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Development of quantitative wastewater surveillance models facilitated the precise epidemic management of COVID-19

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HIGHLIGHTS

• Understanding the decay rate of coronavirus in sewage pipes enables the accurate prediction of COVID-19 cases.
• Wastewater surveillance at the communities facilitates the identification of SARS-CoV-2 hidden cases.
• The model developed herein transforms wastewater surveillance into a public health management tool.

GRAPHICAL ABSTRACT

ABSTRACT

Wastewater surveillance serves as a promising approach to elucidate the silent transmission of SARS-CoV-2 in communities. To understand the decay of the coronavirus in sewage pipes, the decay of the coronavirus traveling over 20 km distance of pipeline was analyzed. Based on the decay model, a WWTP and a community model were then proposed for predicting COVID-19 cases in Xi’an and Nanchang city during the COVID-19 outbreak in 2021 and 2022. The results suggested that Monte Carlo simulations estimated 23.3, 50.1, 127.3 and 524.2 infected persons in the Yanta district of Xi’an city on December 14th, 18th, 22nd and 26th of 2021, respectively, which is largely consistent with the clinical reports. Next, we further conducted wastewater surveillance in two WWTPs that covered the whole metropolitan region in Nanchang to validate the robustness of the WWTP model from December 2021 to April 2022. SARS-CoV-2 signals were detected in two WWTPs from March 15th to April 5th. Predicted infection numbers were in agreement with the actual infection cases, which promoted precise epidemic control. Finally, community wastewater surveillance was conducted for 40 communities that were not 100% covered by massive nucleic acid testing in Nanchang city, which accurately identified the SARS-CoV-2 carriers not detected by massive nucleic acid testing. In conclusion, accurate prediction of COVID-19 cases based on WWTP and community models promoted precise epidemic control. This work
1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a global pandemic and is still a public health emergency of international concern. Epidemiological surveillance of this virus has heavily relied on individual testing of clinical samples by nucleic acid-based test. However, this approach was restricted by several factors, such as the inability to track all asymptomatic disease carriers and reporting bias. Instead, wastewater surveillance has been proven to be feasible for the early warning of enteric viruses, such as norovirus, hepatitis A virus, and poliovirus, historically (Hellmér et al., 2014; Asghar et al., 2014). Recently, wastewater surveillance has also been extensively used for the detection of SARS-CoV-2 in communities, as collected wastewater contains viruses excreted from both symptomatic and asymptomatic individuals in a certain catchment. Several recent studies that analyzed SARS-CoV-2 titers in wastewater treatment plants (WWTPs) by RT-qPCR revealed a good correlation between SARS-CoV-2 incidence rates and virus titers in wastewater (Ahmed et al., 2020a; Nemudryi et al., 2020; Martin et al., 2020; Izquierdo-Lara et al., 2021; Jahn et al., 2021). Recent studies also showed the additional value of wastewater surveillance for the detection of SARS-CoV-2 before it is reported by local clinical tests (Xu et al., 2021; Kirby et al., 2022). However, only a few researchers estimated the infected number and guided the public health actions. For instance, Ahmed et al. (2020a) established a model to estimate the number of infected individuals based on the concentration of viral RNA; the model estimated a median range of 171 to 1090 infected persons in the catchment, which is in agreement with clinical observations. Results from Karrhikeyan et al. (2021) also suggested that the wastewater surveillance could provide rapid prevalence assessments of COVID-19 cases by autoregressive integrated moving average (ARIMA) model.

However, uncertainty still existed in the current model. For instance, the transportation of wastewater in municipal pipelines with biofilms would largely affect the variability of viruses. In addition, high water temperature would also result in the decay of the virus (Kitajima et al., 2020). There is a need to understand the persistence of SARS-CoV-2 and its nucleic acid in water and wastewater environments to properly interpret SARS-CoV-2 RNA measurements from wastewater collection systems. Otherwise, the detection might be negative for viruses at low concentrations, which would underestimate the silent transmission of SARS-CoV-2 in the community. Although some researchers investigated the persistence of SARS-CoV-2 in the sewage (Yang et al., 2022), no study has elucidated the decay of viral RNA during its transport in sewage pipelines. Studies are urgently needed to assess viral decay rate due to sorption on biofilm and the pipe network material as well as chemical inactivation.

To address these urgent issues, we aim to understand the decay of the coronavirus in sewage pipelines and develop a WWTP model for accurate estimation of SARS-CoV-2 infected persons in this study. Next, we validate the model in several cities and proposed a practical workflow that combined WWTP and community wastewater surveillance together to reach precise epidemic control of COVID-19.

