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Presence of SARS-CoV-2 in wastewater from handwash stations in selected facilities in Ghana

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Abstract

Background: Following the COVID-19 pandemic, the occurrence of the causative agent, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), in water, has been reported as handwashing was recommended as a non-pharmaceutical tool to limit the spread of the virus. Wastewater from these handwash stations is discarded without proper guidelines and could end up in the environment, serving as a dissemination route for SARS-CoV-2.

Objective: This study investigated the potential role of wastewater from handwash stations in the transmission of SARS-CoV-2 in Ghana.

Methods: A total of 390 water (195 reservoir water and 195 wastewater) samples from handwash stations were collected and analysed from selected schools, commercial banks, and hospitals in Accra, Ghana, between the 13th of June, 2022, and the 25th of August, 2022. Samples were first concentrated using the phase separation method before RNA extraction, and viral nucleic acid was amplified for SARS-CoV-2 detection using RT-PCR (N gene and ORF3a regions). Isolation of SARS-CoV-2 was performed for all 17 samples using VERO E6 cells.

Results: From the RT-PCR analysis, a total of 17 samples (4.4%) were positive for SARS-CoV-2 RNA. All 17 positive samples were wastewater samples. Propagation on Vero E6 cells yielded no cytopathic effect (CPE). Samples from schools had the highest positivity rate (15 out of 17), followed by the hospitals (2 out of 17) and the commercial banks (0 out of 17).

Conclusion: SARS-CoV-2 RNA detected in wastewater has a low likelihood of causing secondary infections in humans; however, the monitoring of SARS-CoV-2 in water matrixes could provide information on viral dynamics in the community.

Keywords: COVID-19, handwash stations, wastewater, reservoir water, GIDC

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INTRODUCTION

SARS-CoV-2 was detected in Ghana on the 12th of March 2020 [1], following its emergence in Wuhan, China, in 2019 [2]. SARS-CoV-2 is highly transmissible, and its transmission mainly occurs through exposure to respiratory droplets of disease carriers generated from sneezing and coughing [3]. Globally, and in Ghana in particular, there was introduction of some non-pharmaceutical measures as part of efforts to curb the spread of the infection. These included wearing face masks, using hand sanitisers, social distancing, a lockdown to restrict movement within some heavily affected parts of the country, a ban on international travel, and handwashing [4]. Over time, some of these restrictions have been eased while others, such as wearing face masks, the use of hand sanitiser and handwashing, are still being practised. Traces of SARS-

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CoV-2 RNA have been detected in different water matrixes [5-7], hence the potential role of water matrixes in the transmission of SARS-CoV-2 in the population. Since the beginning of the COVID-19 pandemic, non-pharmaceutical measures such as handwashing have been recommended to mitigate the spread of the virus. Handwashing is a simple but life-saving practice which sheds the virus down the drain. However, there are no proper guidelines for discarding and treating wastewater generated from these handwash stations, especially in low-income settings where wastewater treatment is inadequate or non-existent. The presence of trace SARS-CoV-2 RNA in water sources and their potential role in disease transmission, coupled with challenges with wastewater treatment in resource-limited settings, emphasises the need to investigate the role of SARS-CoV-2 virus transmission via wastewater. The study determined the presence of SARS-CoV-2 RNA in waste and reservoir water within Accra in the Greater Accra Region of Ghana.

MATERIALS AND METHODS

Study design and sites

This was a cross-sectional study carried out at selected schools, banks, and hospitals in Accra, Ghana, from the 13th of June 2022 to the 25th of August 2022 (Figure 1) based on the high enforcement of hand hygiene as the high human traffic at these sites is heavy. Again, the highest cases of SARS-CoV-2 were recorded in the Greater Accra Region.

Sample collection

Sterile containers were used to collect one litre each of reservoir water (n = 195) used for handwashing and its corresponding raw wastewater (n = 195) from handwash stations. Samples were collected in the mornings and afternoons using the grab sampling technique on each sampling day [8]. Samples were transported at 4℃ immediately to the laboratory and subsequently concentrated using the PEG-dextran phase separation method with slight modifications as described previously by Sharma et al. For each 1-litre sample collected, 500 ml was centrifuged at 1000 g for 20 minutes at 4℃. The supernatants were carefully poured into a 1-liter beaker, and 287 ml of 29% polyethylene glycol (PEG), 39.5 ml of 22% Dextran, and 35 ml of 5 N of NaCl were added. The mixture was stirred vigorously for 1 hour and poured carefully into a separation funnel, mounted on a burette stand in a vertical upright position, and left to stand overnight at 4℃. The funnel was checked for phase separation, and the entire lower phase and interphase were harvested in a drop-wise pattern into a sterile 50 ml centrifuge tube [9].

