The Journal of Infectious Diseases

BRIEF REPORT

Environmental Sampling for Severe Acute Respiratory Syndrome Coronavirus 2 During a COVID-19 Outbreak on the Diamond Princess Cruise Ship

Takuya Yamagishi,[1] Makoto Ohnishi,[2] Nobuaki Matsunaga,[3] Kensaku Kakimoto,[4] Hajime Kamiya,[1] Kiyoko Okamoto,[1] Motoi Suzuki,[1] Yoshiaki Gu,[3] Mikiyo Sakaguchi,[3] Taichi Tajima,[3] Saho Takaya,[5] Norio Ohmagari,[5] Makoto Takeda,[6] Shutoku Matsuyama,[6] Kazuya Shirato,[6] Naganori Nao,[6] Hideki Hasegawa,[7] Tsutomu Kageyama,[7] Ikuyo Takayama,[7] Shinji Saito,[7] Koji Wada,[8] Retsu Fujita,[9] Hiroki Saito,[10] Keiji Okinaka,[11] Mathew Griffith,[12] Amy Elizabeth Parry,[12] Brenda Barnetson,[13] James Leonard,[13] and Takaji Wakita[2]; and Taskforce for the COVID-19 Cruise Ship Outbreak

[1]Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan, [2]National Institute of Infectious Diseases, Tokyo, Japan, [3]Antimicrobial Resistance Clinical Research Center, National Center for Global Health and Medicine, Tokyo, Japan, [4]Field Epidemiology Training Program, National Institute of Infectious Diseases, Tokyo, Japan, [5]Disease Control and Prevention Center, National Center for Global Health and Medicine, Tokyo, Japan, [6]Department of Virology III, National Institute of Infectious Diseases, Tokyo, Japan, [7]Influenza Virus Research Center, National Institute of Infectious Diseases, Tokyo, Japan, [8]Graduate School of Public Health, International University of Health and Welfare, Chiba, Japan, [9]Graduate School of Medicine, International University of Health and Welfare, Tokyo, Japan, [10]Department of Emergency and Critical Care Medicine, Yokohama City Seibu Hospital, St Marianna University School of Medicine, Yokohama, Japan, [11]Division of General Internal Medicine, National Cancer Center Hospital East, Chiba, Japan, [12]Health Emergency Program, World Health Organization Western Pacific Regional Office, Manila, Philippines, and [13]Department of Health Services, Princess Cruises, USA

During a COVID-19 outbreak on the Diamond Princess cruise ship we sampled environmental surfaces after passengers and crew vacated cabins. SARS-CoV-2 RNA was detected in 58 of 601 samples (10%) from case cabins 1–17 days after cabins were vacated but not from noncase cabins. There was no difference in detection proportion between cabins of symptomatic (15%, 28/189; cycle quantification [Cq], 29.79–38.86) and asymptomatic cases (21%, 28/131; Cq, 26.21–38.99). No SARS-CoV-2 virus was isolated from any of the samples. Transmission risk of SARS-CoV-2 from symptomatic and asymptomatic patients may be similar and surfaces could be involved in transmission.

Keywords. COVID-19; SARS-CoV-2; cruise ship; Diamond Princess; environmental sampling.

Cruise ships are known to be vulnerable to outbreaks of certain infectious diseases, such as norovirus, and environmental analysis has often found sanitary conditions aboard inspected ships to be inadequate [1]. Between January and February 2020, a novel coronavirus disease (COVID-19) outbreak occurred on the Diamond Princess cruise ship [2, 3]. Following arrival in Yokohama, Japan, passengers were isolated in their cabins for 14 days (Supplementary Box A). By 20 April, 712 COVID-19 cases had been detected among the 3713 passengers and crew (19%), with 13 deaths. We examined the role of surfaces, wastewater, and air in transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) during this outbreak.

METHODS

We performed environmental sampling on the Diamond Princess cruise ship on 22–23 February 2020, prior to disinfection of the vessel and while some passengers and crew members remained on board. We obtained specimens from cabins in which confirmed COVID-19 cases had stayed (case cabins), cabins with no confirmed case at any time (noncase cabins), and common areas.