2. Materials and methods

2.1. Effect of transportation of wastewater on viral activity

In this study, to analyze the virus decay along the long-distance municipal pipeline, two recirculating water systems (RWS) were set up. The system consists of two 80 m$^3$ tanks (with 60 m$^3$ of synthetic wastewater) and a pipeline loop with a total length of 100 m. The synthetic wastewater was prepared as described previously (Klatt and LaPara, 2003). A mixture of gelatin, polyoxymethylene-sorbitan monooleate, and starch was prepared in filter-sterilized tap water to simulate the polysaccharide, protein, and lipid components of municipal wastewater. The water temperature of the two RWS was monitored throughout the study, with the temperatures respectively maintained at 12.0 ± 2 °C and 28.0 ± 2 °C throughout the duration of the experiment. The time required for one circulation is approximately 15 min. Avian infectious bronchitis virus (IBV), a gamma coronavirus obtained from chicken feces from a previous study was used (Fu et al., 2020). Briefly, IBV was seeded into the wastewater with 10$^4$ GC/L (final concentration). Once the IBV was seeded into the tank, it would travel 9600 m in 24 h. We sampled 200 mL wastewater when the travel distance reached 5, 10, 15, and 20 km (sampling at 12.5, 25, 37.5, and 50 h, respectively) to evaluate the effect of transportation of wastewater on the viral activity. Once the experiment was finished, the inoculated wastewater was disinfected by dioxide chlorine for 60 min before discharging into the sea. Meanwhile, to evaluate the effects of biofilms on the viral activity in the municipal pipeline, IBV was also seeded into the two RWS on day 60. Wastewater was also sampled at the above travel distance and discharged into the sea after 50 h.

2.2. Calculations of decay rate of IBV in wastewater pipeline

IBV RNA concentrations (GC/L) at various temperatures or different travel distances were used to calculate the decay rate. The observed IBV RNA concentrations were linearized using the natural log (ln)-transformation of the IBV RNA concentrations, as shown in Eq. (1) (Chick, 1908). These values and their associated travel distances were used to calculate the first-order decay rate constants in units per kilometer by linear regression in the R package. The fit of the regression and the appropriateness of the linear model were assessed by the root mean square error (RMSE) and R$^2$ value, which assesses the fit by measuring the distance of the observed values from the fitted line.

$$\log \left( \frac{C_d}{C_0} \right) = -kd$$

where $C_0$ and $C_d$ are the IBV RNA concentrations of GC in the wastewater at travel distances of 0 and d km, respectively, and k is the decay rate constant. The distance required to achieve a 90 % (one log) reduction (D90) was calculated using Eq. (2).

$$D_{90} = -\log(0.1)$$

After the mean k values were estimated for each treatment, the values were log10-transformed, and linear regression was used to characterize the effects of different treatments on the first-order decay rate constant. Two-way ANOVA with Tukey’s multiple comparison tests and paired t-tests were performed to evaluate the effect of treatment factors (biofilm matrix and temperature) on the decay of IBV RNA. All statistical analyses were performed in the R package, and a P value <0.05 was considered statistically significant.

2.3. Clinical data collection

Data on COVID-19 infection in Xi’an, and Nanchang city were obtained from the National Health Commission of the People’s Republic of China released in public between December 10th 2021, and April 18th 2022. The infection cases were detected by several rounds of massive nucleic acid testing highlights the viability of wastewater surveillance for outbreak evaluation and identification of hidden cases, which provides an extraordinary example for implementing precise epidemic control of COVID-19.
covering all of the citizens in studied regions, which included all of SARS-CoV-2 positive carriers. The epidemiological information includes the demographic and clinical characteristics of individual cases. Previous studies suggested that for omicron variant or delta variant, the majority of viral shedding occurred 1–5 days after the infection (Araf et al., 2022; Shiehzadegan et al., 2021). Thus, we estimated the actual number of infection cases as the confirmed number of cases plus the cases detected in the next 1–5 days.

2.4. Sewage sampling and pasteurization in Xi’an city

We collected wastewater with intervals of 4 days from December 1st 2021 to April 18th 2022 from the influent of a WWTP in Yanta district of Xi’an city and a manhole in three communities. To reduce the variability of grab sampling, 200 mL of wastewater samples were collected in the morning peak (8 am to 10 am) at 15-min intervals for 2 h and pooled together. Upon initial receipt, samples were placed in the biosafety cabinet with UV for 10 min and then pasteurized in a 60 °C water bath for 60 min to inactivate the virus. Pasteurized samples were then used for viral precipitation, and the remaining samples were stored at 4 °C.

2.5. RNA extraction

RNA extraction was performed as described by Wu et al. (2020). Briefly, the wastewater samples (100 mL) were first centrifuged at 4750g for 5 min. The supernatant was mixed with 8.0 g of PEG8000 and 4.7 g of sodium chloride to final concentrations of 10 % and 1 M, respectively. The mixture was incubated at 4 °C overnight with 100 rpm agitation, followed by centrifugation at 10,000g for 2 h at 4 °C to pellet the virus particles. The pellet was then resuspended in 200 μL of phosphate buffered saline (PBS). RNA was extracted using QIAamp Viral RNA Mini Kits (Qiagen) according to the manufacturer’s instructions with a final elution volume of 50 μL. Meanwhile, IBV was used as a molecular process control to monitor the efficiencies of the RNA extraction and reverse transcription quantitative PCR (RT-qPCR). Briefly, 50 μL of the virus concentrate was spiked with 2.1 × 10^5 copies (2 μL of a viral stock) of IBV and subjected to RNA extraction as described above. A working stock of SARS-CoV-2 strain hCoV-19/Jiangxi/20210709-3 was isolated from a patient by the Nanchang Centre for Disease Control and Prevention. Afterwards, gamma irradiation (Cobalt-60 using an MDS Nordion Irradiator) with 5 Mrad dose was used to ensure the biosafety during handling of SARS-CoV-2 in a biological safety level 2 (BSL-2) laboratory. The number of irradiated SARS-CoV-2 and IBV in working stock was counted by the epifluorescence enumeration method (Patel et al., 2007). Then both viruses were diluted in different concentrations and subjected to RNA extraction to evaluate the correlation of recovery rates between the two viruses.

