Araştırma Makalesi/Original Article
Makale Dili "İngilizce"/Article Language "English"

Türk Hijyen ve Deneysel Biyoloji Dergisi

Determination of the presence of SARS-CoV-2 in wastewater samples taken from wastewater treatment plant in Ankara province (October 2020-April 2021)

Ankara ili atık su arıtma tesisinden Ekim 2020-Nisan 2021 tarihleri arasında gönderilen atık su numunelerinde SARS-CoV-2 varlığının tespiti

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ABSTRACT

Objective: COVID-19 is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that has spread globally. Although respiratory droplets and aerosols are the primary routes of transmission, the detection of SARS-CoV-2 RNA in the feces of symptomatic and asymptomatic individuals has raised the possibility of fecal-oral transmission and demonstrated the potential for monitoring the virus in municipal wastewater. In this context, wastewater-based epidemiology (WBE) has gained importance as an early warning approach that can help monitor viral circulation in communities and identify changes in infection prevalence. This study aimed to investigate SARS-CoV-2 RNA in municipal wastewater in Ankara and evaluate the relationship between viral concentrations in wastewater and the course of the pandemic.

Methods: The study was conducted at the Ankara Central (Tatlar) Wastewater Treatment Plant, which serves eight central districts of Ankara. A total of 23

ÖZET

Amaç: COVID-19, şiddetli akut solunum yolu sendromu koronavirüsü 2'nin (SARS-CoV-2) neden olduğu ve küresel ölçekte yayılan bir enfeksiyon hastalığıdır. Başlıca bulaşma yolu solunum damlacıkları ve aerosoller olmakla birlikte, semptomatik ve asemptomatik bireylerin dışkılarında SARS-CoV-2 RNA'sının saptanması, dışkı-ağız yoluyla bulaşma olasılığını ve şehir atık sularında virüsün izlenebilirliğini gündeme getirmiştir. Bu kapsamda atık su temelli epidemiyoloji (wastewater-based epidemiology, WBE), toplumdaki virüs dolaşımının izlenmesine ve enfeksiyon yaygınlığındaki değişimlerin belirlenmesine yardımcı olan bir erken uyarı yaklaşımı olarak önem kazanmıştır. Bu çalışmada, Ankara'daki şehir atık sularında SARS-CoV-2 RNA'sının araştırılması ve atık sulardaki virüs konsantrasyonlarının salgının seyriyle ilişkisinin değerlendirilmesi amaçlanmıştır.

Yöntem: Çalışma, Ankara'nın sekiz merkez ilçesine hizmet veren Ankara Merkez (Tatlar) Atık Su Arıtma Tesisi'nde gerçekleştirilmiştir. Ekim 2020-Nisan 2021

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Geliş Tarihi / Received : 14.10.2025
Kabul Tarihi / Accepted : 20.04.2026

DOI ID : 10.5505/TurkHijyen.2026.61224

Şenses Ergül Ş, Eriş Zİ, Semizoğlu Ö, Karadeniz Dursun F, Namlı Bozkurt EN, Özcan N. Determination of the presence of SARS-CoV-2 in wastewater samples taken from wastewater treatment plant in Ankara province (October 2020-April 2021). Turk Hij Den Biyol Derg, 2026; 83(3): 239 - 250

influent wastewater samples were collected from the plant between October 2020 and April 2021. The samples were transported to the laboratory under cold-chain conditions, and viral particles were concentrated using the polyethylene glycol (PEG) 8000 precipitation method. RNA was extracted using the IDEXX Water DNA/RNA Magnetic Bead Kit. SARS-CoV-2 RNA was analyzed by real-time reverse transcription-polymerase chain reaction using the IDEXX Water SARS-CoV-2 RT-PCR Test and a Rotor-Gene Q instrument. Quantification was performed using a three-point standard curve prepared with the ATCC VR-3276SD synthetic SARS-CoV-2 RNA standard over a range of 10^3-10^5 copies.

Results: SARS-CoV-2 RNA was detected in 13 (56.5%) of the 23 wastewater samples examined. Viral concentrations in the positive samples ranged from 13.67 to 628.65 copies/L. The highest concentration was detected in October 2020, whereas the lowest was detected in January 2021. SARS-CoV-2 RNA was not detected in samples collected between January 6 and March 18, 2021, coinciding with a decline in the number of cases reported nationwide. In late March and April 2021, SARS-CoV-2 concentrations in wastewater increased again in parallel with the rise in reported case numbers.