RNA Extraction and Detection of SARS-CoV-2 using RT-PCR

RNA extraction on the concentrated samples was done using QIAmp RNA extraction kit from Qiagen (QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany) and Real-time Polymerase Chain Reaction (RT-PCR) of SARS-CoV-2 was performed using Veri-Q nCoV-OM (MiCo BioMed Co., Ltd, Gyeonggi, Republic of Korea 13499) detection kit according to manufacturer protocols.

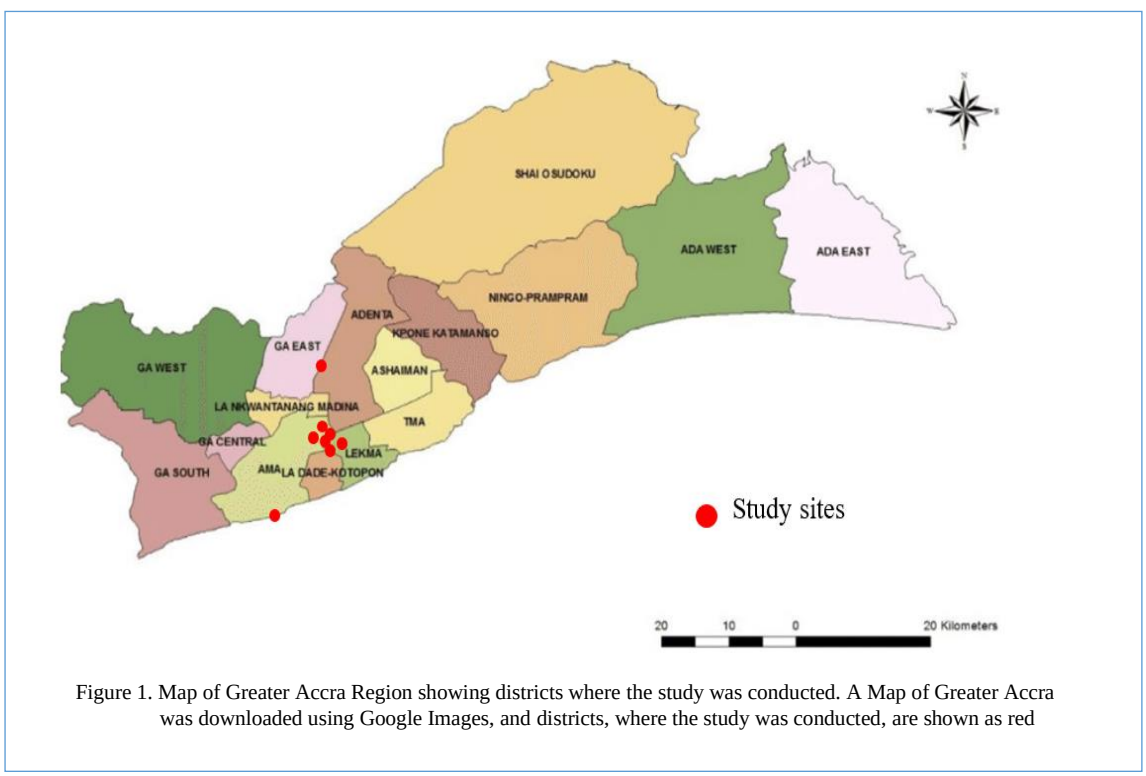

Figure 1. Map of Greater Accra Region showing districts where the study was conducted. A Map of Greater Accra was downloaded using Google Images, and districts, where the study was conducted, are shown as red

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Detection and Cultivation of SARS-CoV-2

Virus isolation was attempted for all positive samples using Vero E6 cells. Cells were cultured in complete Dulbecco Modified Eagle's Medium (cDMEM) as previously described by Rimoldi et al. (2020). The media was changed and replaced with fresh 5 mL DMEM (with no FBS/antibiotic Na-pyruvate and L-Glutamine) before infection attempts. VERO E6 cells were infected using 2 mL of eluent from processed contaminated water samples. Inoculated cells were incubated at 37°C, 5% CO2 atmospheric pressure for 72 - 96 hours. The cells were examined for cytopathic effect (CPE) every 24 hours.