Recovery rate (%) = virus concentration calculated by the standard curve of RT-qPCR/virus abundance before RNA extraction.

The recovery rate of spiked IBV during every RNA extraction was then used to normalize the viral load of SARS-CoV-2 in wastewater.

For quantification of SARS-CoV-2 RNA by RT-qPCR, the N1 region of the nucleocapsid gene was targeted using the primers and probes as well as the PCR conditions described by Chu et al. (2020). The one-step N gene RT-qPCR was carried out for 45 cycles using the TaqMan Fast Virus one-step Master Mix with a 20 μL reaction mixture (Thermo Fisher, USA). The plasmid carrying N1 target was provided by the Chinese Center for Disease Control and Prevention. The concentration of plasmid was calculated using the Qubit dsDNA HS assay kit (ThermoFisher, USA) and the copy number was obtained based on the length and Avogadro’s number. The plate-specific standard curves were generated by ten-fold dilution of the plasmids containing N1 gene from 10⁷ to 100 copies μL⁻¹. The final concentration of SARS-CoV-2 in the wastewater was measured in genome copies (GC) per L, which was calculated by dividing the viral RNA concentration by the amount of the tested raw sewage sample (100 mL):

\[
\text{Virus load (copies/100 mL) = \frac{\text{Viral concentration} \times \text{total RNA volume}}{\text{volume for qPCR}}} \times \text{Recovery rate of spiked IBV}
\]

where viral concentration is the virus load calculated by plate-specific standard curve.

The genotype of delta and omicron variant were further confirmed by commercial RT–qPCR detection kits (BioGerm, Shanghai, China). IBV was detected by one-step detection kit targeting ORF1ab gene (Shanghai Xinyu Biotech, China). RT–qPCR amplifications were performed in 40 μL reaction mixtures using iTaq™ Universal Probes One-Step Reaction Mix (Bio–Rad Laboratories, Richmond, CA). All RT–qPCRs were performed in triplicate. For each qPCR run, a series of three positive and negative controls were included.

2.6. Monte Carlo simulation to estimate the infection number in the community and district

Although individual shedding in saliva, sputum, stool, and urine all contribute to the virus load in wastewater, herein we only examined the probable contributions of stool and urine to the virus load of wastewater, as a previous study suggested that the virus loads in saliva and sputum are 3 log₁₀ lower than those in stool and urine (Crank et al., 2022).

The amount of SARS-CoV-2 RNA copies/g in feces was modeled as a log-uniform distribution from 2.56 to 7.67 log₁₀ copies/g, as observed in a previous study (Rose et al., 2015). The daily stool mass per person was modeled as a normal distribution with a mean of 211 g as reported by Rose et al. (2015). Meanwhile, we assumed that the virus load in urine followed a distribution with a mean of 2.91 log₁₀ copies/mL and an average urination amount of 1500 mL (Table S1). Based on the data from Xi’an in 2020, the average water use is 135 L/person/day. Meanwhile, based on previous studies, the latent period for Delta and Omicron variant were 1–5 days with 3 days in average (Thompson et al., 2021). Thus, actual infection cases on specific day were calculated based on the reported cases on the wastewater sampling date plus the ones detected in the next 1–5 days.

The sampled WWTP in the Yanta district has a catchment of approximately 900,000 persons (capita). We first used Eq. (4) to obtain the corrected SARS-CoV-2 RNA copies without decay:

\[
\text{Log (corrected RNA copies/liter wastewater)} = \text{Log (RNA copies detected by qPCR/liter wastewater)} + k \times d
\]

where \(k\) is the decay rate of CoV RNA at 12 °C with mature biofilm in pipes, \(d\) is the pipe distance from community to WWTP (details see supplementary Table S1).

Then, we used the above assumed virus load in stool and urine as input parameters and considered the virus decay in the pipes, and the predicted number of persons infected can be calculated by the following Eq. (5) (WWTP model):

\[
\text{Persons infected} = \frac{\text{Corrected RNA copies/liter wastewater}}{\text{Gene target dilution factor}} + \frac{\text{RNA copies/liter wastewater}}{\text{dilution factor}}
\]

where 1.5 is the average urine amount per person per day (1.5 L).

