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BRIEF REPORT

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Investigation of the use of PCR testing prior to ship boarding to prevent the spread of SARS-CoV-2 from urban areas to less-populated remote islands

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Abstract: Preventing coronavirus disease (COVID-19) outbreaks and the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from urban areas to less-populated remote islands, many of which may have weak medical systems, is an important issue. Here, we evaluated the usefulness of pre-boarding, saliva-based polymerase chain reaction (PCR) screening tests to prevent the spread of SARS-CoV-2 from Tokyo to the remote island of Chichijima. The infection rate on the island during the study period from September 1, 2020 to March 21, 2021 was 0.015% (2/13,446). Of the 8,910 individuals tested before ship boarding, seven tested positive for COVID-19 (PCR tests of saliva samples). One was confirmed positive by subsequent confirmatory nasopharyngeal swab testing. Based on the testing results, positive cases were denied entry onto the ship to prevent the spread of COVID-19 from Tokyo to Chichijima. This study demonstrated that implementing pre-boarding PCR screening tests is a useful strategy that can be applied to other remote islands with vulnerable medical systems.

***Keywords*:** pre-boarding screen, COVID-19, saliva-based polymerase chain reaction

Introduction

Coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), resulted in more than 800,000 deaths globally between December 2019 and August 2020. Human-to-human transmission readily occurs, which has led to the spread of COVID-19 to almost all continents of the world. The World Health Organization (WHO) declared COVID-19 a public health emergency of international concern on January 30, 2020 and a pandemic on March 11, 2020. Since then, the high rates of SARS-CoV-2 transmission have been of great concern, as rapid human-to-human transmission may lead to COVID-19 cluster formation. The first outbreak of COVID-19 on a cruise ship was reported on the Diamond Princess cruise ship, wherein a passenger who had disembarked from Hong Kong tested positive for COVID-19 on February 1, 2020. The ship, with 3,711 passengers and crew members, was quarantined immediately after reaching Japanese waters on February 3, 2020. Over the next month, more than 700 people on board were infected, and for weeks, the ship was the site of the largest outbreak outside of China (*1*).

This incident suggests that spending long periods with infected individuals in closed spaces increases the risk of infection significantly (*2*). Outbreaks occur easily on vessels due to individual's proximity and a high proportion of older people, who tend to be more vulnerable to the disease.

Although a high risk of transmission arose from the episode of the Diamond princess, there are areas and islands where water liners are the only possible means to reach the area. Therefore, in the case of these islands that can only be reached by ship, it is necessary to consider quarantine measures, such as isolating COVID-19-positive cases before boarding. Chichijima is the largest island in the Ogasawara Islands, located about 1,000 km south of Tokyo and inhabited by approximately 2,100 residents (*3*).

The only way to reach Chichijima is by ship, taking approximately 24 h from Tokyo. (Figure 1, Table S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=49*). The island has only one clinic, which is not fully equipped to manage severe cases of COVID-19. Individuals on the island suspected of having a SARS-CoV-2 infection (persons with symptoms such as fever and cough) undergo rapid lateral flow antigen testing, which requires a nasopharyngeal swab and no special equipment. Subsequently, positive individuals are transferred to a specified hospital in Tokyo. In the case of negative individuals who are strongly suspected of having SARS-CoV-2 infection, specimens are transported to the mainland for testing by polymerase chain reaction (PCR). Suspected cases who test positive are transferred to the mainland using Self Defense Force helicopters to prevent the spread of the virus. To reduce the risk of outbreaks and keep the island safe from the pandemic, it is necessary to screen passengers traveling from the mainland to Chichijima Island using PCR tests prior to boarding.

Following this concept, the Tokyo Metropolitan Government conducted SARS-CoV-2 PCR testing using the saliva samples of all passengers who planned to board a regular ship from Tokyo to Chichijima between September 1, 2020 and March 31, 2021. Studies have evaluated the epidemiological and laboratory findings of SARS-CoV-2, the public health response to SARS-CoV-2 in Canada, and telemedicine in quarantined areas using simulation models in Australia (*4,5*). However, to our knowledge, this is the first study to evaluate the strategies for identifying infected individuals before they travel from large urban cities, where the community spread of SARS-CoV-2 is ongoing, to remote islands. Although studies on screening tests at airport quarantine stations have been conducted, this study elucidated the importance of extensive PCR testing of individuals with suspected SARS-CoV-2 infection who are asymptomatic, thereby facilitating quarantine measures.