Cases were defined as any person on board the vessel who had at least 1 oropharyngeal specimen that tested positive for SARS-CoV-2 by real-time reverse transcriptase polymerase chain reaction (rRT-PCR). Cases were categorized as symptomatic or asymptomatic based on their presentation at the time of sample collection. For case cabins, we randomly selected cabins in which confirmed symptomatic or asymptomatic COVID-19 cases had stayed. To understand the duration and survivability of SARS-CoV-2 on surfaces, we also selected case cabins according to the last date on which any person was in the cabin. Case cabins had been disinfected by 5% hydrogen peroxide spraying prior to sampling (14–15 February 2020), including some of those that were sampled. To understand the contribution of airborne transmission, we selected noncase cabins next to a case cabin or at least 3 cabins away from a case cabin. To understand the contribution of wastewater, we also included noncase cabins located below case cabins. We swabbed diverse surfaces in cabins and common areas (Supplementary Box B).

For sampling, we used polyester-flocked oropharyngeal specimen collection swabs moistened with viral transport medium (VTM). We swabbed areas (4×5 cm^2) in 3 directions. We placed swabs into VTM and kept them frozen at −80°C until testing at National Institute of Infectious Diseases (NIID), Japan. In addition, a second sampling of surfaces from part of the SARS-CoV-2 RNA-detected items was conducted on 27 February 2020 for viable virus isolation, with samples stored at 4°C and transferred directly for laboratory isolation.

Received 14 May 2020; editorial decision 13 July 2020; accepted 17 July 2020; published online July 21, 2020.

Correspondence: Takuya Yamagishi, MD, PhD, Infectious Disease Surveillance Center, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku, Tokyo 162–8640, Japan (tack-8@niid.go.jp).

The Journal of Infectious Diseases® 2020;XX:1–5

DOI: 10.1093/infdis/jiaa437

We obtained air samples (50 L/min for 20 minutes) from cabins by placing 2 air samplers (Airport MD8, Sartorius) in 7 random cabins on the bed and on the toilet seat. Collection was performed through a special gelatin filter (type 175, Sartorius; T1 phage capture rate, 99.99%; effective filtration area, 38.5 cm^2). After collection, the sample was put in the gelatin filter in the original package, checked, and stored at −80°C until testing at NIID (typically at least 14 days).

Samples were tested by rRT-PCR. We attempted viral isolation from some samples in which viral RNA had been detected by rRT-PCR and from the second round of sampling. Samples were mixed with Dulbecco's modified Eagle medium supplemented with standard concentrations of penicillin G, streptomycin, gentamicin, amphotericin B, and 5% fetal bovine serum. These were inoculated on confluent VeroE6/TMPRSS2 cells [4]. Culture medium at 0 or 48 hours postinfection was collected, diluted 10-fold in water, and boiled for 5 minutes. An rRT-PCR assay was performed to quantify the increased amount of coronavirus RNA with a MyGo Pro system (IT-IS Life Science) [5].

The median highest and lowest temperature in Yokohama between 3 February and 27 February 2020 were 13.0°C (range, 6.5–18.5°C) and 5.5°C (range, 0.0–9.3°C). The median highest and lowest humidity were 73% (range, 41%–98%) and 40% (range, 17%–76%).

We calculated the prevalence of surfaces positive for SARS-CoV-2 and used the Fisher exact test to evaluate the difference in SARS-CoV-2 detection between cabins with symptomatic and asymptomatic cases. We considered 2-tailed $P < .05$ statistically significant, and used Bonferroni correction. This investigation did not require institutional ethics review as a public health investigation under Japanese Infectious Disease and Quarantine Law.

RESULTS

Overall, SARS-CoV-2 RNA was detected in 58 (10%) of 601 environmental samples (Table 1). SARS-CoV-2 RNA was detected in approximately two-thirds of all case cabins swabbed, while it was not detected in any noncase cabins. Except for 1 sample from an air hood in a corridor, SARS-CoV-2 RNA was not detected in samples swabbed in common areas. SARS-CoV-2 RNA was not detected in any air samples.

SARS-CoV-2 RNA was most often detected on the floor around the toilet in bathrooms (39%, 13/33; cycle quantification [Cq], 26.21–37.62) and bed pillows (34%, 11/32; Cq, 34.61–38.99) (Table 2).

In case cabins occupied by symptomatic cases, SARS-CoV-2 RNA was detected in 15% (28/189) of samples tested, with Cq values ranging from 29.79 to 38.86. SARS-CoV-2 RNA was detected in 21% (28/131) of samples from case cabins with asymptomatic cases, with a range of Cq values from 26.21 to 38.99. All but 2 case cabins had 2 occupants before the room was vacated. The remaining 2 cabins had 1 and 3 occupants.