For a single community, the virus load will be first diluted in the community. The dilution factor equals the number of individuals in the community \(\times\) daily water use per person. Thus, the infection cases can be modeled by the following Eq. (6) (community model):

\[
\text{Persons infected} = \frac{\text{RNA copies/liter wastewater}}{\text{dilution factor}}
\]

The Monte Carlo simulation was built in Excel (Microsoft, Redmond, WA) with ModelRisk version 6.0. The number of iterations per simulation was 10,000. The root mean square error (RMSE) was used as a criterion for selecting probability distributions based on goodness of fit. Maximum population densities estimated in the simulations. Sensitivity analyses were graphically represented by tornado charts (Vose, 1996). Plots were
automatically generated in ModelRisk by selecting predicted infection cases as the output. For the tornado plot, a rank order correlation was used, which provided a statistical measure of the correlation between the five most important inputs and output generated values. The predicted infection cases are reported as the median and 95% confidence interval (CI) determined by bootstrapping the model with 100 experiments of 1000 draws each. The predicted cases with or without the consideration of virus decay were compared with one-way ANOVA, followed by Tukey’s post hoc test using the R package.

2.7. Validation of the WWTP model in Nanchang city

To validate the effectiveness of the WWTP model on the prediction of SARS-CoV-2 infection cases, we conducted weekly wastewater surveillance on two WWTPs in Nanchang city from November 28th 2021 to April 18th 2022. The sampling and detection methods were described previously. Meanwhile, wastewater from 40 communities (with population coverage ranging from 1500 to approximately 40,000) that were not 100% covered by massive nucleic acid testing was also sampled on March 28th, April 1st, April 4th and April 7th 2022, respectively.

3. Results

3.1. Effect of transportation of wastewater on viral activity

To evaluate the effect of transportation of wastewater on viral activity, we seeded avian infectious bronchitis virus (IBV) into the influent of two recirculating water system (RWS) with virus concentration of 6.03 ± 0.20 log_{10} gene copies (GC)/L. The water temperature of the two systems was monitored throughout the study, with the temperatures maintained at 12.0 ± 2 °C and 28.0 ± 2 °C throughout the duration of the experiment. In pipelines without biofilms, the results showed that high temperature (28.0 °C) significantly reduced the viral RNA abundance by 2.18 log_{10} GC/L after experiencing 20 km travel distance, while only a 1.68 log_{10} GC/L reduction was observed in the pipeline at 12.0 °C (Fig. 1). The mean first-order decay rate constants (k) were 0.0799/km (R^2 = 0.9570) at 12 °C to 0.12/km at 28 °C (R^2 = 0.9572) (Table S2). The average IBV RNA D90 (distance required for one log_{10}-reduction) was 12.51 and 8.34 km for experiments conducted at 12 °C and 28 °C, respectively.

Next, we further examined the effect of the biofilm matrix in the pipeline on IBV decay when it passed through the pipeline. We first evaluated biofilm formation during the experiment. The number of sessile bacteria on day 0 for the two RWS averaged 11 cfu/cm². For low-temperature wastewater, the number of sessile bacteria increased from 3.53 log_{10} cfu/cm² on day 15 to 5.19 log_{10} cfu/cm² and reached a plateau on day 60 (Fig. S1). Likewise, the bacterial counts in the biofilm remain stable in the high-temperature RWS after 60 days’ operation. The above observations suggested that for both low- and high-temperature RWS, biofilms would be mature after 60 days. As most municipal pipelines have >60 days of operation, we seed IBV at day 60 into two RWS to examine the effect of the biofilm matrix in the pipeline on the viral activity. The decay rate constant was 0.22/km and 0.12/km for the pipeline with mature biofilm at 28.0 °C and 12.0 °C, respectively (Table S2). The estimated IBV RNA D90 was 8.34 km at 12 °C and 4.54 km at 28 °C. Based on the above results, we adjusted the input parameters previously proposed by Ahmed et al. (2020b) to predict infected persons by Monte Carlo simulation.

3.2. Detection of SARS-CoV-2 delta variant in wastewater samples of Xi’an city

A passenger was detected as SARS-CoV-2 positive in the airport of Xi’an city on 9th December 2021. Afterwards, a massive COVID-19 outbreak caused by delta variant B.1.617.2 occurred in Xi’an city from 9th December 2021 to January 14th 2022, with a total of 2053 cases. Infection cases were found in 13 districts of Xi’an city, of which 40.26% were found in Yanta district (Fig. 2). The infection case in Yanta district was initially found in December 12th, and daily infection cases peaked on December 27th with 95 cases then decreased and reached zero on January 14th. For the three communities under wastewater surveillance in Yanta district, a total of five cases were identified in Community One, which were diagnosed on December 20th, 22nd, 25th, 29th and 31st, respectively. No SARS-CoV-2 was detected in Community Two and Three.