RESULTS

Sampling was carried out in schools, commercial banks, and a COVID-19 treatment centre, Ghana Infectious Disease Centre (GIDC), in the mornings and afternoons of sampling days. A total of 195 samples each were obtained from reservoir and wastewater at hand washing stations, with 196 and 194 samples collected in the mornings and afternoons, respectively. A total of 64 samples were collected from banks, while 68 and 258 samples were from GIDC and schools, respectively. Table 1 shows the distribution of samples collected and the SARS-CoV-2 detection rates. A total of 15 out of 258 (5.8%) samples processed from schools tested positive for SARS-CoV-2 RNA, and two samples from the GIDC tested positive (2.9%) for SARS-CoV-2 RNA. None of the samples collected from the banks tested positive for SARS-CoV-2 (Table 1). It was observed that more samples collected in the morning were positive for SARS-CoV-2 RNA than those collected in the afternoon (p = 0.011). Of the 196 samples collected in the morning, 7.1% (n = 14) tested positive for SARS-CoV-2 RNA, and 1.5% (n = 3/194) tested positive for SARS-CoV-2 RNA for samples collected in the afternoon (Table 1). Again, a significant association was observed between sample types and SARS-CoV-2 positivity, with contamination occurring only in wastewater samples (8.7%, p = 0.001). However, we were not able to recover the virus from all RNA-positive samples. There was a significant difference in the SARS-CoV-2 positivity rate between samples collected from the schools in the morning and afternoon (Figure 2). Samples collected from the schools in the morning (12 out of 130, representing 9.2%) had a higher SARS-CoV-2 detection rate compared to samples collected from the schools in the afternoon (3 out of 128, representing 2.3%) (Table 1). There was no significant association between SARS-CoV-2 detection at GIDC and sampling time (Figure 2). Out of 34 samples collected in the morning at GIDC, 5.9% (n = 2) tested positive for SARS-CoV-2. None of the samples collected in the afternoon tested positive for SARS-CoV-2.

Table 1. Distribution of SARS-CoV-2 positivity rates by sample characteristics

Characteristics		SARS-CoV-2		
Sample site	Samples Processed	Number of positive samples	%	p-value
Schools	258	15	5.8%	0.103
*GIDC	68	2	2.9%	
Banks	64	0	0%	
Sample time				
Morning	196	14	7.1%	0.011
Afternoon	194	3	1.5%	
Sample type				
Reservoir water	195	0	0%	0.001
Wastewater	195	17	8.7%	
Total	390	17	4.4%	

*GIDC: COVID-19 treatment centre

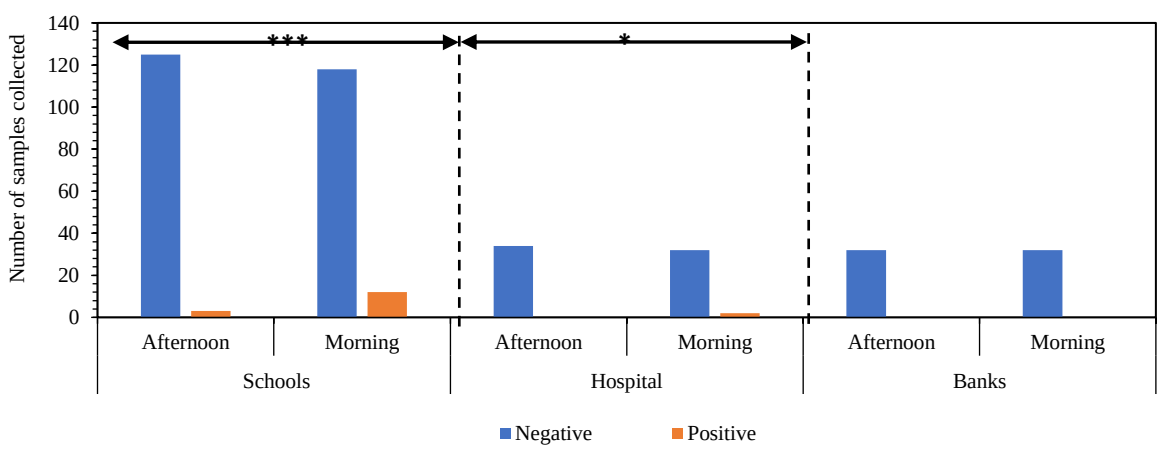

Figure 2. Detection of SARS-CoV-2 RNA based on sampling time at various study sites

*** denotes p-value < 0.001 and * denotes p-value > 0.1

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No sample from the bank tested positive for SAR-CoV-2 in the morning or in the afternoon (Table 1).