Conclusion: The findings demonstrated that SARS-CoV-2 RNA could be detected in municipal wastewater and that WBE could serve as an effective complementary approach to outbreak surveillance. Considering that chemicals such as detergents and disinfectants in wastewater may interfere with viral detection, increasing sample volumes and improving concentration methods are recommended. Routine implementation of wastewater-based epidemiological surveillance may contribute to the early detection of pathogens with outbreak potential and the evaluation of the effectiveness of public health interventions. Standardized and systematic studies are needed to integrate this approach into epidemiological surveillance systems.

Key Words: SARS-CoV-2, wastewater, virus, outbreak

tarihleri arasında tesisten toplam 23 giriş suyu örneği alınmıştır. Soğuk zincir koşullarında laboratuvara ulaştırılan örneklerdeki virüs partikülleri polietilen glikol (PEG) 8000 çöktürme yöntemiyle yoğunlaştırılmıştır. RNA izolasyonu IDEXX Water DNA/RNA Magnetic Bead Kit kullanılarak gerçekleştirilmiş; SARS-CoV-2 RNA'sı, IDEXX Water SARS-CoV-2 RT-PCR Test ve Rotor-Gene Q cihazı kullanılarak gerçek zamanlı ters transkripsiyon-polimeraz zincir reaksiyonu yöntemiyle analiz edilmiştir. Nicel değerlendirme, ATCC VR-3276SD sentetik SARS-CoV-2 RNA standardı kullanılarak 10^3-10^5 kopya aralığında hazırlanan üç noktalı standart eğri üzerinden yapılmıştır.

Bulgular: İncelenen 23 atık su örneğinin 13 (%56,5)'ünde SARS-CoV-2 RNA'sı saptanmıştır. Pozitif örneklerdeki virüs konsantrasyonları 13,67-628,65 kopya/L arasında değişmiştir. En yüksek konsantrasyon Ekim 2020'de, en düşük konsantrasyon ise Ocak 2021'de belirlenmiştir. 6 Ocak-18 Mart 2021 tarihleri arasında alınan örneklerde SARS-CoV-2 RNA'sı saptanmamış ve bu dönem ülke genelindeki vaka sayılarındaki azalmayla paralellik göstermiştir. Mart sonu ve Nisan 2021'de vaka sayılarının artmasına paralel olarak atık sulardaki SARS-CoV-2 konsantrasyonlarının yeniden yükseldiği gözlenmiştir.

Sonuç: Şehir atık sularında SARS-CoV-2 RNA'sının saptanabildiğini gösteren bulgular, WBE'nin salgınların izlenmesinde kullanılabilecek etkili bir tamamlayıcı yaklaşım olduğunu ortaya koymuştur. Atık sularda bulunan deterjan ve dezenfektan gibi kimyasalların virüsün saptanmasını güçleştirebileceği dikkate alınarak örnek hacminin artırılması ve yoğunlaştırma yöntemlerinin geliştirilmesi önerilmektedir. Atık su temelli epidemiyolojik izlemin rutin olarak uygulanması, salgın potansiyeli taşıyan patojenlerin erken dönemde saptanmasına ve halk sağlığı müdahalelerinin etkinliğinin değerlendirilmesine katkı sağlayabilir. Bu yaklaşımın epidemiyolojik izlem sistemlerine entegrasyonu için standartlaştırılmış ve sistematik çalışmalara gereksinim bulunmaktadır.

Anahtar Kelimeler: SARS-CoV-2, atık su, virüs, salgın

INTRODUCTION

Coronavirus disease-2019 (COVID-19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) belonging to the species SARS-related coronavirus in the subgenus Sarbecovirus of the family Coronaviridae containing enveloped viruses with positive sense single stranded RNA (1). COVID-19 was first detected in Wuhan, China in December 2019. This disease was later reported in 114 countries and therefore the World Health Organization declared COVID-19 as a pandemic on 11th March 2020 (2,3). As of 29th March 2023, the disease has affected 761,402,282 individuals and 6,887,000 deaths were reported worldwide (3,4).