From December 1st 2021, in the Yanta district, we monitored SARS-CoV-2 in the inlet municipal wastewater of a WWTP and a manhole of a community in this district (Fig. S2). Considering IBV is also a coronavirus which is consistent with SARS-CoV-2 virus in nucleic acid extraction procedures, IBV was spiked in for normalization of the SARS-CoV-2 virus load. Results suggested that the recovery rate of IBV and SARS-CoV-2 ranged from 10.43% to 35.7% without significant difference (p < 0.01, Table S3), suggesting IBV has the potential to serve as process control. Wastewater surveillance was then conducted on December 1st, 5th, 14th, 18th, 22nd, 26th, and 31st of 2021. The first SARS-CoV-2-positive water sample was detected on December 14th with an average virus load of 3.83 × 10^5 GC/L for N genes; at that time, two clinical COVID-19 cases attributed to the delta variant had been identified in Yanta district, but none

Fig. 1. Mean decay curves of IBV RNA over travel distance (km) in untreated wastewater pipeline without (A) or with biofilm (B). The measurements were linearized on first-order decay, in which the natural log (ln)-transformed measured concentration at each time point was divided by the concentration at time zero. The error bars (SD) are omitted for better readability.
from the communities. Samples collected on December 18th, 22nd, and 26th showed an ongoing outbreak of COVID-19 during this period, harboring average virus loads of $6.89 \times 10^2$, $1.54 \times 10^3$ and $4.56 \times 10^3$ GC/L, respectively. Subsequent genotyping assays of wastewater samples confirmed delta variant. Afterwards, wastewater sampling was not conducted until January 14th 2022 due to a strict lockdown of the whole city.

In the monitored Community one, SARS-CoV-2 was first detected on December 18th with an average virus load of $1.42 \times 10^4$ GC/L; two days after the wastewater data were reported, two infection cases had been identified in a resident of the community. Samples collected on December 22nd from this same sewershed also retrieved SARS-CoV-2 with concentrations of $1.66 \times 10^4$ GC/L. Another five new cases were diagnosed on December 26th, respectively. Afterwards, the concentration of SARS-CoV-2 dropped to a level below the detection limit on January 14th 2022. For another two communities, none SARS-CoV-2 signal was detected both in clinical and wastewater samples.

### 3.3. Prediction of the actual infection cases by the WWTP model

Meanwhile, based on latent period of most SARS-CoV-2 infection cases, actual infection cases were calculated based on the reported cases on the wastewater sampling date plus the ones detected in the next 1–5 days. Thus, we estimated the actual infection cases on December 14th (2–19 cases), December 18th (19–106 cases), December 22nd (114–238 cases), and December 26th (243–420 cases) (Table 1). As average wastewater temperature is 12.0 °C, we further corrected the viral load by using the decay rate under low temperature for modeling. Given the uncertainty and variations of the input parameters, the WWTP model estimated a median value of 23.3, 50.11, 127.3, and 524.2 infected persons in the catchment basin on December 14th, 18th, 22nd and 26th, respectively (Fig. S3), which is in reasonable agreement with clinical infection cases at the above four time points. However, when the decay of SARS-CoV-2 in pipeline was not considered, the predicted cases were significantly lower than actual number ($p < 0.05$, Table S4), suggesting an underestimation effect of prediction.

### 3.4. Community model to estimate the actual infection cases

As summarized in Table 1, the Monte Carlo simulation estimates a median number of 3.68 (0.86–9.97, 95 % CI) infected persons on December 18th and 5.13–13.68, 95 % CI) on December 26th 2021 in the monitored community. This is consistent with the clinical observation; two cases were reported until December 18th, while three additional cases were found on December 26th. The sensitivity analysis indicated that the estimated number of infections and prevalence were strongly correlated with the log_{10} SARS-CoV-2 RNA copies in stool, followed by the RNA copies detected in urine and the amount of feces/person/day. The model was least sensitive to the travel distance of wastewater (Fig. S4).

### Table 1

| Sampling point | RNA copies/L | Estimated infection cases$^a$ | Predicted infection cases | Median (95 % CI) |
|----------------|--------------|-------------------------------|---------------------------|-----------------|
| WWTP in Yanta district | | | | |
| 14-Dec-2021 | $3.83 \times 10^2$ | 8 (2–19) | 23.3 (5.33–63.83) |
| 18-Dec-2021 | $6.89 \times 10^2$ | 64 (19–106) | 50.11 (11.4–139.62) |
| 22-Dec-2021 | $1.54 \times 10^3$ | 118 (114–238) | 127.3 (29.1–347.5) |
| 26-Dec-2021 | $4.56 \times 10^3$ | 272 (243–420) | 524.2 (118.5–1436.1) |
| Community one | | | | |
| 18-Dec-2021 | $1.42 \times 10^4$ | 2 | 3.68 (0.86–9.97) |
| 26-Dec-2021 | $1.66 \times 10^4$ | 5 | 5 (1.13–13.68) |
| Community two | | | | |
| 18-Dec-2021 | ND | 0 | NA |
| 26-Dec-2021 | ND | 0 | NA |
| Community three | | | | |
| 18-Dec-2021 | ND | 0 | NA |
| 26-Dec-2021 | ND | 0 | NA |

$^a$ Cases detected in the next three days were indicated, the number in the bracket indicated the number of detected cases in the next 1–5 days; ND: not detected; NA: not applicable.
3.5. Application of the WWTP model on the early-warning and monitoring of COVID-19 outbreak in Nanchang city

To validate whether WWTP model is also robust for monitoring COVID-19 outbreak caused by omicron variant B.1.1.529, we selected another inland city, Nanchang that experienced COVID-19 outbreak from March to April 2022. Nanchang city was divided by Gan River into West and East regions (Fig. S2). We sampled wastewater in Honggutan WWTP (covering West urban region) and Qingshanhu WWTP (covering East urban region) in Nanchang city from December 2021 to April 2022 to check the sensitivity of wastewater surveillance for early warning of the community SARS-CoV-2 infection (Fig. S2).