The range of time between the last occupant vacating a case cabins and detection of SARS-CoV-2 RNA was 1–17 days, and rates of positivity decreased with time (Supplementary Table). Areas where SARS-CoV-2 RNA was detected at least 14 days

Table 1. Detection of SARS-CoV-2 RNA During a COVID-19 Outbreak on the Diamond Princess Cruise Ship, by Cabin and Area

	Samples Tested, No.		SARS-CoV-2 RNA Detected, No. (%)	
Sample	Cabins	Items	Cabins	Items
Surface samples				
Cabins of people with confirmed COVID-19	33	330	21 (64)	57 (17)
Cases with symptoms before disembarkation	19	189	10 (53)	28 (15)
Cases without symptoms before disembarkation	13	131	10 (77)	28 (21)
Case symptom status unknown	1	10	1 (100)	1 (10)
With 5% hydrogen peroxide spraying[a]	8	79	5 (63)	9 (11)
Without 5% hydrogen peroxide spraying	25	251	16 (64)	48 (19)
Cabins of people without confirmed COVID-19	16	160	0 (0)	0 (0)
Shared areas				
Medical clinic		20		0 (0)
Restaurants on 5th deck		24		0 (0)
Other		53		1 (2)[b]
Total	49	587	21 (43)	58 (10)
Air samples				
Cabins of people with confirmed COVID-19	4	8	0 (0)	0 (0)
Cabins of people without confirmed COVID-19	3	6	0 (0)	0 (0)
Total	7	14	0 (0)	0 (0)

[a]Spraying with 5% hydrogen peroxide was conducted 1 to 10 days after infected persons had left the cabins.

[b]Hood of air outlet in the corridor.

Table 2. Detection of SARS-CoV-2 RNA in Cabins Occupied by People With Confirmed COVID-19 During the Diamond Princess Cruise Ship Outbreak, by Swabbed Item and Presence of Symptoms

	Total		Symptomatic[a] (19 Cabins)			Asymptomatic[b] (13 Cabins)			
Item	Samples Tested in Cabins, No.	SARS-CoV-2 Detected, No. (%)	Samples Tested in Cabins, No.	SARS-CoV-2 Detected, No. (%)	Cq Value	Samples Tested in Cabins, No.	SARS-CoV-2 Detected, No. (%)	Cq Value	*P* Value[c]
Floor around the toilet in the bathroom	33	13 (39)	19	5 (26)	29.79–37.02	13	7 (54)	26.21–37.62	.15
Pillow	32	11 (34)	18	6 (33)	34.61–38.84	13	5 (38)	36.31–38.99	1.00
Phone	33	8 (24)	19	2 (11)	31.93–37.74	13	6 (46)	33.09–37.95	.04
Table	34	8 (24)	19	5 (26)	34.25–37.87	14	3 (21)	36.28–37.85	1.00
TV remote control	33	7 (21)	19	4 (21)	30.35–37.29	13	3 (23)	35.58–38.53	1.00
Chair arm	33	4 (12)	19	2 (11)	36.91–38.86	13	2 (30)	37.29–38.17	1.00
Toilet flush button	33	2 (6)	19	2 (11)	36.71–38.13	13	0 (0)	…	.50
Toilet seat	33	2 (6)	19	1 (5)	36.10	13	1 (3)	37.25	1.00
Light switch	33	1 (3)	19	0 (0)	…	13	1 (3)	38.02	.41
Doorknob	33	1 (3)	19	0 (0)	…	13	1 (3)	37.93	.41
Total	330	57 (17)	189	28 (15)	29.79–38.86	131	28 (21)	26.21–38.99	.14

[a]Cabins with symptomatic cases were those with at least 1 symptomatic case, and cabins with asymptomatic cases were those with no symptomatic cases before sampling.

[b]One case cabin occupied by an infected passenger with unknown symptoms was excluded. In this cabin, SARS-CoV-2 RNA was detected only from the floor around the toilet (Cq 33.33).

[c]*P* values evaluated the proportion of SARS-CoV-2 detection between cabins of symptomatic and asymptomatic cases.

after the cabin was vacated included the floor around the toilet and bed pillows. The lowest Cq values were detected on samples taken 4 (Cq, 26.21) and 7 (Cq, 29.79) days after cabins were vacated, both obtained from the floor around the toilet.