In December 2019, cluster of pneumonia like disease cases with symptoms including fever, difficulty in breathing, cough and invasive lesion on both lungs were reported from Wuhan, China (4). First COVID-19 case was reported in Turkey on 11th March 2020. SARS-CoV-2 is a member of the family Coronaviridae that consists of a group of enveloped viruses with single-stranded RNA genome, which cause diseases ranging from common colds to acute respiratory distress syndrome (5). The virus is transmitted from person to person through droplet infections i.e. when infected person is in close contact with another person. For the patients infected with SARS-CoV-2, various gastrointestinal symptoms such as diarrhea, vomiting and abdominal pain are also being reported in addition to fever and respiratory symptoms (6).

The primary routes of viral transmission (SARS-CoV-2) was considered to be through droplet infections and person to person close contact, but later it is evident from various published studies that there is increasing possibility of fecal-oral transmission, however, environmental conditions that could facilitate faecal oral transmission is not yet clear (7,8).

There has been growing evidence of gastrointestinal symptoms caused by SARS-CoV-2 infections and the presence of viral RNA not only in feces of infected individuals but also in wastewater. In January 2020, first report of detection of SARS-CoV-2 in faeces, has made it clear that human wastewater might contain this virus. This may illustrate the probability of environmentally facilitated transmission, mainly the sewage, however, environmental conditions that could facilitate faecal oral transmission is not yet clear (1). Studies have shown the survival of SARS-CoV-2 in stool for up to 4 days (9). Viral shedding of SARS-CoV-2 in stool samples occurs in a substantial proportion of patients, making faecal-oral transmission plausible. Furthermore, detection in stool samples or anal swabs can persist long after negative respiratory testing (10).

The growing corpus of scientific evidence highlights the potential of wastewater-based epidemiology (WBE) as a useful approach for determining disease incidence in different populations. WBE offers valuable insights and addresses surveillance challenges that routine clinical monitoring encounters. Human excretions, comprising urine, feces, mucus, saliva, etc., are a significant anthropogenic contributor to wastewater, primarily directed into septic tanks and sewer lines. These excretions, especially from infected individuals, may contain biological markers of the causative agents of various diseases, including respiratory viruses. Sewer lines are the first point in the wastewater drainage and treatment system where the detection of viruses can occur, often before the diagnosis of clinical cases. Sewer systems transport the drainage water and waste matter to wastewater treatment plants (WWTPs) for treatment before releasing it into the environment. Despite the apparent usefulness of wastewater-based monitoring, it does not provide insights into individual disease severity. Nevertheless, it offers the advantage of being unbiased, as it is not influenced by variations in access to clinical testing and health-seeking behavior (11).

Wastewater-based epidemiology (WBE) serves as an important tool to trace the circulation of viruses in a community, providing opportunities to estimate their prevalence, genetic diversity, and geographic distribution (12,13). The importance of wastewater surveillance is also highlighted by its ability to

detect low levels of viruses; this can happen when the number of infected cases is decreasing following public health interventions, which has been the case for successful examples such as poliovirus eradication programs (14). It is also useful to determine when a new virus is introduced into a population (15,16) or when fluctuations occur due to changes in seasons or precipitation (17-19). Thus, such a surveillance strategy can be useful as an "early warning" system (13) to determine if re-introduction of SARS-CoV-2 had occurred in a community. The aim of the present study was to investigate the presence of SARS-CoV-2 in wastewater samples during the outbreak in Ankara Province.

MATERIAL and METHOD

Ankara was selected as pilot city for this study. Samples were collected from the influent of the treatment plant serving the 8 central districts. The wastewater samples used in this study were collected from Ankara Central (Tatlar) Waste Water Treatment Plant. The wastewater of 8 central districts (Cankaya, Kecioren, Yenimahalle, Mamak, Etimesgut, Sincan, Altindag and Golbasi) are collected in this Plant. (Figure 1). During the study, wastewater samples collected between October 2020 and April 2021 were used.