The first COVID-19 case was identified in the West region of Nanchang city on March 15th 2022 in an airline passenger arrived two days ago. Next, three rounds of massive nucleic acid testing were conducted in the whole city in the next five days. COVID-19 cases were continuously detected in the following 30 days. The number of infected cases rose significantly in the next seven days and peaked on March 24th, after which the new infection number gradually decreased until March 28th (Fig. 3A, B). Then, second wave of outbreak occurred from March 28th to April 16th due to a new imported case. From March 15th to April 15th 2022, a total of 780 cases were registered in the city (Fig. S2).

To check the sensitivity of wastewater surveillance, we conducted weekly sampling in the WWTP in West and East region, respectively. In West region, the first SARS-CoV-2-positive water sample was detected on March 15th 2022 with an average virus load of 1.89 $\times$ 10$^3$ GC/L. Samples collected from March 22nd evidenced an outbreak of COVID-19 during this period, harboring a virus load of 1.34 $\times$ 10$^4$ GC/L. The concentration of SARS-CoV-2 dropped to 1.58 $\times$ 10$^2$ GC/L on March 28th. However, the virus load increased to 6.92 $\times$ 10$^2$ GC/L on April 4th, after which the virus load dropped below the detection limit on April 11th. This is consistent with the trend of the second wave of COVID-19 outbreak. As average wastewater temperature is 14.0 °C during the outbreak, we further corrected the viral load by using the decay rate under low temperature for modeling. WWTP model estimated 28.64, 215.0, 6.3 and 12.3 cases on March 15th, 22nd, 28th and April 4th 2022, which were close to the actual number of cases (Table 2).

Based on the SARS-CoV-2-positive carrier's travel path, hidden transmissions might also have occurred in the East region of the city. Thus, wastewater surveillance was also simultaneously conducted in WWTP for the East region (covered 1.45 million residents) (Fig. 3B). Wastewater surveillance gave positive results on March 16th and March 23rd with concentrations at 1.14 $\times$ 10$^2$ and 1.13 $\times$ 10$^3$ GC/L, respectively. Viral load dropped into 3.46 $\times$ 10$^2$ GC/L on March 30th, and further declined into 6.24 GC/L on April 5th. Subsequently, we estimated 5 (2.33–13.8, 95 % CI), 61.1 (14.07–168.3, 95 % CI), 18.3 (4.4–51.9, 95 % CI) infected persons on March 16th, March 23rd, March 30th and April 5th based on the WWTP model, respectively. On April 13th, WWTP showed negative results although one infected person was still detected sporadically. Except for March 23rd, there is no significant difference between predicted and actual infected number.

| Sampling point | RNA copies/L | Estimated infection cases$^a$ | Predicted cases Median value (95 % CI) |
|---------------|-------------|-------------------------------|--------------------------------------|
| WWTP in East region | | | |
| 16-Mar-2022 | $1.14 \times 10^2$ | 3 (3–11) | 5 (2.33–13.8) |
| 23-Mar-2022 | $1.13 \times 10^3$ | 19 (9–41) | 61.1 (14.07–168.3) |
| 30-Mar-2022 | $3.46 \times 10^2$ | 12 (4–15) | 18.3 (4.4–51.9) |
| 5-Apr-2022 | 62.4 | 6 (6–7) | 3.7 (0.8–5.3) |
| 13-Apr-2022 | ND | 1 | NA |
| WWTP in west region | | | |
| 15-Mar-2022 | $1.89 \times 10^3$ | 35 (12–102) | 28.64 (6.5–78.39) |
| 22-Mar-2022 | $1.34 \times 10^4$ | 202 (72–230) | 215 (26.7–544.1) |
| 28-Mar-2022 | $1.58 \times 10^2$ | 12 (7–40) | 6.3 (1.42–17.41) |
| 4-Apr-2022 | $6.92 \times 10^2$ | 36 (24–78) | 12.3 (2.8–33.6) |
| 11-Apr-2022 | ND | 1 | NA |
| 18-Apr-2022 | ND | 1 | NA |

$^a$ Cases detected in the next three days were indicated, the number in the bracket indicated the number of detected cases in the next 1–5 days; ND: not detected; NA: not applicable.