Materials and Methods

Data collection

Saliva collection kits were distributed free of charge to those traveling to Chichijima from the mainland one week before embarking. Tests were not conducted among people traveling from Chichijima to the mainland. Saliva collection, a non-invasive and simple procedure, could be performed by the passengers themselves, with a small exposure risk to the person collecting the sample (*6*). Saliva samples self-collected at least 24 h prior to boarding were used for the screening test. All room temperature specimens were sent by post to the SB Coronavirus Inspection Center Corp. (Tokyo, Japan), a diagnostic company, after which the SARS-CoV-2 Direct Detection RT-qPCR Kit (Takara Bio Inc., Shiga, Japan) was used for testing per the manufacturer's instructions (*7*). We collected written questionnaires from those who agreed to have their saliva samples tested; and the questionnaires contained several questions to ascertain their general characteristics. Those who did not provide consent

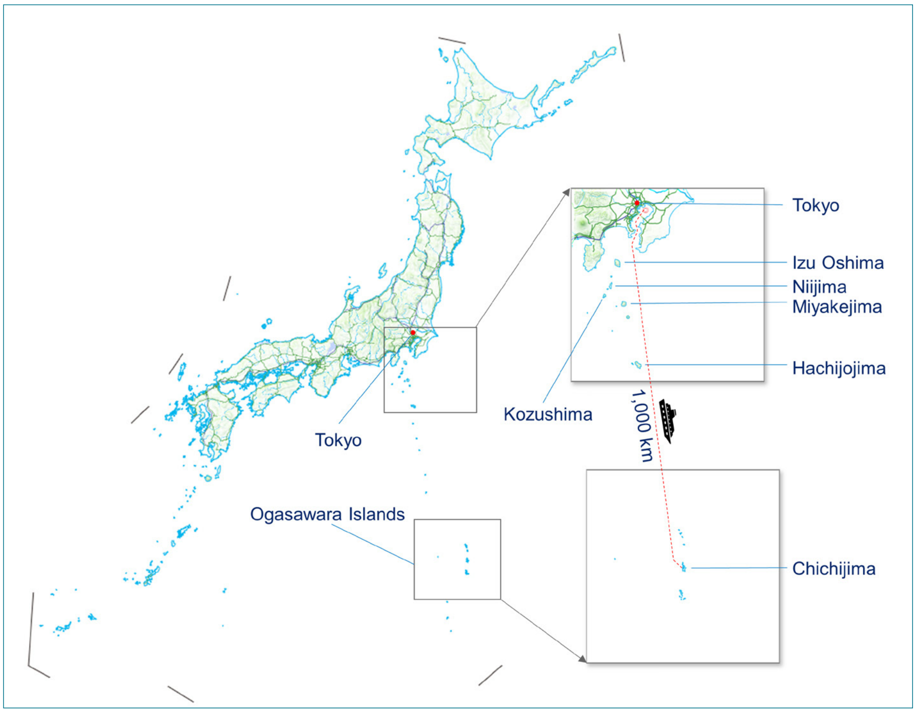

Figure 1. Location of Chichijima and the route from Tokyo to Chichijima.

were not included in the survey. Vaccinations were not yet available in Japan during the study period. During the seven-month study period, 34 round trips were conducted. Among the 11,372 passengers attempting to board the ship, 8,910 agreed to undergo PCR testing before boarding (testing rate, 78.4%) (Figure 2).

Ethics approval

Prior to testing, all saliva samples were processed to avoid identifying specific individuals and recovering personal data. Participants had the opportunity to refuse to participate in the study through the website. This study was approved by the Institutional Review Board of the National Center for Global Health and Medicine (approval number: NCGM-G-003678-00, date of approval: August 5, 2020). The study was in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical analysis

Descriptive statistics are presented as mean ± standard deviation or counts (percentages). Statistical analyses were performed using SPSS version 25 (IBM Corp., Armonk, NY, USA).