No viable virus could be isolated from the 58 samples with SARS-CoV-2 RNA detected by rRT-PCR or the 18 samples obtained in the second sampling.

DISCUSSION

Following the COVID-19 outbreak in the Diamond Princess cruise ship, we detected SARS-CoV-2 RNA on environmental surfaces of cabins of symptomatic and asymptomatic COVID-19 cases up to 17 days after the cabins had been vacated. Although we were unable to isolate the virus from any of the samples, our findings have implications for outbreak prevention and control strategies as well as disinfection procedures.

Our findings suggest that environmental surfaces may have played a role in transmission of the virus. SARS-CoV-2 RNA was detected on multiple surfaces of case cabins, most often on bed pillows and the floor around the toilet in the bathroom, for up to 17 days, longer than previously reported [6]. SARS-CoV-2 has been detected in oral swabs, anal swabs, blood, tears, conjunctivae, and sputum [7, 8]. Our finding of SARS-CoV-2 RNA on bed pillows may have come from coughing, nasal drainage, or tears during sleep. This suggests that cleaning of linens before use by another person and safe transfer and cleaning of used lines are important to prevent contact transmission of SARS-CoV-2. The RNA detected on the floor around the toilet may have come from stool or from respiratory secretions. Lower rates of RNA detection in samples from surfaces with high frequency of hand-touching (eg, doorknobs) may be due to good hand hygiene practices, frequent cleaning, or surface material [9]. As with health care settings, where patient hand hygiene guidance is essential to prevent health care-associated infections, education on good hand hygiene is critical for stopping SARS-CoV-2 transmission on cruise vessels.

Another important finding is that there was no difference in surface contamination between cabins of cases who were symptomatic and asymptomatic. The asymptomatic cases may have been pre- or postsymptomatic. Nevertheless, it is evident surface contamination occurred in rooms occupied by persons who were classified as being asymptomatic at the time they vacated their cabins. Studies have shown evidence of asymptomatic and presymptomatic transmission [10, 11]. Asymptomatic transmission presents a substantial challenge for public health because isolation of only symptomatic patients will not interrupt transmission chains.

Our findings also imply that simple cleaning procedures of the environment can remove the virus from surfaces and reduce transmission. In addition to the low proportion of RNA detection in the samples mentioned above (eg, door knobs), RNA was detected from only 1 sample in common areas, a ceiling vent that may have been difficult to reach during cleaning. For environmental cleaning during the quarantine, standard disinfectant with hydrogen peroxide as the active ingredient was used, and the frequency of disinfection was increased with a focus on areas of highest foot traffic (personal communication, J. Leonard, 19 March 2020). The contribution of environmental surfaces to transmission might be limited by periodic cleaning using hydrogen peroxide products or other products active against SARS-CoV-2. Interestingly, SARS-CoV-2 RNA

was detected in case cabins that had been disinfected by hypochlorite spraying. Although the spraying of hydrogen peroxide could structurally disinfect SARS-CoV-2 [12], removing the virus by wiping environmental surfaces may be safer during outbreaks.

The duration of viable SARS-CoV-2 on environmental surfaces is still not fully determined. Although the chance of detecting viral RNA decreased with time, we could not isolate viable virus. Other human coronaviruses can persist on hard surfaces at room temperature for up to 9 days [6]. A recent study indicated that SARS-COV-2 had varying viability on different surfaces and was similar to SARS-COV-1 under experimental conditions, with the virus surviving on plastic and stainless steel for over 72 hours [9]. The high Cq values in most of our positive samples suggests low-level contamination of the environment after the COVID-19 cases vacated the cabins, potentially explaining why no virus was isolated. Alternatively, there may have been aspects of the sampling, storage, transport, or isolation methods that complicated isolation success.

We could not find evidence of air transmission in this study. One study reported that the virus was viable for up to 3 hours in the air [9], and viral RNA was detected from air sampling of airborne infection isolation rooms in a general ward [13]. Our sampling was conducted only after cases left the cabin, potentially complicating viral isolation. Viral load in the air, if it existed, would be higher when cases were in the cabin. Another reason could be the relatively short sampling time (20 minutes); studies reporting viral RNA detection from the air have sampled for 30 minutes [14] or 4 hours [13]. Detection of SARS-CoV-2 RNA from a single air vent in the ceiling of a corridor was more likely the result of a projectile droplet, but this suggests that the virus could flow beyond 1 m in a condition with limited airflow. Some respiratory pathogens, such as influenza virus or SARS-CoV-1, have been reported to transmit beyond 1 m in some circumstances [15]. Alternatively, stopping the air recirculation aboard the ship may have prevented airborne transmission in the common area or between the cabins. The possibility of airborne transmission of SARS-CoV-2 and the effect of stopping air recirculation in the cruise ship during the COVID-19 outbreak need further study.