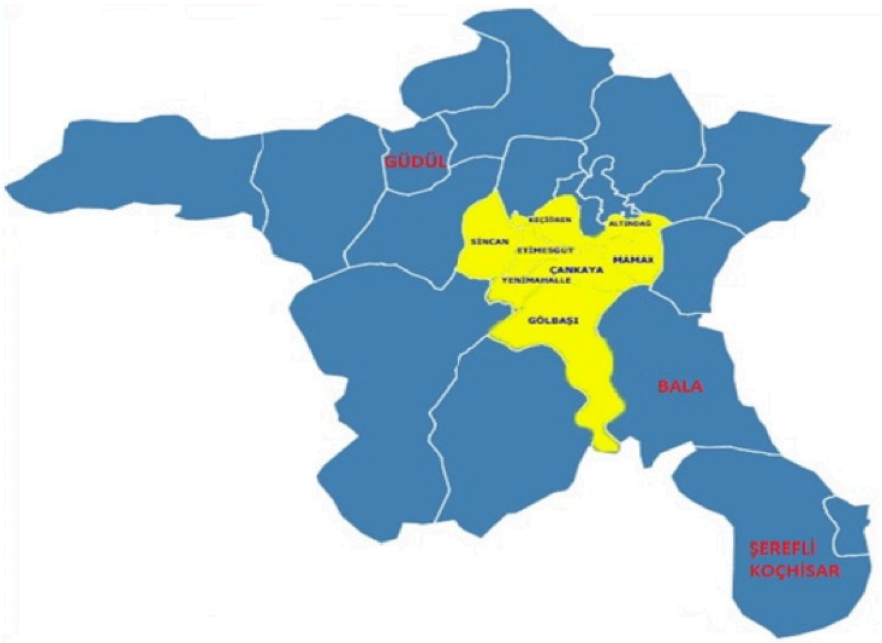

Figure 1. In the area marked with yellow, 8 central districts connected to the Ankara Central (Tatlar) Wastewater Treatment Plant are shown

Detection of SARS-CoV-2 from wastewater samples PEG 8000 method was used. The applicability of the method was firstly tested by artificially inoculated wastewater samples with virus strain produced by cell culture method at National Virology Refrence Laboratory of General Directotae of Public Health. During analyses biosafety and biosecurity protocols determined by CDC for SARS-CoV-2, was followed (Figure 2).

