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Pilot Implementation of SARS-CoV-2 Wastewater Surveillance on Cruise Ships Arriving at the Port of Piraeus from June to November 2021 †

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Abstract: This study evaluated the usefulness and validity of wastewater SARS-CoV-2 monitoring in passenger ships. Wastewater grab samples (n = 54) were collected from seven cruise ships arriving at the port of Piraeus from June to November 2021. Samples were analyzed for the presence of SARS-CoV-2 RNA with an RT-PCR based method. Results were compared against the number of cases detected on the ships before arrival. It was demonstrated that sewage monitoring can detect the presence of SARS-CoV-2, even with few cases on board. Future efforts should focus on the collection of more representative samples to increase the consistency and validity of the investigated practice.

Keywords: wastewater-based epidemiology (WBE); SARS-CoV-2; COVID-19; passenger ships

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1. Introduction

In the era of the COVID-19 pandemic, SARS-CoV-2 wastewater surveillance has been widely utilized to monitor disease spread at the community level. This practice involves sampling wastewater influent from wastewater treatment plants (WTPs) followed by molecular analysis to detect the virus and determine the concentration of SARS-CoV-2 RNA, a parameter that is used as an indicator of disease burden at the community. However, there are very few reports on the usage of sewage monitoring to capture the virus's circulation on passenger ships. The aim of this study was to evaluate the usefulness and validity of sewage SARS-CoV-2 monitoring in passenger ships, by implementing a short-term wastewater surveillance programme on cruise vessels arriving at the port of Piraeus from June to November 2021.

2. Material and Methods

Seven cruise ships from two cruise industry companies participated in this study. Single wastewater grab samples (500 mL) were collected from the influent of the ship's sewage treatment plants. Samples were collected on the day of arrival at the port of Piraeus and were transported to the laboratory of Hygiene and Epidemiology (University of Thessaly) at 4 °C. Samples were processed within 48 h after collection. For SARS-CoV-2 detection, we applied a concentration method based on polyethylene glycol precipitation of the virus from 105 mL of wastewater, followed by high-speed centrifugation. RNA extraction was performed on a KingFisher Flex system (ThermoFisher Scientific, Waltham, MA, USA) using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Applied Biosystems™, Waltham, MA, USA). For real-time reverse transcription polymerase chain reaction (RT-PCR), the TaqPath™ COVID-19 CE-IVD RT-PCR Kit (Applied Biosystems™)

(targets three different SARS-CoV-2 specific genomic regions; ORF1ab, the spike protein gene and the nucleocapsid protein gene) was used following the manufacturer's instructions, on a validated QuantStudio™ 5 real-time PCR system (ThermoFisher Scientific). Furthermore, for each sample, a 10-1 dilution was also analyzed, and both samples and their dilutions were analyzed in duplicate. SARS-CoV-2 RNA concentration in each sample was calculated using the N gene cycle threshold (Ct) and expressed as genome copies per mL. Further information can be found in a previous publication [1]. Data on the number of cases detected on the ship before arrival were extracted from reports that were acquired from the port health authority of Pireaus.

3. Results

From 29 June to 8 November 2021, a total of 54 wastewater samples from seven cruise ships were collected and analyzed for the presence of SARS-CoV-2 RNA. Nine samples (16.7%) had detectable concentrations ranging from 1302 to 166,000 genome copies/mL. The first SARS-CoV-2-positive sample (ship 1) was collected on 6 August 2021 and confirmed, the surveillance findings reported a total of seven cases on board for the specific voyage. The consequent sample for ship 1, which was collected on 13 August, was also positive although no new cases were reported. However, the concentration dropped from 10,416 to 3348 genome copies/mL. The particular ship was monitored until 5 October, and SARS-CoV-2 RNA was not detected in any other sample, while a total of six more cases (in three distinct voyages) were detected from clinical surveillance for the corresponding period. For ship 2, all three samples tested were found positive, and in all three voyages at least one positive case was found. It is noteworthy that considerable variance in RNA concentrations was observed (83,328, 2464 and 1302 genome copies /mL for the three samples). Out of fourteen samples analyzed for ship 3, three consecutive samples were found positive, all in October 2022, with no identified cases during that period. For this particular ship, only one case was identified (on 30 July 2021) throughout the period of the study. One out of six samples was positive for ship 4 in the absence of any confirmed cases. In the positive sample, a surprisingly high concentration (166,600 genome copies/mL) was measured. For all other monitored ships, all samples were negative, which is in agreement with clinical surveillance that did not identify any cases. In Table 1, an overview of the sewage testing results in correspondence with conventional surveillance data is presented.

Table 1. Number of wastewater samples tested for SARS-CoV-2, number of samples found positive and total number of cases per ship for the study period.

Ship	Total Number of Samples Analyzed	Total Number of Samples Found Positive	Total Number of COVID-19 Cases for the Study Period *
Ship 1	15	2	15
Ship 2	3	3	5
Ship 3	14	3	3
Ship 4	6	1	0
Ship 5	4	0	0
Ship 6	4	0	0
Ship 7	8	0	0

* as reported by the port health authority of Piraeus.

4. Discussion and Conclusions

This pilot study yielded promising results on the usefulness of wastewater SARS-CoV-2 monitoring as a surveillance tool on passenger ships. The ship reported seven COVID-19 cases during a voyage was timely identified through the analysis of a single wastewater sample, and it is noteworthy that the identification of SARS-CoV-2 RNA preceded the confirmation of cases indicating that, under certain circumstances, wastewater epidemiology can provide evidence of an outbreak earlier than conventional surveillance including screening of all passengers and crew by RADT. The results were less consistent when few

cases (one to three) were detected on board. Although on several occasions, wastewater samples were found positive even with only one or two identified cases, there were instances where COVID-19 cases occurred, but the virus was not detected in sewage. In the vast majority of voyages where no cases were reported, sewage samples were also negative, with few exceptions. In particular, no cases were reported on board when the corresponding sewage sample was negative for 40 out of 45 voyages. Several factors may explain the observed inconsistency, but, most likely, it is related to sampling technique limitations and the variance in viral shedding. Typically, wastewater epidemiology surveillance systems at the community level incorporate automatically collected 24 h composite samples, resulting in the acquisition of more representative samples. Usually, the measured concentrations are highly correlated with reported cases [2]. However, it is more challenging to implement this approach on passenger ships as it requires special equipment and technical adjustments, as well as due to the high turnover of passengers. Additionally, inter-individual viral shedding in feces may vary greatly [3], and this variance profoundly affects the results in wastewater samples when very few cases are prevalent on board. These same factors can also explain the reason why RNA concentrations were not correlated with the number of cases in the present study. Nonetheless, our results demonstrate that sewage monitoring can detect the presence of SARS-CoV-2 on board, and future efforts should focus on the integration of standardized sampling techniques to increase the consistency and validity of the investigated practice.

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