Correlation of levels of respiratory viruses in wastewaters with public health records and evaluation of normalising factors suitability

RNA quantification data of SARS-CoV-2, IAV, and RSV in wastewater were normalised with somatic coliphages quantification data, PMMoV, crAssphage, and various physico-chemical parameters (chemical and biological oxygen demand, total nitrogen, phosphorus, inflow, electrical conductivity) and Spearman's correlation was calculated with reported clinical cases of infections caused by the aforementioned respiratory viruses. Data from October 2021 to February 2023 were used. Moreover, Spearman's correlations were made with clinical case data reported the same day of sewage sampling (week delay 0) as well as from previous and subsequent weeks (week delay −5 to 5).

Regarding SARS-CoV-2 correlations, COVID-19 cases in Spain were no longer reported from April 2022 in patients below 65 years. Thus, three different correlation studies were performed: a) using all available data from October 2021 to February 2023; b) using only data prior to April 2022 (Fig. 4); or c) using data collected after April 2022. It was observed that in analyses a) and c) the correlations between SARS-CoV-2 RNA and reported clinical cases were neutral or very weak, so they were not included in this work due to its irrelevance. On the contrary, analysis b) (when all clinical cases during the pandemic were being reported) resulted in extraordinarily high ($r > 0.8$, p-value < 0.05) correlations (Fig. 4B). Furthermore, correlations were slightly stronger or equal when comparing virus quantification data in wastewater with clinical case data from the week following water sampling date (delay = 1) compared to data from the same week (delay = 0). Moreover, normalising the wastewater data with microbiological or physico-chemical parameters showed that the weakest correlations were obtained when adjusting RNA quantification data with coliphage counts ($r = 0.434$, p-value < 0.05, delay = 0) and crAssphage RNA levels ($r = 0.572$, p-value < 0.05, delay = 0). Notably, the strongest correlation was obtained by applying no delay and adjusting obtained virus genomic copies per litre with WWTP inflow (m^3/day) ($r = 0.866$, p-value < 0.05), even though, in general terms, correlations were slightly stronger when applying a one-week delay.

As for Influenza virus, strong Spearman's correlations ($r > 0.7$) were observed between IAV genomic copies in wastewater and reported clinical cases (Fig. 5B). Interestingly, the strongest correlations were observed when comparing virus quantification data in wastewater with clinical cases data from the week following water sampling (delay 1). The correlation of unadjusted IAV genomic copies per litre with clinical cases was $r = 0.799$ (p-value < 0.05, delay = 1). Additionally, after normalising the wastewater data with microbiological or physico-chemical parameters, it was found that the lowest correlations were

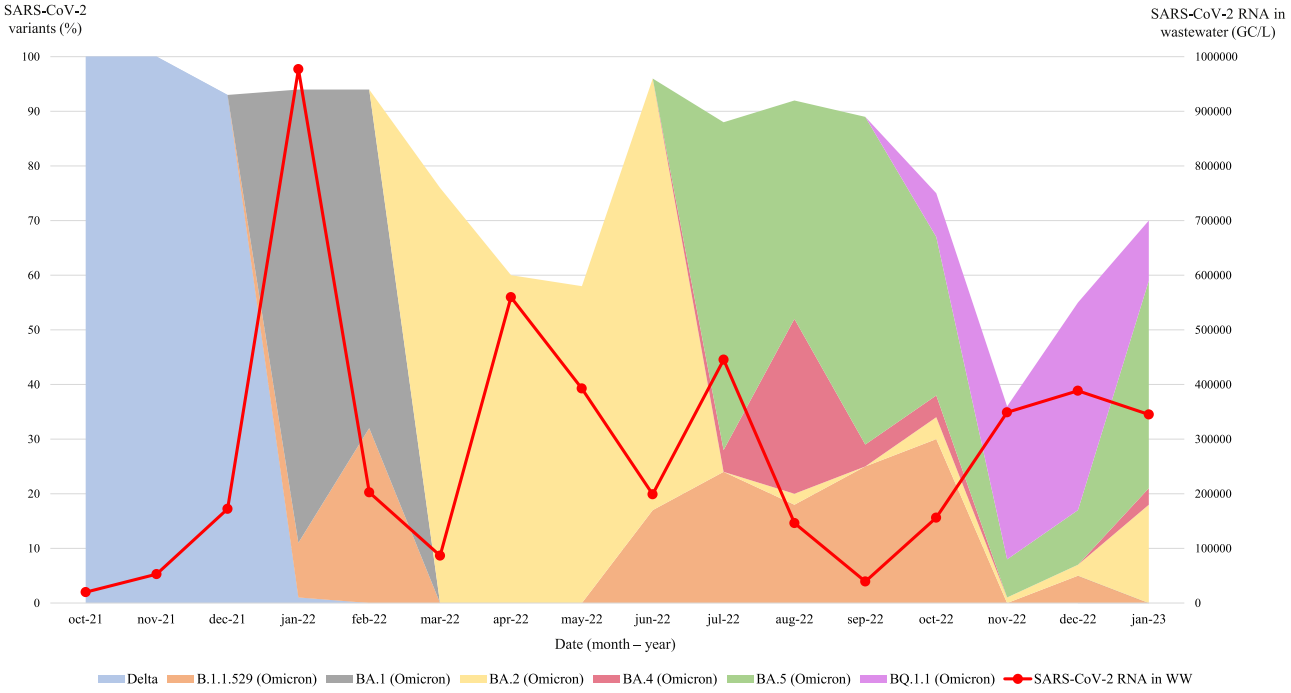


Fig. 3. RT-qPCR results for SARS-CoV-2 RNA levels in influent wastewater samples (red line) and percentage of variants of concern obtained via sequencing analysis with Freyja software from October 2021 to January 2023.