DISCUSSION

In this study, we sought to detect the presence of SARS-CoV-2 in water from handwash stations in selected facilities in the Greater Accra Region of Ghana. Since the beginning of the pandemic, SARS-CoV-2 has been detected in various water sources, particularly in wastewater. The COVID-19 pandemic in Ghana has been largely driven by cases from the Greater Accra region. It was imperative to determine this for Ghana. We collected samples from selected schools, a COVID-19 treatment centre and banks. Throughout the study, we collected a total of 390 samples, 190 from wastewater and their corresponding reservoir water. From this, the SARS-CoV-2 detection rate was 4.4 % (n = 17/390). We detected SARS-CoV-2 RNA in wastewater but not in reservoir water from handwash stations in Ghana. However, a viable virus was not recovered from cell cultures. The detection of SARS-CoV-2 RNA in wastewater is in line with growing evidence across the world [5-6,10-11], where SARS-CoV-2 has been detected in water matrixes. Additionally, the null detection of SARS-CoV-2 RNA in reservoir water reported in this study is similar to findings reported by Rimoldi et al. and Rosiles-González et al. [11-12]. This has several implications for public health and surveillance. The sampling site categories indicated that wastewater samples from schools had the highest SARS-CoV-2 RNA detection rate (5.8%), followed by the hospitals (2.9%), with samples from the banks recording no SARS-CoV-2 RNA [Table 1].

The COVID-19 vaccination program in Ghana currently covers people aged 15 and older, and most children in basic schools fall below this age bracket [13]. The lack of COVID-19 vaccination in most basic school children could explain why SARS-CoV-2 RNA was detected in high numbers in samples from schools. In accordance with these findings, an increase in COVID-19 cases involving children in Accra (18% to 20%) was reported in June 2022 [14]. This increase was related to outbreaks in schools in Accra, Ghana [15]. The significant association ($p < 0.001$) between samples collected in the morning and afternoon at the schools could be due to students' handwashing habits at specific times of the day rather than the temperature of the environment. Although studies have found that coronaviruses are affected by temperature [16-17], it was observed during the period of this study that there were high interactions and high compliance to hand hygiene among students in the morning, during morning break periods, compared with the afternoon in the schools even though compliance levels were not measured.

Hospitals are at the forefront of the treatment of diseases, including COVID-19. It is therefore not surprising that SARS-CoV-2 RNA was detected in samples from the hospital, albeit at a low level (2.9%) (Table 1) despite rigorous disinfection exercises. Interestingly, SARS-CoV-2 was detected in wastewater in the morning at the hospital but not in the afternoon. Considering that this hospital is a COVID-19 treatment centre, such low-level detection was interesting. It underscores the importance of the disinfection regime at the facility.

The null detection of SARS-CoV-2 RNA in samples processed from the commercial banks could be attributed to the combined use of alcohol hand rubs and handwash stations used in hand hygiene in all banks captured in this study. The synergetic action of the alcohol hand rubs and handwashing at the handwash stations could be accounting for the elimination of viral RNA in wastewater generated from these handwash stations. COVID-19 cases in the Ghanaian populace have generally remained low as compared to other countries, and with 28.7% of the total population estimated to be fully vaccinated [14], the low numbers of SARS-CoV-2 RNA detected are in conformance with the general case burden in the country. The inability to isolate viable, infective SARS-CoV-2 virus from culture even though SARS-CoV-2 RNA was amplified on RT-PCR suggests that surfactants used in handwashing are effective in disrupting virion structure and inactivating the virus; thus, SARS-CoV-2 RNA detected in wastewater has a low likelihood to retain its infectivity before contact cause infections in humans.

Conclusion

This study found that while SARS-CoV-2 RNA may be present in wastewater, it may not pose a significant risk for transmission through this route. Nevertheless, continued surveillance of wastewater samples is essential in tracking the prevalence of the virus and identifying potential outbreaks. Vaccination of children under 15 years in Ghana should be considered and implemented since the data illustrates that there was higher positivity at the basic schools (children between 4 - 16 years) compared to the other study sites.

DECLARATION

Ethical consideration

The study was approved by the Scientific and Technical Committee of the Noguchi Memorial Institute for Medical Research: STC Paper 6(4) 2021-22 (the 25th of May, 2022)

Consent to publish

All authors agreed to the content of the final paper.

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Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author contributions

Conceptualisation: IAA, MAAP; Funding acquisition: IAA, MAAP; Methodology: IAA, MAAP, KWCS, MOA, ID, NAAN; Resources: IAA; Investigation: MOA, ID, EOD; Data curation: MOA, ID; Data analysis: MOA, MAAP, IAA; Manuscript development: MOA, IAA, MAAP; Manuscript review and editing: MOA, IAA, MAAP, KWCS, NAAN, JN, EOD, JOC; Supervision: IAA, MAAP

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Availability of data

Data for this work is available upon reasonable request from the corresponding author.

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