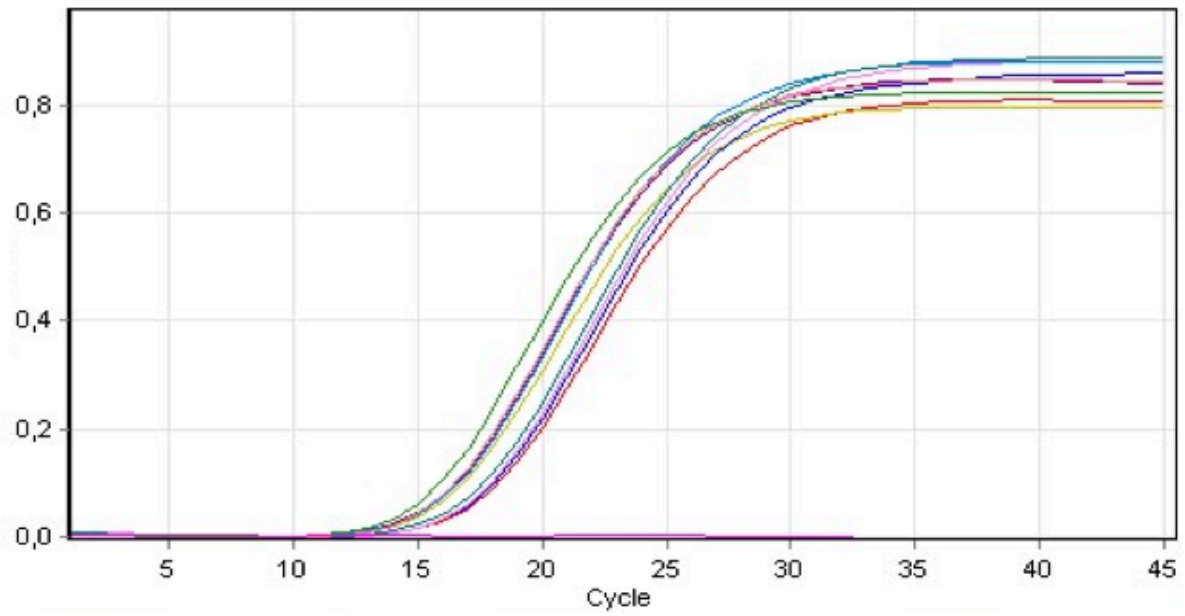

Figure 2. Applicability of PEG 8000 method in the laboratory

During the study, the wastewater samples were delivered to the laboratory on the day it was taken, in a plastic sample container, in the cold chain. Samples were obtained as 1 L from the inlet of the wastewater plant. The outer surface of the sample containers containing waste water was decontaminated by wiping with 70% alcohol. As the first step of the analyses, wastewater samples were incubated at 60°C for 1.5 hours. After incubation, before starting the concentration process of the wastewater, it was cooled to 2-8°C and mixed by turning it upside down. Then, samples were centrifuged for 30 min in a swinging bucket rotor at 4700xg at 4°C. The resulting supernatants were taken into a clean tube and 3.5 g of PEG 8000 and 0.788 g of NaCl were added. Sample tubes were centrifuged at 12000xg for 120 minutes at 4°C in a fixed angle rotor. The supernatant formed at the end of the process was removed without damaging the pellet, and the pellet was dissolved in 400 µL of nuclease-free water and was ready for genetic analysis.

SARS-CoV-2 RNA isolation was carried out in accordance with the instructions of the manufacturer of the IDEXX Water DNA/RNA Magnetic Bead Kit (IDEXX 98-0014719-00, Germany). This process was followed by Real-Time PCR analysis performed with IDEXX Water SARS-CoV-2 RT PCR kit (IDEXX 98-0014718-00, Germany) and Qiagen RotorGeneQ instrument (Qiagen, Germany). In addition, quantitation was performed with the standard ATCC VR-3276SD Quantitative Synthetic SARS-CoV-2 RNA: ORF, EN of SARS-CoV-2, and parallel dilutions containing 10^3-10^5 copies were prepared in accordance with the manufacturer's instructions, and the analysis was performed by creating a three-point standard curve. During the study all analyses were performed in parallel.

RESULTS

The applicability of PEG 8000 method to detect SARS-CoV-2 in waste water samples were performed in 4 replicates each composed of 2 parallel studies (Figure 2). Afterwards, total of 23 waste water samples obtained from Ankara Central (Tatlar) Waste Water Treatment Plant between October 2020 and April 2021 were analyzed in means of SARS-CoV-2

presence. Of the analyzed 23 samples, 13 of them were found positive. In Figure 3, the results of SARS-CoV-2 anaylsis of samples were shown. Positive samples were quantified with ATCC VR-3276SD quantitative synthetic SARS-CoV-2 RNA. In Figure 4, the quantitative evaluation of SARS-CoV-2 presence in analysed wastewater samples were presented. As it can be seen from Figure 4, the SARS-CoV-2 concentration of the positive wastewater samples were between 13,67-628.65 copies/L. While the minimum concentration was determined on January 2021, the maximum SARS-CoV-2 concentration was detected on October 2020. As it was shown on Figure 4 the amount of SARS-CoV-2 detected in wastewater was started to decrease since mid-November 2020, and it was on an upward trend since mid-March 2021. In addition, negative results for SARS-CoV-2 were obtained in the wastewater samples sent to the laboratory between 06.01.2021 and 18.03.2021.

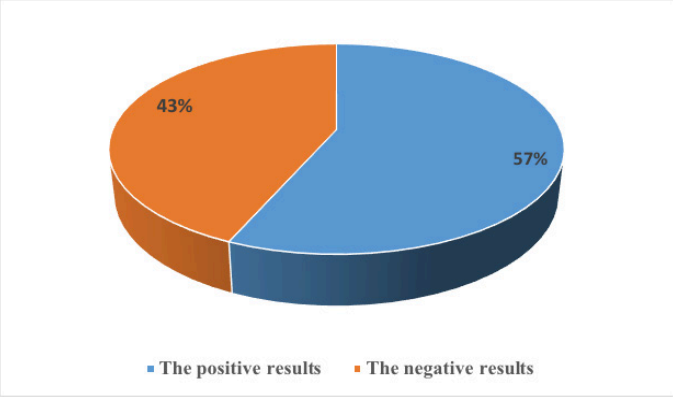

Figure 3. Real-Time PCR investigation for the presence of SARS-CoV-2 in wastewater samples taken from Ankara water treatment plant between October 2020 and April 2021