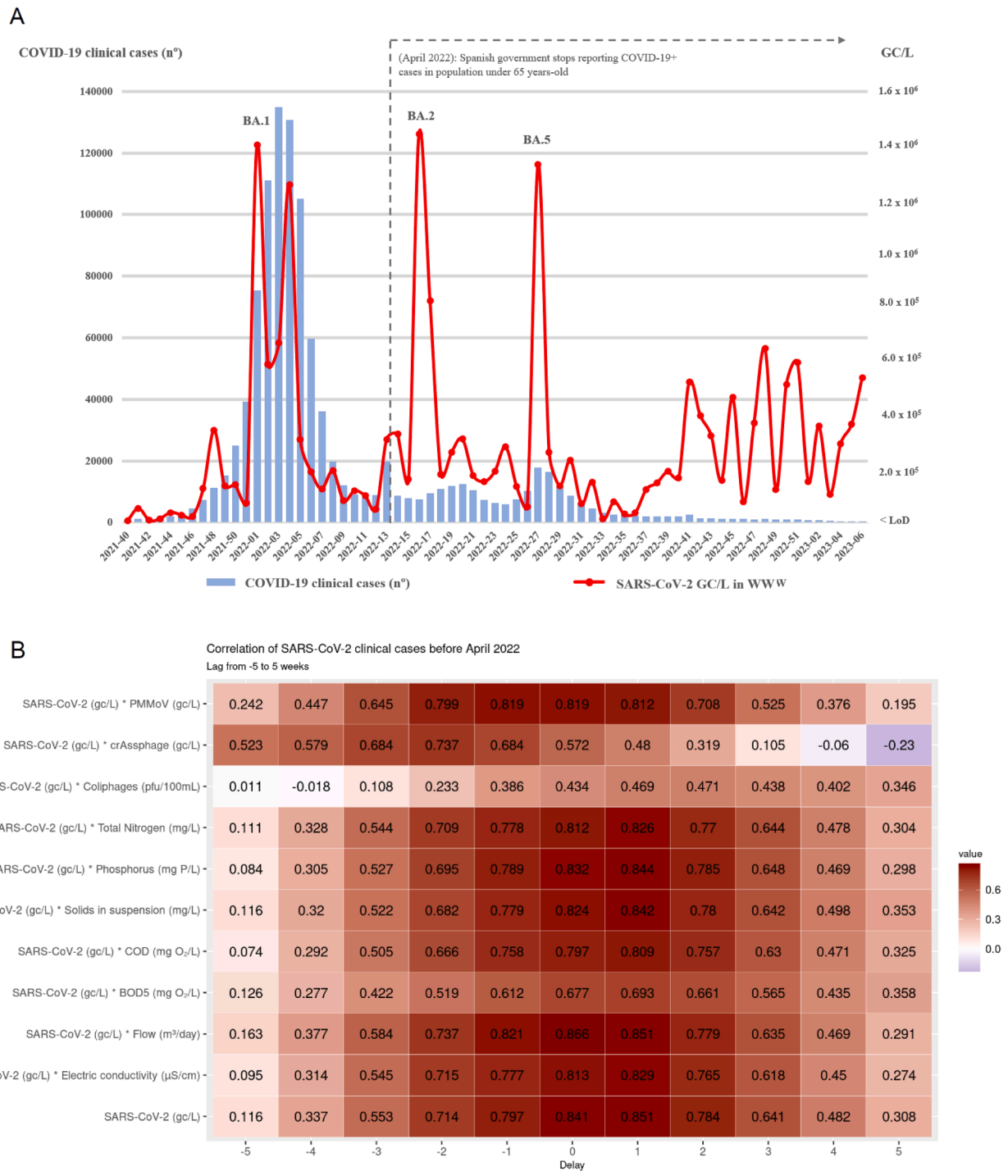


Fig. 4. A) Representation of COVID-19 clinical cases (blue) and SARS-CoV-2 RNA quantification data (GC/L) (red) in wastewaters; B) Spearman's Correlation analysis between COVID-19 clinical cases and SARS-CoV-2 RNA quantification data in wastewater covering the period before April 2022. < LoD: below the limit of detection.

obtained when adjusting water PFU/L of coliphages, while the strongest correlation ($r = 0.808$, p-value < 0.05) was obtained by applying a one-week delay and adjusting virus quantification data with WWTP inflow (m^3/day). Furthermore, in order to estimate the minimum viral concentration in sewage to detect clinical cases in the Valencian region, the relationship between the number of clinical cases on the week following sewage samples collection of IAV and the detection rate of its RNA in wastewater samples was analysed. Those weeks with less than 110 clinical cases reported resulted in 0 % positive wastewater samples for IAV RNA ($n = 33$). As the number of clinical cases increased to a range of 110 to 205 cases per week ($n = 12$), the proportion of positive wastewater samples for IAV RNA significantly increased to 44.5 %. Notably, when the clinical cases surpassed 205 ($n = 22$), all wastewater samples tested positive for IAV RNA, indicating a 100 % detection rate.

In the case of RSV, moderate correlations ($r > 0.6$, p-value < 0.05) were observed between RSV quantification data in wastewater and reported clinical cases without applying any delay to the latter (Fig. 6B). The correlation between unadjusted RSV GC/L and clinical cases was $r = 0.668$ (p-value < 0.05). Notably, the most robust correlations were observed when comparing virus quantification data in wastewater with