Fig. 3. Daily COVID-19 cases in the West region (A) and East region (B) of Nanchang city from March to April 2022 and the daily timeline of key events in response to COVID-19 outbreak in Nanchang city (C). The virus loads of different sampling times are indicated in bar graph.
3.6. Application of round community wastewater surveillance for finding the hidden cases

After four rounds of massive nucleic acid testing, the lockdown of SARS-CoV-2-negative communities were canceled, while the lockdown of SARS-CoV-2-positive communities were extended for one week. However, we found that in 40 communities, not whole community was covered by four rounds of massive nucleic acid testing. To further find SARS-CoV-2 carriers in these communities, four rounds of community wastewater surveillance was initiated respectively on March 28th, April 1st, April 4th and April 9th. However, SARS-CoV-2 signal was found in wastewater of one community (Community A in Fig. S2B) on March 28th, after which Community model estimated 2 (1.13–3.54 95% CI) cases. On April 1st, SARS-CoV-2 signal was also found in wastewater of another community (Community B). We predicted 3 (2.63–5.68, 95% CI) cases based on virus concentration in wastewater. Fifth round of massive nucleic acid testing also confirmed above prediction later on; 2 and 3 cases were found in above two communities (Table S5). Unexpectedly, three infected cases were also respectively found in another three communities (Community C, D, and E) in fifth round of massive nucleic acid testing, suggesting the sensitivity of two rounds of wastewater surveillance was 62.5% (5 out of 8 positive cases). SARS-CoV-2 signal of above three communities turned positive until April 4th. On April 9th, the signals of SARS-CoV-2 became negative for all sampled communities after 4th round of community wastewater surveillance. Thus, except for SARS-CoV-2 positive communities, the lockdown of the majority of communities were canceled.

4. Discussion

In this study, we investigated whether the presence of SARS-CoV-2 in WWTP and community can be used as an early warning for COVID-19 infections. Although the detection of clinical samples remains the gold standard for disease surveillance and tracking, such data are limited due to factors such as sampling bias and inability to track asymptomatic disease carriers. The advantage of wastewater surveillance is that infectious agents are excreted in the urine and feces of infected individuals regardless of disease symptom severity. In this study, we employed both WWTP and community wastewater surveillance under different scenarios. Wastewater surveillance in WWTP is a promising approach for early warning for a WWTP catchment basin, while surveillance on the community level facilitates the accurate identification of hidden cases in blocks. For instance, Deng et al. (2022) established a territory-wide sewage surveillance system in Hong Kong by setting up 112 stationary sampling sites covering the majority of downtown districts in Hong Kong. The combination of WWTP and community wastewater surveillance have achieved successful early warning in Hong Kong (Xu et al., 2021) and served as a decision-making tool for the preparedness and responses to the COVID-19 outbreak in communities (Deng et al., 2022), which transforms wastewater epidemiology into a public health management tool.

However, at present, no study has investigated the survival of CoVs during their passage through the sewer pipe network, which affects the accurate estimation of infection cases via wastewater surveillance. Current evidence suggests that CoVs do not survive well in aqueous environments relative to norovirus, which can persist for months (Crank et al., 2022). Herein, we used IBV as a surrogate CoV to analyze its decay under different treatments. Our study examined the survival of IBV in a stimulated sewer pipe under different treatments, which provided critical information to understand the survival of CoVs during their passage through the sewer pipe network. The results suggested that the biofilm matrix and temperature appear to be important factors affecting the decay of IBV, resulting in the degradation of CoVs by up to a 4.8 log10 reduction. Equations describing the linear regression fit for the decay of CoVs were summarized. The fit of each linear model was reasonable, with R2 values ranging from 0.927 to 0.957. The decay rate increased more rapidly with increasing temperature in the pipeline having a mature biofilm. A previous study also examined the decay rate of murine hepatitis virus (MHV) and gamma-irradiated SARS-CoV-2 (Ahmed et al., 2020b). Their results suggested that there was no statistically significant decay at lower temperatures (4°C and 15°C) irrespective of the virus type and water source, but the decay at higher temperatures (25°C and 37°C) was significantly faster than that at lower temperatures. Notably, their results also showed that SARS-CoV-2 RNA decayed faster in untreated wastewater than in tap water, indicating the role of the matrix in accelerating virus decay (Ahmed et al., 2020b). Combined with the above factors, we developed a modified model to estimate the number of infection cases for both communities and WWTPs level based on the viral RNA concentration in wastewater.

Endogenous biomarker is essential to process control of RNA extraction of SARS-CoV-2. PMMoV and CrAssphage displayed less variation and were traditionally used in wastewater samples (Greenwald et al., 2021). However, the use of PMMoV and CrAssphage for normalization of viral load of SARS-CoV-2 in wastewater is still challenging, as both viruses have different performance during the nucleic acid extraction process relative to SARS-CoV-2. (CrAssphage is DNA virus and PMMoV is non-enveloped virus). IBV is a non-pathogenic coronavirus for human, which is ideal as endogenous biomarkers for SARS-CoV-2. Afterwards, we obtained the viral load in wastewater and established the WWTP models.

By using the developed models, we evaluated the robustness of the models in Xi'an city and Nanchang city, which experienced outbreaks caused by two different SARS-CoV-2 variants. Both cities were inland city experienced similar scales of COVID-19 outbreaks. Overall, the WWTP model was robust for both variants, indicating the applicability of this approach. This is probably due to the wide range of input parameters covering the virus load in stool and urine. In Xi'an city, modeling results suggested that developed models were robust for predicting the infected cases in both metropolitan and communities. Moreover, SARS-CoV-2 was detected 4 days earlier than the clinical data were reported in Xi'an city, suggesting that SARS-CoV-2 has circulated in the local communities before massive testing started on December 18th. Likewise, the WWTP model also provided an accurate estimation for the "real-time" infected cases in Nanchang city. Meanwhile, community wastewater surveillance was also conducted in the 40 communities that were not 100% covered by massive tests to identify the omitted hidden cases. Out of 40 communities, two communities were SARS-CoV-2 positive after four rounds of massive testing.