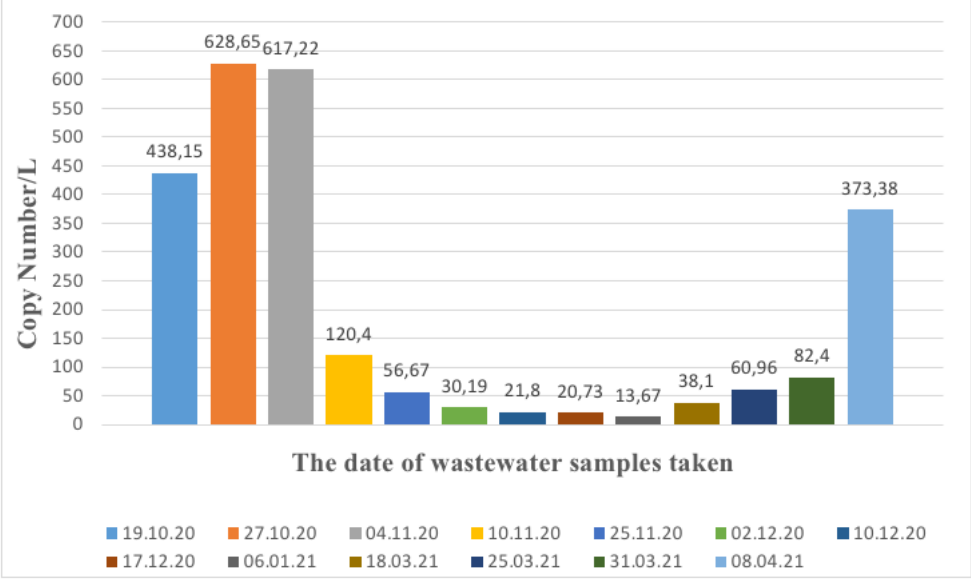

Figure 4. The quantitative evaluation of SARS-CoV-2 presence in analysed wastewater samples

DISCUSSION

Sewage systems, where instant data related to public health can be collected, are considered advantageous when appropriate markers are selected to monitor viral epidemiology. Wastewater Based Epidemiology (WBE), based on biological, chemical monitoring of the sewage system, has been applied in communities worldwide to study the emergence and epidemiology of pathogenic viruses such as polioviruses, noroviruses and hepatitis A (20,21). The reason for this is shown as; patients not applying to the hospital and the number of cases that have their diseases at home may be quite high (22). When monitoring for SARS-CoV-2 in wastewater was first proposed, the prevailing scientific opinion was that the virus might not be present in wastewater at sufficiently high concentrations, and that detection of both the virus itself and single-stranded RNA (ssRNA) in wastewater could be highly variable (20).

The ongoing global pandemic of coronavirus disease 2019 (COVID19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been a public health emergency of international concern (23,24). It provides a meaningful opportunity to test the hypothesis that WBE can be used to detect and manage communicable disease transmission in populations (18). SARS-CoV-2 ribonucleic acid (RNA) has been reported to be detected in feces of not only symptomatic but also asymptomatic patients. These clinical observations suggest that municipal wastewater in affected communities may contain the virus. WBE is therefore a promising approach to understand the prevalence of viruses in a given wastewater treatment plant catchment population, because wastewater contains viruses excreted from symptomatic and asymptomatic individuals in a catchment (21) Although the main transmission routes of SARS-CoV-2 are known as inhalation of infective droplets and direct contact (20,25), another important transmission route is the fecal distribution as a result of SARS-CoV-2 replication in the gastrointestinal system (26-29) . The virus causes epidemics by the fecal-oral route, which results in many sporadic cases and can even be fatal (21,30,31).

Many research groups around the world have taken action to monitor SARS-CoV-2 RNA in wastewater for this purpose (18). Several studies have reported the presence of SARS-CoV-2 in municipal wastewater in countries including Australia (21), Spain (26), Italy (28), the Netherlands (29), Japan (30) and Türkiye (31). These studies were planned either to monitor the virus in wastewater, to obtain information about the distribution and course of the epidemic in the country, and to take necessary measures in the light of the data obtained (32,33). According to the literature; although virus concentration methods varied among studies, it has been reported that the duration of SARS-CoV-2 shedding in stool samples is longer than in respiratory and serum samples (34).