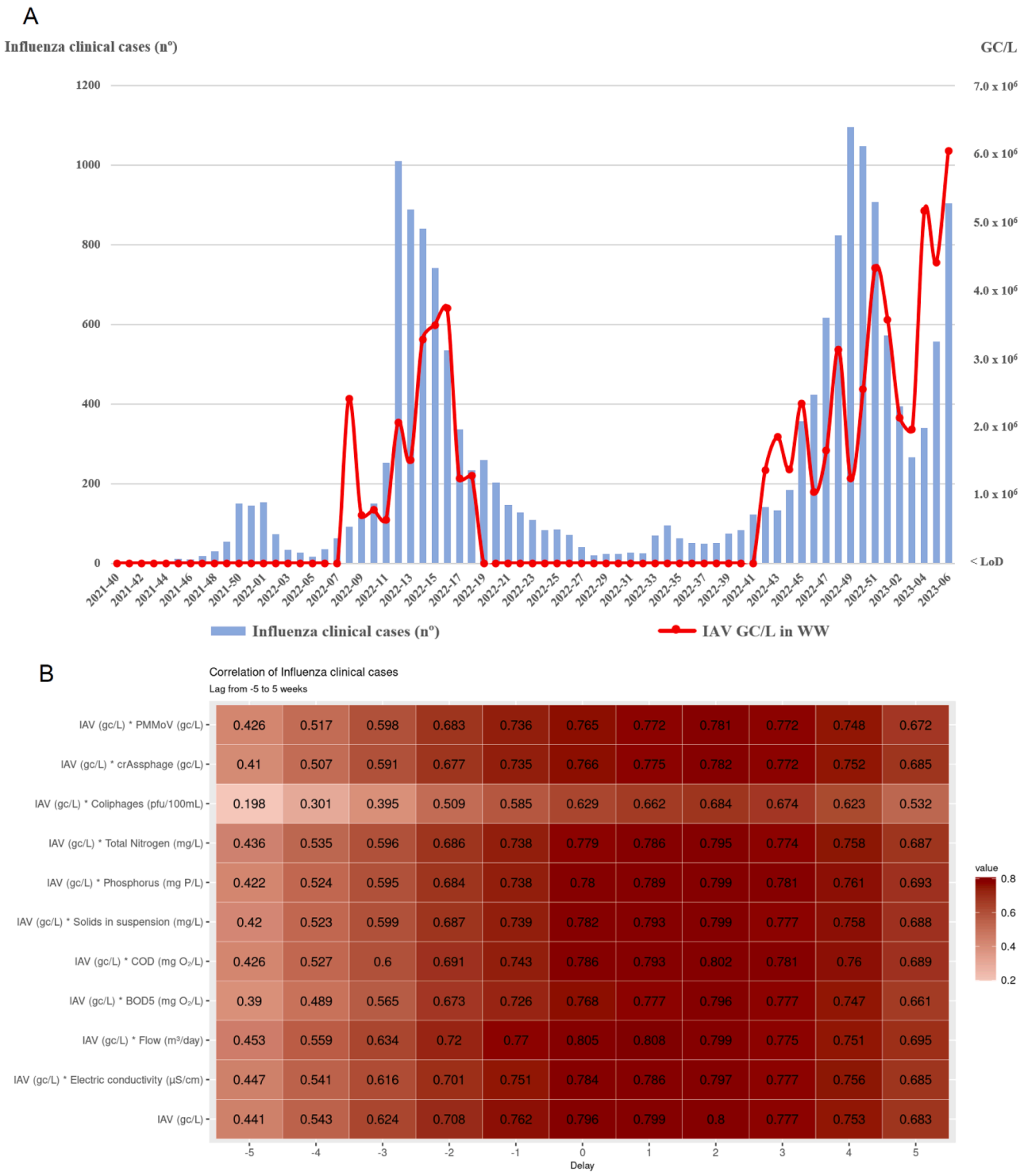


Fig. 5. A) Representation of reported Influenza virus positive clinical cases (blue) and IAV RNA quantification data (GC/L) (red) in wastewaters; B) Spearman's correlation analysis between Influenza virus positive clinical cases and IAV RNA quantification data from October 2021 to February 2023. < LoD: below the limit of detection.

clinical case data collected three weeks after water sampling (delay 3). Similar to the other analysed respiratory viruses, the weakest correlation was observed when adjusting water GC/L with the coliphages PFU/L data ($r = 0.53$, delay = 0) and the strongest correlation was obtained when applying a one-week delay on raw clinical cases data ($r = 0.706$, p-value < 0.05) and adjusting RSV RNA quantification data with the WWTP inflow (m^3/day) ($r = 0.705$, p-value < 0.05). Moreover, the minimum number of clinical cases required for RSV RNA to be detected in wastewater samples collected from the Valencian Region of Spain was estimated regarding clinical cases data of the third week following wastewater sample collection (which yielded the strongest correlations). When the weekly clinical cases were 40 or less ($n = 37$), only two positive wastewater samples for RSV RNA were observed, indicating a 5.4 % detection rate. Subsequently, as the number of clinical cases increased to a range of 40 to 210 per week ($n = 18$), half of the wastewater samples tested positive for RSV RNA, accounting for 50 %. Finally, when the