Results and Discussion

As of March 2021, among the seven individuals who tested positive for COVID-19 during the screening, one was confirmed positive in a subsequent confirmatory PCR test at a COVID-19 designated hospital in Tokyo; the remaining six tested PCR-negative for COVID-19 with the confirmatory PCR tests performed two to three days later. To understand the discrepancies between the results of the screening and confirmatory tests, we checked the cycle threshold (Ct) values of the PCR tests and found that all values were at the borderline of the detection limit ($Ct < 40.0$). This suggests that the cases were either false positives or that the number of viral copies was below the lower limit of detection at the time of the confirmatory test. Hence, these seven individuals did not board the vessel.

As of March 1, 2021, only two confirmed cases of COVID-19 had been reported on Chichijima Island. The first was a passenger who had a negative PCR test result at the time of boarding. As there was a 7-day interval between pre-boarding screening and diagnosis, the patient must have been in the "window period" at the time of screening. In the second case, a resident of the island was in close contact with the aforementioned passenger and was infected while residing on the island. During this period, Ogasawara Village required all visitors to record their temperature, wear a mask, and restrict large groups of visitors. For those who wished to consult the doctor for symptoms of acute infection, the doctor conducted medical interviews and antigen tests. If the antigen test results were positive, the patients were transported from Chichijima to Tokyo. In addition, the number of people on board the ships was reduced by 30-50%, and the ships were regularly ventilated and sterilized. Disinfectants were also made available in

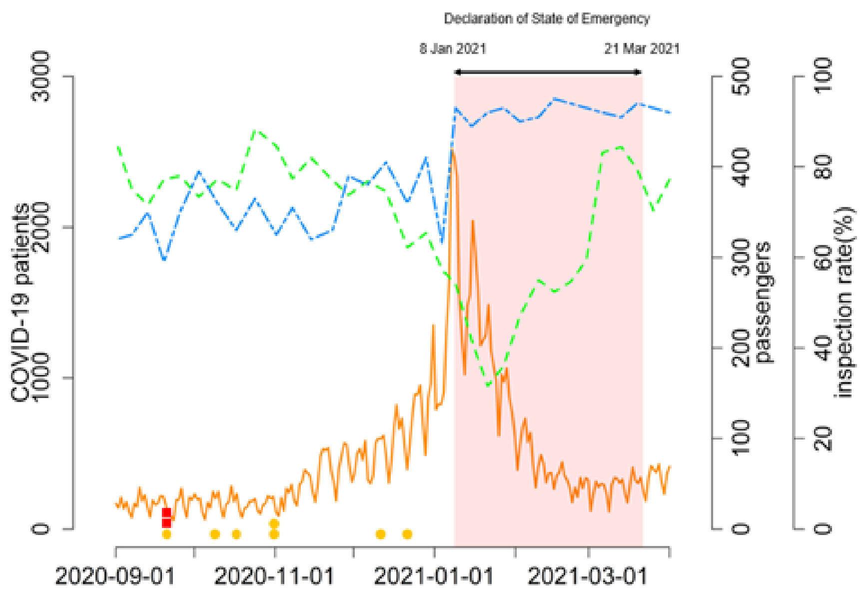

Figure 2. The orange line indicates the coronavirus disease (COVID-19) cases that occurred in Tokyo between September 1, 2020 and March 31, 2021. The green line indicates the number of passengers traveling from Tokyo to Chichijima, and the blue line indicates the testing rate of saliva samples by polymerase chain reaction (PCR) before boarding the vessel. The red squares indicate COVID-19 cases that occurred in Chichijima, and the orange circles indicate patients who tested positive for COVID-19 using saliva samples subjected to PCR prior to boarding. The period of declaration of a state of emergency is indicated by the orange block.

restaurants, employees wore masks and gloves when serving customers, and seats were regularly wiped and disinfected.