In a study performed in Australia (21) it was reported that two out of six samples of waste water taken within a six-day period, were found positive in means SARS-CoV-2. Also it was emphasized in the study that one of the biggest challenges of quantitative determination of the viral RNA concentrations measured in the sewage is to predict the actual numbers of cases in the community (21). Similarly, the percentage of SARS-CoV-2 positive waste water samples were found as; 43% in the present study (Figure 3).

According to recent studies, there is evidence that SARS-CoV and MERS-CoV survive under different environmental conditions. The first reports of SARSCoV RNA in wastewater were obtained from studies conducted at different hospitals in Beijing during the 2003 SARS outbreak. It was reported that the coronavirus could survive in wastewater from a few hours to a few days, depending on the temperature and the amount of solid matter. As it was stated in the previous studies the coronavirus persists in raw wastewater for 2 days at 20◦C and for 14 days at 4◦C. However, it was reported that suspended solids in wastewater provide protection against the virus. Therefore the presence of coronavirus in raw

wastewater more than pre-treated wastewater is stated to be related with its preservation by suspended solid material in raw waste water (25).

SARS-CoV-2 viral ssRNA fragments detected in the wastewater system is accepted as the indicator of the virus entering the sewage system through human feces and urine (20). In the researches performed to qualify SARS-CoV-2 ssRNA, it was reported that the amount of it is almost close to the amount in sputum, was tested positive in stool samples at a rate of 67% (34, 35). The data found is important because active replication of viruses within the gastrointestinal system is known as a significant factor affecting the spread of infective virions through wastewater (20).

In a previous study, virus concentrations of the samples taken from patients at hospital and the wastewater system of the hospital, were compared. It was mentioned that virus concentrations of samples taken from patients were even higher (>106 copies mL-1) compared to ssRNA of the wastewater system. Therefore, it is not surprising that hospital wastewater tests positive for SARS-CoV ssRNA, but not for infective virions (34). Besides, the lower concentration of infective virions in hospital wastewater can be attributed to the lower detection limit due to the heavy use of disinfectants and various surfactants

Randazzo et al. (26) reported that SARS-CoV-2 RNA were detected in wastewater samples taken during the earliest stages of the epidemic in Valencia, Spain when communicated cases in that region were only incipient. Also the data in the study showed that the wastewater viral RNA context increased rapidly and anticipated the subsequent ascent in the number of declared cases (26).

Various virus concentration techniques have been developed and applied for optimum recovery of viruses from wastewater. When developing and refining virus concentration methods, it is crucial to consider factors such as recovery efficiency, simplicity, cost, time taken to process wastewater samples, analysis of sample volume, availability of reagents, and scalability. Among the several virus concentration methods (e.g. adsorption to an electronegative filter membrane in the presence of a cation or an electropositive filter membrane, ultrafiltration, centrifugation, ultracentrifugation, precipitation using aluminum hydroxide adsorption or activated charcoal or moringa seeds or Skimmed-Milk, PEG, magnetic nanoparticle separation), PEG precipitation is widely used for concentrating viruses in wastewater due to its simplicity, cost-effectiveness, and efficiency. The PEG method is well established for its ability to concentrate viral particles and its cost-effectiveness. While PEG precipitation is slightly more time-consuming due to the centrifugation and precipitation steps, it offers high recovery rates for non-enveloped viruses like norovirus. On the other hand, the NMP method is faster and requires minimal handling, with its efficiency being especially notable for enveloped viruses, such as SARS-CoV-2, although the mechanism responsible this improved performance was not assessed in this study (27).

Quantitative determination of the amount of SARS-CoV-2 in wastewater is still important for wastewater-based epidemiological research. Of the analyzed 23 samples taken in Ankara between October 2020 and April 2021 in the study, 13 of them were found positive. While the minimum concentration (13,67 copies/mL) of SARS-CoV-2 was determined on January 2021, the maximum SARS-CoV-2 concentration (628,65 copies/mL) was detected on October 2020. In literature it was also mentioned that the presence of chemicals like detergents, disinfectants etc. in wastewater can negatively affect virus viability. Both positive and negative effects of the composition of waste water should be considered during epidemiological researches.