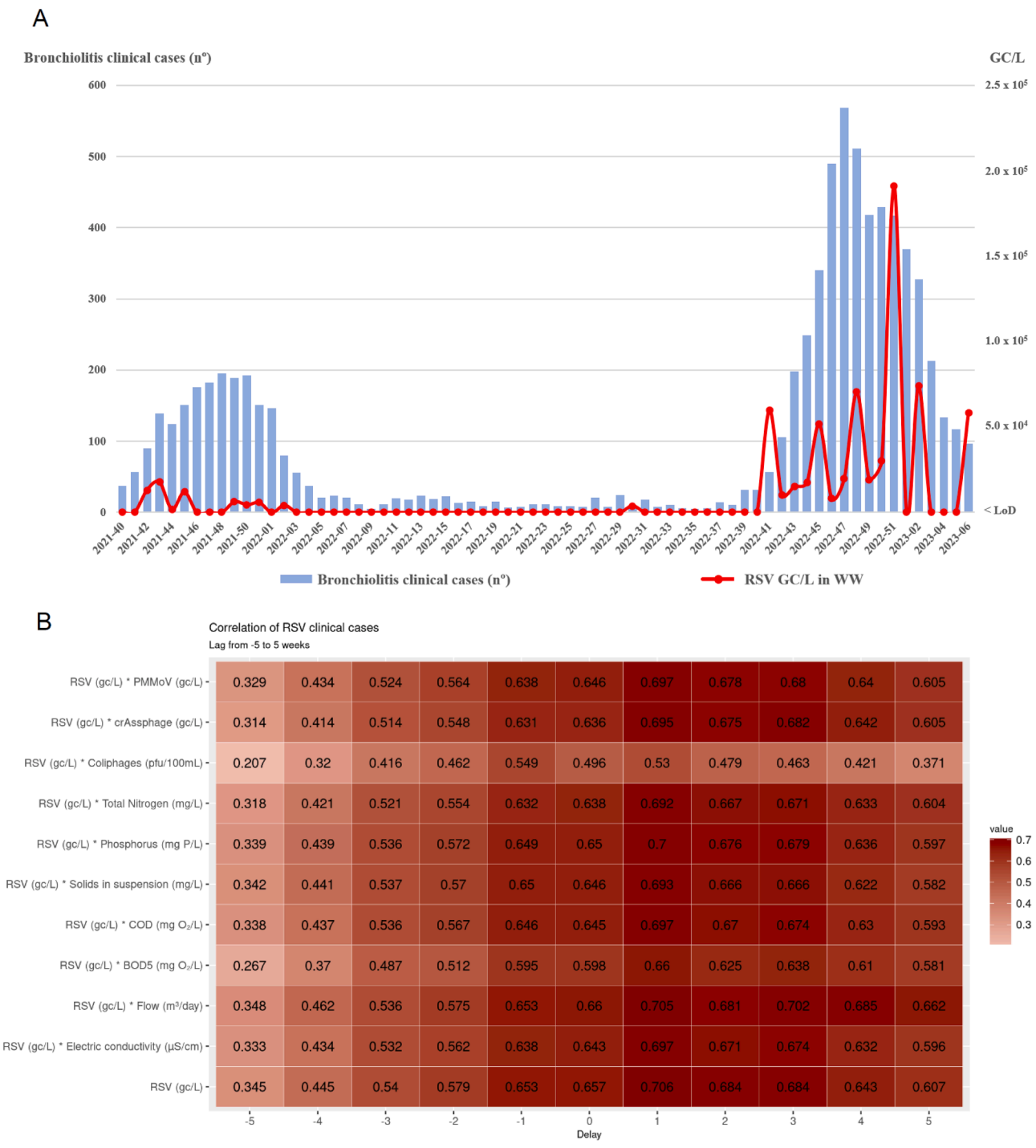


Fig. 6. A) Representation of reported bronchiolitis clinical cases caused by respiratory syncytial virus (RSV) (blue) and RSV RNA quantification data (GC/L) (red) in wastewaters; B) Spearman's correlation analysis between reported RSV positive clinical cases and RSV RNA GC/L in wastewaters from October 2021 to February 2023. < LoD: below the limit of detection.

number of clinical cases surpassed 210 (n = 11), all the wastewater samples tested positive for RSV RNA, indicating a 100 % detection rate.

3.4. Mathematical approximation for respiratory viral illnesses' cases prediction based on virus quantification data in sewage waters

In order to analyse the robustness of the correlations obtained in the previous section and to rule out the spurious nature of these correlations, a model for predicting clinical cases from the quantification of the genetic material of each virus in wastewater was performed by the Random Forest algorithm. For each respiratory virus, predictive models were performed. In the case of RSV and IAV, the clinical data were five-times randomly separated into two sets, "training" (n = 40) and "test" (n = 29), to fit the Random Forest models (RF1-RF5), while for SARS-CoV-2 clinical data were separated in "training" and "test" with a n = 12 each because of the limited data. Five different Random Forest models were run for each virus depending on data set selection: A) complete: with all the available data, both the standardised concentrations and the standardisation factors themselves; B) standardised: only including as variables the virus concentration values standardised according to

biological or physicochemical factors; C) standardised with delay: including the data with the delay that offered the best correlation with the clinical cases; D) standardised concentrations with and without delay; E) standardised concentrations together with the standardisation factors and with and without applying delay.

For SARS-CoV-2, the model with the least error in its estimates (mean error of 18,629.9 clinical cases) was the one that included the normalised virus concentrations and the number of clinical cases from the same week and the week after the wastewater sampling. It should be noted that because the model had to be modelled with few data (due to underreporting of all positive clinical cases), the model has a low reliability (Fig. 7).

In the case of IAV, the model that gave the least error in its estimates (mean error of 108.5 clinical cases) was the one that included the normalised virus concentrations and the number of clinical cases in the week after the wastewater samples were taken (Fig. 8). Furthermore, it was observed that the most relevant factor when introducing error in the model was the one composed of the IAV quantification data corrected for flow rate; modifying or removing this parameter increases the error in the model predictions by 4 %.

With regard to RSV, the model that gave the least error in its estimates (mean error of 52.3 clinical cases) was the one that included the values of all the normalised virus concentrations, the values of the normalisation factors themselves, and the number of clinical cases in the same week and in the third week after wastewater sampling (Fig. 9). In addition, the most important factors introducing error into the composite model were the unnormalised RSV GC/L values themselves, followed by normalisation with crAssphage and flow rate (Fig. 9). Finally, even if the models demonstrate the ability to predict clinical cases, they may not be fully functional given the limited dataset size, but serve well to perform an exploratory analysis of all the data variables. It would be necessary to collect more data from more years and with more frequency to have highly-reliable predictive models, but this study demonstrates its viability as well as describing which variables would be the most interesting when implementing these models in subsequent works.

4. Discussion

In this study, we have undertaken a multifaceted analysis centered around three key objectives. Initially, the prevalence and temporal patterns of human pathogenic enteric and respiratory viruses within wastewater samples were assessed. Subsequently, duplex RT-qPCR and Freyja sequencing approaches were performed to identify SARS-CoV-2 Variants of Concern. Finally, the alignment of our findings with public health records was explored, while also evaluating diverse indicators for the normalisation of data. These interrelated investigations collectively contribute to a comprehensive understanding of viral dynamics in wastewater, their implications for public health, and the methodological nuances essential for accurate data interpretation.

4.1. Occurrence and trends of human pathogenic viruses detected in wastewater

Overall, the results obtained for the prevalence of enteric viruses in wastewater are in line with existing literature (Cuevas-Ferrando et al., 2022; Kitajima et al., 2014; La Rosa et al., 2010; Haramoto et al., 2018). Prevalence of 100 % or nearly 100 % was observed for RVs, HAstVs and HuNoV GI and GII. Notably, the mean concentration of HuNoV GI was three logs GC/L lower than that of the GII genotype. HAV and HEV, on the other hand, were only detected in 3.77 % and 22.64 % of the samples analysed, respectively, with average concentrations of ~3 logs GC/L. In general, these results agree with those described in Corpuz et al., 2020 review paper but show lower HAV detection rates than those recently described in Argentina (Fantilli et al., 2023). However, the HuNoV GI concentrations obtained in this work (~4 logs GC/L) are much lower than those included in the aforementioned review (9 logs GC/L), and would be more in line with the results of other published works (Farkas et al., 2018).