The incidence of COVID-19 among residents and visitors was 0.015% (2/13,446) (Figure 3). Although 24 h are required to reach Chichijima, and there is a high risk of infection on board, the percentage of infected people in Chichijima was relatively low compared with that of other islands in Tokyo. Furthermore, the cumulative number of infected persons and the ratio of infected persons to the total population in Tokyo during the study period were 100, 172, and 0.71%, respectively, with the infection rate in Chichijima being much lower than that in Tokyo (*8,9*). Among the 8,910 individuals tested before boarding the ship during this study, seven tested positive with saliva-based PCR testing and one with subsequent nasopharyngeal swab testing. The low rate of infection may imply that the low number of SARS-CoV-2-positive people in Japan might have led to this result. However, another remote island, which had direct flights from Tokyo and no special quarantine system, reported several cases of COVID-19 daily (*10*). On an island as remote as Chichijima, preventing COVID-19 outbreaks is imperative. PCR testing before boarding the vessel to Chichijima may be a crucial screening measure in preventing those outbreaks.

Data pertaining to the general characteristics of the participants are presented in Table 1. Interestingly, even during the pandemic, the major reason for boarding was tourism (48.2%), followed by business (33.1%), residence (11.4%), and homecoming (5.4%). There were more men (n = 5,836) than women (n = 3,021), and the average age was 43.3 ± 16.7 years. The average body temperature at the time of examination was 36.2 ± 0.4oC. There were 76 (0.85%) participants who reported symptoms on the day before boarding, but they all tested negative for COVID-19.

Cruise ships carry a large number of people in confined spaces with relatively higher homogeneous mixing over a period of time that is longer than that for any other mode of transportation. Thus, cruise ships present a unique environment for the transmission of human-to-human infections, including SARS-CoV-2. Several outbreaks of diseases on cruise ships have been reported in the past. An outbreak of a severe acute respiratory infection occurred on a cruise ship off Brazil in February 2012 that caused 16 hospitalizations and one death (*11*). In May 2009, a dual outbreak of the influenza A virus subtype H1N pandemic (swine flu) and influenza A (H3N2) occurred on a cruise ship. Among the 1,970 passengers and 734 crew members, 82 (3.0%) were infected with the swine flu, and 98 (3.6%) were infected with the influenza A (H3N2) virus (*12*).

Although pre-boarding PCR testing was successful, as was evident from the small number of COVID-19 cases reported on the island, the following study limitations should be considered. First, since the pre-boarding PCR test was voluntary, only 78.4% of the passengers agreed to undergo the test. The reasons for not undergoing the test were not clear, but fear of being identified as infected and not being allowed to board the ship may have been one major reason. It is noteworthy that during the state of emergency (January 8, 2021 to March 21, 2021), the percentage of pre-boarding PCR testing increased significantly from 71.8% to 92.3% (Student's t-test, p = 0.003) (Figure 2), indicating that the state of emergency declaration may have led to behavioral changes. Second, the sensitivity and specificity of saliva tests have been reported to be 86.4% (95% confidence interval [CI]: 82.8%-89.4%) and 97.0% (95% CI: 95.0%-98.3%), respectively (*13*). Moreover, as the specimens were self-collected, there was a risk of improper collection, which may have