In a research performed with a similar methodolgy in Italy (28), the percentage of SARS-CoV-2 positive results in waste water were also similar with our data. Also in another research, sewage samples of six cities in Netherlands were analyzed in means of SARS CoV-2 RNA. It has been reported that no SARS-CoV-2 RNA was detected, 3

weeks before the first Dutch case was reported and detection of the virus RNA in sewage, even when the COVID-19 prevalence is low, could be achived with concentrations of 2.6 – 30 gene copies per mL (29).

Also in a study performed in Japan (30), SARS-CoV-2 detection methods in wastewater samples were compared and quantity of the virus RNA (copies/L) detected were found to increase with the increase in sample volume filtrated.

The positive correlation between the concentration of SARS-CoV-2 gene copies in wastewater and the number of confirmed clinical cases reinforces the potential of wastewater surveillance as an early indicator of community infection levels (36).

Sewage surveillance relies on the assumption that there is a, at least semi-, quantitative relation between the circulation of SARS-CoV-2 in the population and the concentration of SARS-CoV-2 RNA in wastewater. The number of reported COVID19 cases is an underestimation of the true number of infected individuals, as not everybody is tested. Hence, the reported laboratory-confirmed case numbers are an underestimate of the number of virus shedders in the population. The number of people shedding virus in the population will depend on the number of infections and the duration (number of days) over which each individual sheds RNA. Seroprevalence studies provide more complete data on the number of infected individuals and demonstrate that reported case numbers based on rhinopharyngeal swab testing can, depending on the testing policy, vastly underestimate the true percentage of the population that is infected with SARS-CoV-2. Infectious disease models have been generated to estimate the number of infectious individuals in a population, extrapolating via the case fatality ratio or infection fatality ratio (https://ourworldindata.org/covid-models). Seroprevalence data and epidemiological model prevalence estimates have not been used in sewage surveillance studies to relate to SARS-CoV-2 wastewater concentrations, but would provide better estimates of the true number of infectious individuals in a population. In future modelling approaches, these prevalence data can be used as priors, which can be updated using wastewater-based estimates, or vice versa (37).

In Türkiye, the number of clinical cases were announced as; two hundred thousand in November 2020 and decreased to around one hundred thousand at the end of December, followed by a decrease to fifty thousand in January and February of 2021 considering the number of cases reported on the official website of the Ministry of Health (38). Also it was reported that the number of cases, was started to rise since the beginning of March, reached four hundred thousand in April according to the SARS-CoV-2 test results conducted throuhout the country.

Considering the sampling dates of the wastewater samples used in this study; it has been observed that the change in the number of cases across the country is also reflected in the results of the SARS-CoV-2 analysis in wastewater (Figure 3). Low SARS-CoV-2 concentration was found in the wastewater sample sent on January 06, 2021. Negative results were obtained in the wastewater samples sent to our laboratory from mid-January to early March, in consistence with the decreasing in the clinical cases. In parallel with the high number of clinical cases in October and November of 2020 and in March and April of 2021, high copy numbers of SARS-CoV-2 were determined in wastewater samples analysed.

In conclusion; although the data obtained in this study are similar to the data in the literature, the study is original as it includes data from the Ankara region. When both the study results and current literature are evaluated, it is evident that the number of cases and the virus concentrations in the wastewater can be harmonized and utilized in epidemiological outbreak studies. However, it should be taken into consideration that the virus concentration in wastewater may be reduced due to the inhibitory effect of chemicals such as detergents and disinfectants, present in wastewater, and therefore larger sample volumes should be analyzed to overcome the challenges of detectability.

The studies conducted to detect SARS-CoV-2 have demonstrated the important role of WBE as a simple, cost-effective, and non-invasive approach for monitoring the spread of SARS-CoV-2 within communities (39). Moreover WBE is particularly valuable for early warning of disease outbreaks and for assessing the effectiveness of public health interventions, as previously shown for enteric viruses, such as norovirus, hepatitis A virus, and poliovirus, and now for SARS-CoV-2. Nevertheless, further systematic research is required covering aspects from effective sampling and preservation to data interpretation. In the absence of a vaccine or efficient treatment, cost-effective epidemiological surveillance strategies remain urgently needed. Future studies should investigate seasonal variations in viral shedding, optimize detection methods to improve sensitivity, and explore the relationship between wastewater viral loads and disease transmission in order to enhance pandemic preparedness and response. Routine implementation of WBE tool would significantly strengthen communities' preparedness against new or re-emerging viral outbreaks.

ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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