Regarding the distribution of enteric viruses, only a slight increase in the concentration of RVs was noted during the winter months. As in other studies, despite the reported seasonality of infections caused by this type of viruses, specially RVs (which are more common in winter),

	Data set				
	A	B	C	D	E
Training	6559.9	6691.9	6,462.7	5,617.3	6,844.4
Test	23,367.9	21,344.0	21,701.0	18,629.8	20,453.5
Training %OM	0.191	0.195	0.188	0.164	0.2
Test %OM	0.681	0.622	0.633	0.543	0.596

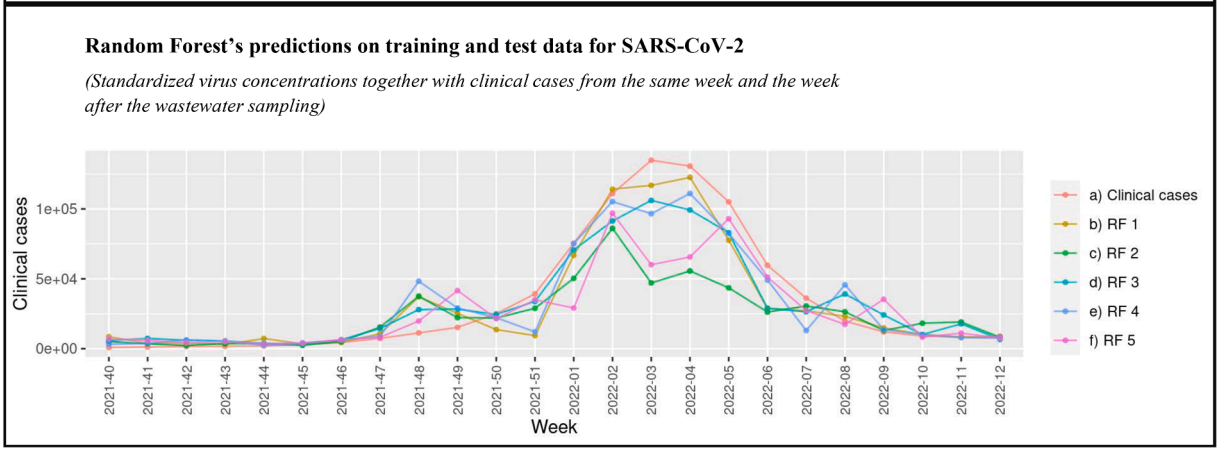
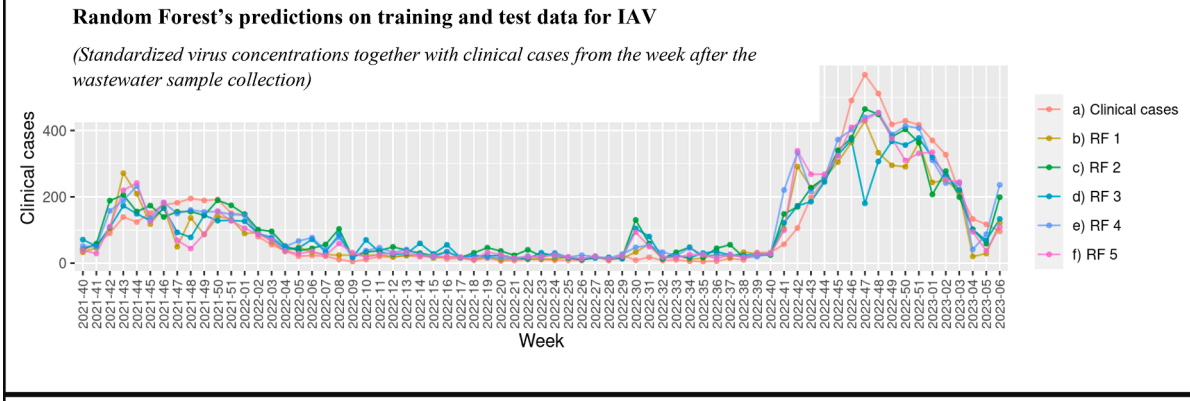


Fig. 7. Top: mean absolute error and its percentage over the mean of clinical cases for the "A-E" data sets; Bottom: Five Random Forest's predictions on training and test data for D) data set (standardised virus concentrations taking into account the number of clinical cases from the same week and the week after the wastewater sampling) for SARS-CoV-2 versus reported "Clinical cases" in the Valencian Region.

	Data set				
	A	B	C	D	E
Training	51.9	67.0	64.2	58.2	49.5
Test	125.6	130.4	108.5	110.6	110.9
Training %OM	0.215	0.277	0,266	0.241	0.205
Test %OM	0.52	0.54	0.449	0.458	0.459



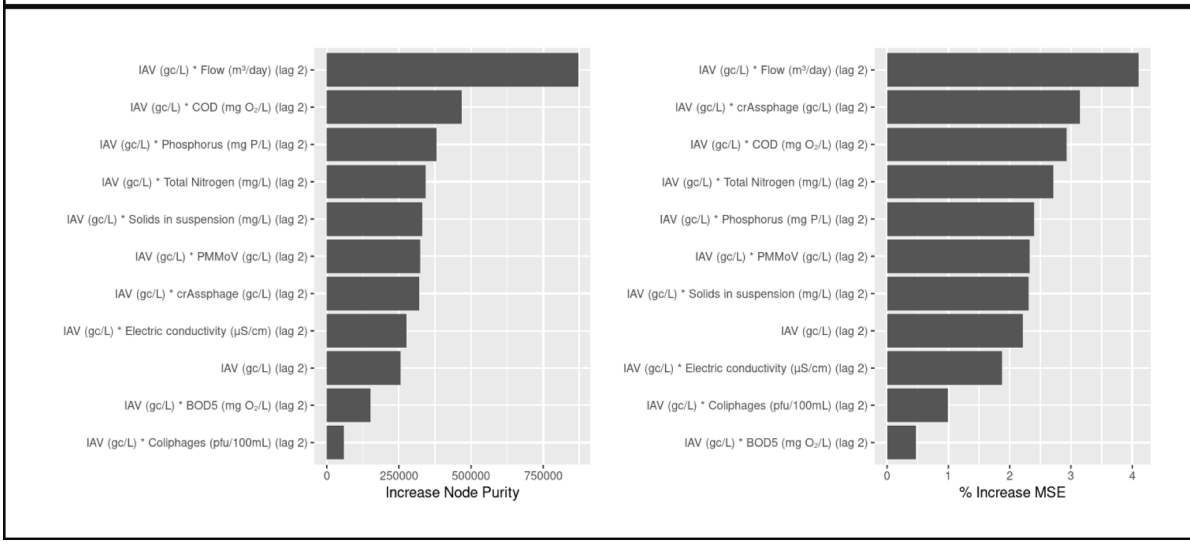


Fig. 8. Top: mean absolute error and its percentage over the mean of clinical cases for the "A-E" data sets; Middle: Random Forest's predictions on training and test data for C) data set (standardised virus quantification data and delayed clinical cases: including the data with the clinical cases from the week following wastewater sample collection) for IAV. Bottom: Importance of factors affecting Random Forest's predictions expressed as Increase in node purity and percentage increase on mean standard error (% MSE).