Above results suggested that quantitative wastewater surveillance could play essential roles in the initial, development and closure stages during the COVID-19 epidemics. In the initial stage, wastewater surveillance could provide early signals and promote the rapid response for tracing the carriers. In the development stage, quantitative wastewater surveillance could estimate the number of infected persons and give promptly feedback regarding the efficiency of the control measures. At the closure stage, combination of WWTP and community wastewater surveillance could confirm whether there are the omitted infected cases not tested, which promoted the precise cancelling of the community lock down.

Based on above results, we further proposed a new strategy to contain and eliminate the SARS-CoV-2 outbreak by employing wastewater surveillance together with regional lockdown to reach a precise epidemic control during a large outbreak (Fig. 4). Firstly, routine wastewater surveillance is conducted twice a week in WWTP to monitor the early signals of SARS-CoV-2. Once WWTP sample is positive, the “real-time” number of infected persons was predicted. Meanwhile, an emergent reaction was triggered: lockdown measures were taken for the catchment basin covered by WWTPs and massive nucleic acid tests was conducted to trace the majority of the carriers. After four rounds of massive nucleic acid tests and three rounds of community wastewater surveillance, the lockdown can be canceled if both wastewater surveillance and massive tests are negative, which can minimize the disturbance of daily live. The lockdown is only extended for a few communities.

There are three limitations of this study. First, SARS-CoV-2 was replaced by IBV to understand the dynamics of CoV in the wastewater pipeline. Ideally, it would be much better to use intact SARS-CoV-2 seeding in the pipeline; however, this is not possible due to high biological safety level requirements for working with SARS-CoV-2 in a pilot-scale RWS.
Therefore, a comparative study using deactivated SARS-CoV-2 as seeding material is needed to assure the reproducibility of the modeling. However, the results from Ahmed et al. (2020b) showed that the decay rates of MHA and SARS-CoV-2 were comparable across all treatments with no significant difference, indicating the feasibility of using other CoVs for such analysis. Nevertheless, comprehensive comparative studies are needed to reach a consensus on the surrogate virus used in modeling.

Another limitation is that there is also significant uncertainty and variations in the model input parameters, especially for the distribution of SARS-CoV-2 RNA concentrations in stool and urine and the volume of excreta produced to the sewer system. This paper is largely limited to currently available data, and future modeling with updated input parameters with more specific and representative data would further improve the accuracy. However, uncertainty and variations do not preclude the usefulness of the Monte Carol simulation to infer the person infected. Despite the high variability within and between individuals, both the community model and the WWTP model achieved reasonable predictions for the infection cases.

Finally, it is also worth noting that the sensitivity of wastewater surveillance is not 100%. Our study found that when wastewater was SARS-CoV-2 negative in WWTP, we still are not able to guarantee that there is no sporadic case in the catchment basin. In Nanchang, results also showed that three SARS-CoV-2-positive communities were not first detected by wastewater surveillance. Thus, rapid and sensitive detection process for SARS-CoV-2 would further improve the wastewater-based early-warning (Chen et al., 2021; Zhu and Zhou, 2022). Additionally, due to limitations of labor, we only monitored SARS-CoV-2 in WWTP once a week or every 4 days, which missed a few significant trendlines. Previous studies suggest that the minimum sampling frequency for SARS-CoV-2 wastewater surveillance is at least twice a week (Zheng et al., 2022). In the future, we will sample the WWTP every two days to track the pandemic dynamic and investigate the detection limits of wastewater surveillance in WWTP.

Nevertheless, as omicron variant has high basic reproductive number, wastewater surveillance still has realistic significance. As there is a very high demand for daily necessities, long-term lockdown would have huge impacts on the daily live and supply chain in the downtown (Liu et al., 2022). How to minimize the lockdown regions became an urgent issue. Combination of massive nucleic acid test with wastewater surveillance at WWTP and communities, the health authority could minimize the public health interventions at the whole metropolitan level. Instead, precise epidemic control at the community level could minimize the disturbance for the daily life which would be cost-effective and labor-saving.

5. Conclusion

In this study, to better predict the infection cases, we examined the effects of wastewater transportation in the pipeline on the decay of CoV. Next, a revised model was developed to predict the number of infected
persons in the Xi’an and Nanchang city. Our results clearly showed that most predictions are in reasonable agreement with clinical reports. Finally, the combination of wastewater surveillance and regional lockdown in Nanchang city highlighted the value of quantitative wastewater surveillance for precise epidemic control of COVID-19.

CRediT authorship contribution statement

Songzhe Fu and Zhiguan Qiu designed and analyzed the results. Qingyao Wang, Fenglan He, Rui Wang, and Wen Xia performed the experiments. Wentao Song conducted the epidemiological investigation. Songzhe Fu and Zhiguan Qiu were involved in conceptualizing, writing original draft, revising and editing the manuscript.

Data availability

Data will be made available on request.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.153250.

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