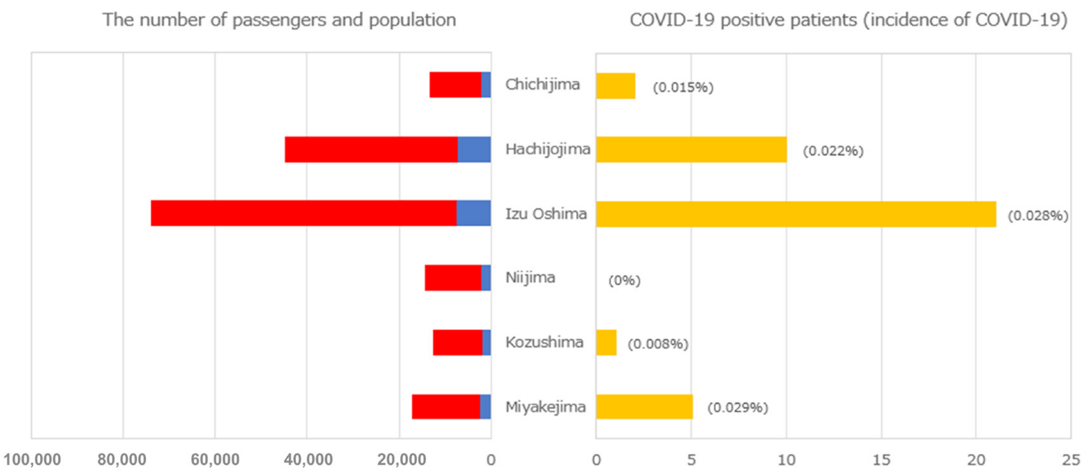

Figure 3. The left figure shows the population and passenger numbers. The blue and red bars indicate the population and passengers, respectively. The right figure shows the number of COVID-19-positive patients and the incidence of COVID-19. The incidence of COVID-19 was calculated as follows: (number of COVID-19 positive cases/total number of passengers and the population) × 100%.

Table 1. Characteristics of the study participants

Characteristics	Values
Total number of passengers expected to travel from Tokyo to Chichijima*	11,372
Total number of passengers who responded to the screening test	8,910
Response rate (%)	78.4
Total number of passengers who boarded the ship†	11,294
Age (years), mean ± SD‡	43.3 ± 16.7
Sex, *n* (%)	
Male	5,836 (65.5)
Female	3,021 (33.9)
Not available	53 (0.6)
Body temperature (°C), mean ± SD	36.2 ± 0.4
Symptoms, *n* (%)	
Cough and phlegm	66 (0.7)
Feeling of fatigue	11 (0.1)
Olfactory impairment	2 (0.02)
Dysgeusia	2 (0.02)
No symptom	8,702 (97.7)
Reasons for visiting the island, *n* (%)	
Residence	1,012 (11.4)
Retreat	477 (5.4)
Business	2,946 (33.1)
Sightseeing	4,299 (48.2)
Others	286 (3.2)

*All prospective passengers, except those aged < 6 years, were considered for testing. †Refers to the actual number of passengers on board, including those aged < 6 years. This included the passengers who canceled their boarding and those who were not allowed to board because they tested positive with the screening test. ‡SD, standard deviation.

increased the number of false negative PCR results. Third, individuals in the early stages of COVID-19 might have been in the "window period" (*14*), wherein the tests might have yielded a negative result. Fourth, no follow-up using a standard PCR was performed 3 or 7 days after arrival on the island to ascertain the number of passengers who may have been in the "window period" or to validate the saliva-based PCR tests. Finally, it is possible that the medical system failed to identify all COVID-19 cases. However, adequate antigen testing can be performed in Chichijima, and when necessary, specimens can be sent to hospitals in central Japan for PCR testing, even if the results of the antigen tests are negative. This approach may be effective in identifying all COVID-19 cases on the island. While there are no simple actions to address the above limitations, it is crucial to educate people that false negative PCR test results are possible. Moreover, standard precautions for COVID-19, including reducing the number of passengers on board by 30% to 50%, regular ventilation and sterilization of the ship, provision of disinfectants in restaurants, wearing of gloves and masks by employees during contact with customers, and disinfection of seats during the cruise, are continuously implemented.

In conclusion, the implementation of PCR screening prior to boarding prevented seven suspected positive cases from visiting the remote island, thus minimizing the number of infected people and the spread of infection on the island. During the study period, many outbreaks occurred on other islands that were visited by passengers from heavily infected areas in Tokyo. In particular, on remote islands where it takes a long time to reach appropriate medical care facilities, the prevention of outbreaks is crucial. Our findings indicate that for islands with vulnerable medical systems, PCR screening prior to boarding should be considered to prevent infected individuals from visiting the island.

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Conflict of Interest: Moto Kimura and Wataru Sugiura have received research grants from SB Coronavirus Inspection Center Corp. Masato Ikeda and Yukumasa Kazuyama are employees of SB Coronavirus Inspection Center Corp.

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