no marked seasonality was detected for any of them (Farkas et al., 2018; Barril et al., 2015). Elevated concentrations of RV were previously observed in Spain (Silva-Sales et al., 2020). RVs are recognized for causing illnesses in children below 5 years old, particularly in neonates up to 2 years old (WHO, 2013). Consequently, the majority of viruses are retained in diapers and do not enter the sewage system. This suggests that the identified RVs could potentially be affecting older children and adults, likely asymptomatic, and thus highly increasing the virus circulation (SantisoBellón et al., 2020). Also, the continuous detection of enteric viruses throughout the year highlights the intricate nature of their transmission and it is unlikely that weather is the sole cause for the widespread occurrence of enteric virus diseases during the winter season. Multiple studies suggest that factors such as the stability and ability of the virus to persist, alternative modes of transmission, the interaction between the virus and host, and changes in human behavior and susceptibility (including hygiene practices, birth rates, and the likelihood of exposure to the environment) could all contribute to the seasonal trend of enteric virus illnesses (Jagai et al., 2012; Atchison et al., 2009; Pitzer et al., 2011). Furthermore, it should be considered that the Mediterranean climate is characterised by less marked seasons, and with the current climate change scenario, the cold period is much shorter.

For respiratory viruses, IAV and RSV were detected at mean concentrations of 4 and 6 logs GC/L, respectively. These mean concentrations were similar to those reported in the US, Canada, and Australia for the same time period for IAV, and were at least one log higher for RSV (Toribio-Avedillo et al., 2023; Hughes et al., 2022; Wolfe et al., 2022; Ando et al., 2023; Mercier et al., 2022). In the case of RSV, peak concentrations concentrated in the winter months, while IAV had a moderate peak in winter and a much more pronounced peak in spring, as recently reported (Wolfe et al., 2022).

Regarding the prevalence and quantification data of SARS-CoV-2 in wastewater, results in this work are in concordance with many others

	Data set				
	A	B	C	D	E
Training	27.7	50.8	44.8	31.3	22.7
Test	69.1	76.9	62.9	53.3	52.3
Training %OM	0.253	0.464	0.41	0.286	0.208
Test %OM	0.632	0.702	0.575	0.487	0.478

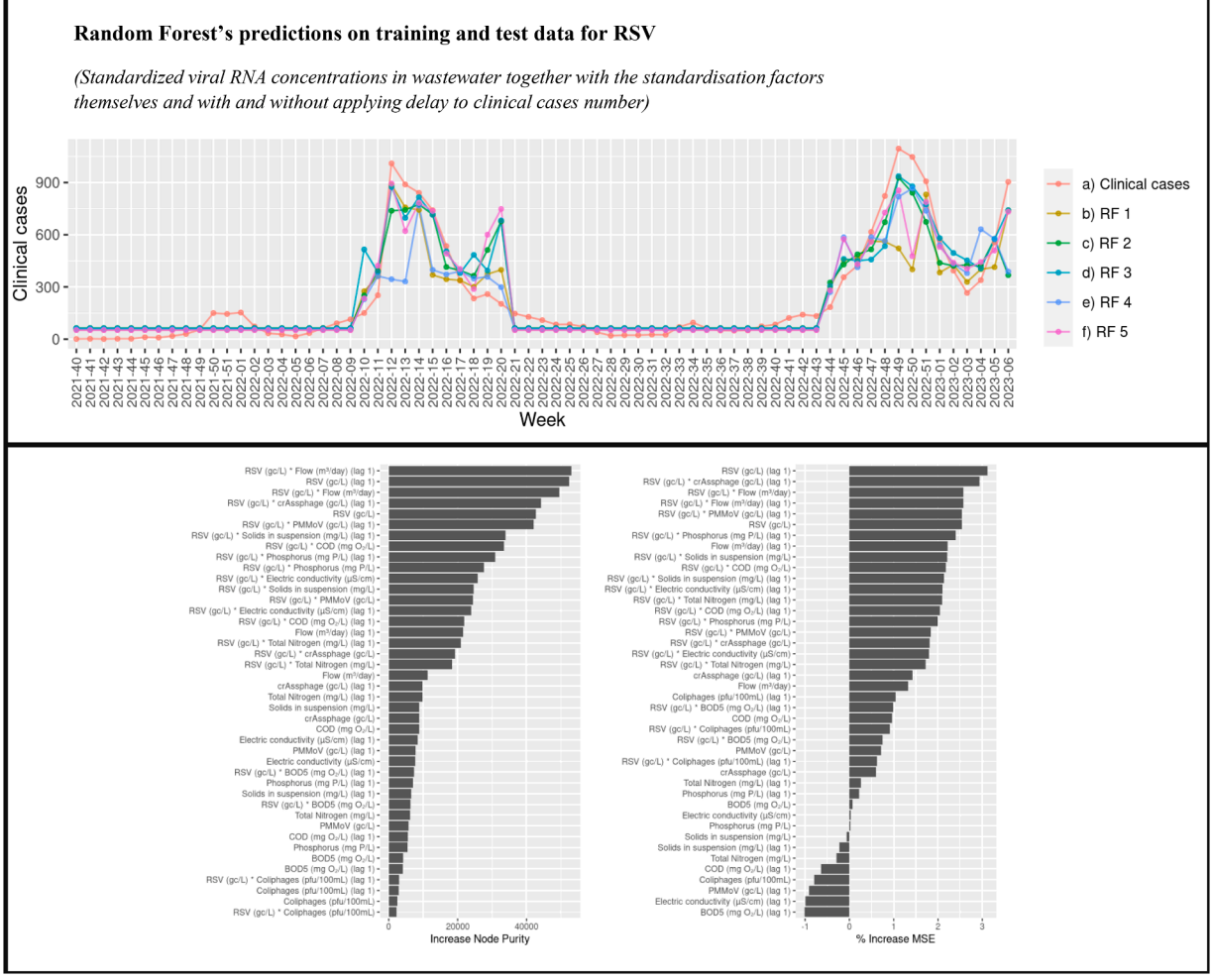


Fig. 9. Top: mean absolute error and its percentage over the mean of clinical cases for the "A-E" data sets; Middle: Random Forest's predictions on training and test data for E) data set for RSV (standardised viral RNA concentrations in wastewater together with the standardisation factors themselves and with and without applying delay to clinical cases number. Bottom: Importance of factors affecting Random Forest's predictions expressed as Increase in node purity and percentage increase on mean standard error (% MSE).

WBE-related works covering different Spanish localities, showing a prevalence of 100 % and an average concentration of 5 logs GC/L throughout the whole sampling period (López-Peñalver et al., 2023; Trigo-Tasende et al., 2023; Mattei et al., 2023).

Finally, the prevalence of crAssphage and PMMoV was assessed due to its proposed role as fecal viral indicator along with the detection of somatic coliphages (Honap et al., 2020; Farkas et al., 2019; Bivins et al., 2020). All three viral indicators were detected in 100 % of the analysed samples, with mean concentrations of 8.09, 5.71, and 7.28 log GC or PFU per liter for crAssphage, PMMoV, and somatic coliphages, respectively. CrAssphage and PMMoV concentrations were in line with existing data (Bivins et al., 2020; Sabar et al., 2022).

Finally, it is essential to consider that the detected concentrations of viruses in wastewater may be influenced by whole-process method variability, fecal shedding that may vary among different variants, heavy rainfall diluting raw wastewater, and population movements (Dhakar et al., 2022; Bivins et al., 2021).

4.2. *Detection of SARS-CoV-2 variants of concern by duplex RT-qPCR and sequencing*

Various global initiatives have emerged to detect SARS-CoV-2 in wastewater during the pandemic, utilising a range of analytical workflows primarily based on RT-qPCR following genetic material preparation and enrichment (Randazzo et al., 2020; Ahmed et al., 2020; La Rosa et al., 2020; Medema et al., 2020; Wurtzer et al., 2020; Wu et al., 2020). As the pandemic progressed, new variants with specific mutations have continuously appeared, some of which have caused worldwide infections.

In addition to RT-qPCR, NGS-approaches using genome sequencing can be used to detect viral mutations, particularly those found in SARS-CoV-2 VOCs and has been recommended as standard procedure for

variant monitoring (Pérez-Cataluña et al., 2022; Crits-Christoph et al., 2021; Prasek et al., 2023).

In the present study, comparison of the lineage designation using duplex RT-qPCR VOC assays to genome sequencing with Oxford Nanopore MinION system and Freyja analysis software showed that the duplex RT-qPCR assays achieved significantly quicker results while maintaining appropriate clinical sensitivity and specificity (EC, 2021). Moreover, RT-qPCR assays allowed the characterisation of samples with low viral loads that would not be possible to process by genome sequencing. However, it should be noted that as the number of new SARS-CoV-2 variants increases, it becomes more difficult to find discriminatory mutations between them, making it difficult to develop new duplex RT-qPCR protocols for the identification of VOCs.

Although the detection of new SARS-CoV-2 variants in wastewater using sequencing methods typically needs higher concentrations of viral particles than PCR-based approaches, identification of samples with Cts over 30 cycles was efficiently performed (Fig. S1 and Fig. 4A). As other studies reported, in mid-December 2021 the Omicron variant overtook the Delta variant, which had been in the majority during the months of October and November 2021 (Pérez-Cataluña et al., 2022; Bar-Or et al., 2021; Crits-Christoph et al., 2021; Izquierdo-Lara et al., 2021; La Rosa et al., 2021; Nemudryi et al., 2020; Rios et al., 2021; Carcereny et al., 2021). The transition from Delta to Omicron occurred rapidly in 2–3 weeks in the month of December (weeks 49 to 51 of 2021), as shown by results obtained by both duplex RT-qPCR and by Freyja software from sequencing data. This Omicron emergence dates fully match the first reports of Omicron clinical cases in the Valencian Region of Spain (week 50 of 2021) (Fig. S3). Interestingly, according to the Spanish Health Ministry, the transition from Delta to Omicron variant in the Valencian Region of Spain took much longer to become clear when looking at clinical case data (6 to 8 weeks) with respect to this study based on wastewater's metagenomic or RT-qPCR data (2–3 weeks) for the same geographical area (Spanish Government, 2022). Also, as stated by the Spanish Health Ministry, Omicron BA.2 and BA.5 variants reached almost 100 % prevalence from the 14th and 28th weeks of 2022, respectively (Spanish Government, 2022). This observation fully matches the two peaks observed in SARS-CoV-2 RNA GC/L in wastewaters from the WWTP analysed in this study by duplex RT-qPCR and Freyja software analyses, which correspond to weeks 16th (April) and 28th (July) of 2022. Thus, the three peaks in SARS-CoV-2 RNA quantification data in wastewater samples (Figs. 3 and 4) correspond to Omicron BA.1, BA.2, and BA.5 variants imposition in the Spanish city of Valencia.

4.3. Correlation with public health records and comparison of indicators for data normalisation

Correlation with clinical data is another key component of WBE whereas measured viral concentrations in wastewater and reported clinical cases of disease should be established, strengthening the proposed methodology. The establishment of these correlations can serve as a validation for a prediction model that accounts for the factors discussed above, providing evidence for the notion that changes of viral concentrations in wastewater will indicate changes in viral disease cases in humans. When infectious agents are linked to non-specific symptoms or asymptomatic patterns, simultaneous monitoring of a wide range of respiratory viruses may be able to shed light on the dynamics of respiratory disease circulation in communities and improve the ability of Public Health authorities to combat seasonal human pathogenic viruses (Toribio-Avedillo et al., 2023; Ahmed et al., 2023; Guido et al., 2016; Britton et al., 2018).

In this context, it is important to analyse the reemergence of respiratory diseases such as the caused by IAV and RSV during the COVID-19 post-pandemic period. As other studies have reported before, the high sanitary restrictions and mask use during the pandemic months caused the prevalence of IAV and RSV to fall drastically among the population (Ando et al., 2023; Eden et al., 2022; Feng et al., 2021). However, once the strict containment measures were lifted, a large increase in cases of disease caused by these two viruses was observed (Ando et al., 2023; Eden et al., 2022; Feng et al., 2021; Emborg et al., 2022; Tempia et al., 2021; Fourgeaud et al., 2021; Boehm et al., 2023). In this sense, RSV and IAV monitoring in wastewater has recently been carried out in the USA, Canada, Spain, and Australia, and the results showed reasonable correlations with clinical cases and outbreaks (Toribio-Avedillo et al., 2023; Hughes et al., 2022; Wolfe et al., 2022; Ahmed et al., 2023; Ando et al., 2023). The RSV and IAV data included in the current research, possess significant potential for informing and shaping public health strategies in response to seasonal respiratory pathogens. This encompasses the dissemination of targeted messages during cold seasons to raise awareness about associated symptoms, the implementation of preventive measures like mask-wearing for vulnerable population, offering guidance to healthcare practitioners for efficient diagnostic triage, and optimising the allocation of resources.

The clinical cases pattern in this study, as in previous studies, nicely matched the viral RNA prevalence pattern in wastewater samples (Toribio-Avedillo et al., 2023; Ahmed et al., 2023). For both IAV and RSV, we found statistically significant associations between the reported confirmed cases and the corresponding quantification data in wastewater data (Figs. 5 and 6). This demonstrates the value of wastewater surveillance for monitoring infectious diseases in the community and supports their future application for monitoring additional respiratory viruses. Our experience is in line with what has been seen in other localities for IAV and RSV (Toribio-Avedillo et al., 2023; Hughes et al., 2022; Wolfe et al., 2022; Ahmed et al., 2023; Ando et al., 2023; Mercier et al., 2022).

As demonstrated by the correlation of respiratory virus in wastewater samples with clinical data, WBE can offer representative samples of the community by collecting population health data. However, sewage flow and faecal load in wastewater are known to vary significantly from day to day (Bertels et al., 2022). Two viruses suggested as indicators of fecal contamination, PMMoV and crAssphage, were quantified from wastewater samples to normalise the SARS-CoV-2, IAV, and RSV concentrations along with somatic coliphage plaque counting (Figs. 4–6). Besides, wastewater physico-chemical parameters data was also used for respiratory virus's concentration normalisation (Figs. 4–6). This was done to account for the many factors capable of changing estimated fecal load over time and potential dilution due to rain. Although viral shedding could be a relevant variable when conducting these type of studies, several publications regarding viral shedding have been published with very variable data that seems to not accurately describe the real shedding rates (Chan et al., 2011; Hughes et al., 2022). Besides, respiratory viruses can also reach sewage from other sources distinct to stool (mucus and saliva). Thus, the viral shedding of each of the analysed viruses has not been taken into account as a variable in drawing conclusions.

According to obtained Spearman's correlations, the association between the SARS-CoV-2, RSV, and IAV titers and clinical case numbers was mainly improved by the physico-chemical parameters-based normalisation (Figs. 4–6). It should also be noted that there was an important improvement in resulting correlations strength when applying a delay on clinical cases data, as there exists a logical frame of time between an individual getting infected and its search for sanitary aid. Interestingly, PMMoV-based normalisation did not significantly alter the correlation, and finally both crAssphage-based and somatic coliphage-based normalisations led to much weaker correlations (Figs. 5–7). These results differ from some works stating crAssphage or PMMoV loads are proper normalising parameters (Wu et al., 2020; Wilder et al., 2021; D'Aoust et al., 2021), but are in line with several of other studies which are reporting that these approaches might not be as consistent as other literature suggests (Gerrity et al., 2021; Jafferali et al., 2021; Langeveld et al., 2023). For example, Ai et al. (2021) states that crAssphage-based normalisation of SARS-CoV-2 titers in

wastewater samples led to much weaker correlation with clinical cases while PMMoV slightly but not significantly improved that correlation. On the other hand, physico-chemical parameters seem to better adjust to the role of normalising factor. In particular, normalising GC/L values by WWTP inflow parameter, which is lately being proposed as the optimal normalising factor (Langeveld et al., 2023), showed outstanding improvements on obtained correlations with clinical data. Interestingly, the fact that virus quantification data in wastewater can be normalised with physico-chemical parameters makes the analysis process cheaper, since, unlike in the case of normalising via biological indicators, a PCR or viral plaque counting step is needless. Besides, our results reflected other physico-chemical parameters (total nitrogen, phosphorus, solids in suspension, electrical conductivity, COD, and BOD5) to also be robust parameters for data normalising, while normalisation based on PMMoV genome concentration suggested a viral rebound in February 2022 that was not observed on the incidence curve and presented lower correlations. Finally, Random Forest results demonstrated that the algorithm can be effectively applied to respiratory virus GC/L in wastewater data to determine realistic clinical trends of respiratory illness in a given community (Figs. 7–9). The results obtained in the clinical case prediction models using Random Forest reinforced the conclusions obtained from the Spearman's correlations: the inflow to the WWTPs seems to be the normalising factor that best fits the predictive model. In addition, the existence of a delay between the onset of infections in the population (translated into increased virus load in wastewater) and the appearance of clinical cases should be taken into account, since people do not go to health centers immediately post-infection. Despite the low reliability, the models demonstrated the existence of shared information between the collected data and the clinical cases, supporting the previous results of the Spearman's correlations. This opens the way to the possibility of the implementation of predictive models as an early warning system. More data is required to train such models, which was not possible to collect to the extent of this text.

5. Conclusions

Overall, this work reinforces the potential of wastewater-based epidemiology as a multi-pathogen monitoring tool. This study shows that it is possible to reliably monitor the confluence of respiratory and enteric viruses and the diseases they cause in the same geographical area, with a great interest in public health. Finally, this work also highlights the high normalising capacity of fecal load normalisation by wastewater physico-chemical parameters, specially WWTP inflow, while raising doubts about the appropriateness of normalising data by quantification of crAssphage, PMMoV, or somatic coliphages.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT (https://chat.openai.com/) in order to improve readability and language. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

CRediT authorship contribution statement

Inés Girón-Guzmán: Writing – review & editing, Methodology. **Enric Cuevas-Ferrando:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Regino Barranquero:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Azahara Díaz-Reolid:** Writing – review & editing, Methodology. **Pablo Puchades-Colera:** Writing – review & editing, Methodology. **Irene Falcó:** . **Alba Pérez-Cataluña:** Writing – review & editing, Methodology, Formal analysis. **Gloria Sánchez:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.121463.

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