No viral RNA was detected from the toilet floor of cabins directly under case cabins. If the virus were transmitted through wastewater systems, we assumed that it would be detected in these cabins because wastewater travels from higher to lower decks on the ship. We thus did not find evidence that SARS-CoV-2 was transmitted through the wastewater system; however, further study is needed to conclusively rule it out.

The strength of our study was the ability to conduct systematic environmental sampling during an outbreak response. Rooms were left untouched for several days after disembarkation of passengers, providing an ideal situation to evaluate persistence of viral RNA. Limitations include, first, the fact that it took approximately 3 hours to bring the specimen to the laboratory due to logistical challenges, which may have affected isolation of the virus. Second, there were potential misclassifications of noncase cabins because the sensitivity of RT-PCR is not satisfactory. Third, some cabins were disinfected before sampling, which might be a reason why viable virus was not isolated.

In conclusion, the environment around COVID-19 cases was extensively contaminated with SARS-CoV-2 during the cruise ship outbreak. Environmental surfaces could have contributed to viral transmission through direct contact, but we found no evidence of transmission through air or wastewater. High rates of environmental contamination in cabins with asymptomatic cases support other evidence that transmission can occur from asymptomatic persons. Cleaning of surfaces with active disinfectant and communication messages demonstrating and emphasizing hand hygiene are essential to interrupting the chain of transmission during outbreaks.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgment. We acknowledge the Ministry of Health, Labor, and Welfare, including quarantine officers and other public health officers, and Dr Hidekazu Nishimura, Sendai Medical Center, who helped to take samples from the environment. We thank all the crew members in the Princess Cruises for their dedicated cooperation; and all staff of the National Institute of Infectious Diseases who worked on the COVID-19 response. We appreciate Dr Mateusz M. Pluncinski for his kind advice on the design and reviewing the manuscript.

Author contributions. T. Y., G. M., and B. B. designed the study. T. Y., K. W., R. F., N. O., Y. G., N. M., M. S., T. T., S. T., H. S., and K. O. sampled from the environment. T. W., M. O., K. O., N. N., K. S., S. M., I. T., S. S., M. T., T. K., and H. H. conducted laboratory testing of the samples. T. Y., M. O., G. M., P. A., and B. B. analyzed the data and developed a draft manuscript. N. O., T. W., M. O., K. K., H. K., M. S., T. K., R. F., and J. L. reviewed the manuscript and provided inputs.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Carling PC, Bruno-Murtha LA, Griffiths JK. Cruise ship environmental hygiene and the risk of norovirus infection outbreaks: an objective assessment of 56 vessels over 3 years. Clin Infect Dis **2009**; 49:1312–7.

2. Kakimoto K, Kamiya H, Yamagishi T, Matsui T, Suzuki M, Wakita T. Initial investigation of transmission of COVID-19 among crew members during quarantine of a cruise ship - Yokohama, Japan, February 2020. MMWR Morb Mortal Wkly Rep **2020**; 69:312–3.
3. Yamagishi T, Kamiya H, Kakimoto K, Suzuki M, Wakita T. Descriptive study of COVID-19 outbreak among passengers and crew on Diamond Princess cruise ship, Yokohama Port, Japan, 20 January to 9 February 2020. Euro Surveill **2020**; 25:2000272.
4. Matsuyama S, Nao N, Shirato K, et al. Enhanced isolation of SARS-CoV-2 by TMPRSS2- expressing cells. Proc Natl Acad Sci U S A; **2020**; 117:7001–3.
5. Corman VM, Eckerle I, Bleicker T, et al. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill **2012**; 17:20285.
6. Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J Hosp Infect **2020**; 104:246–51.
7. Xia J, Tong J, Liu M, Shen Y, Guo D. Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. J Med Virol **2020**; 92:589–94.
8. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA **2020**; 323:1843–4.
9. van Doremalen N, Bushmaker T, Morris DH, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med **2020**; 382:1564–7.
10. Rothe C, Schunk M, Sothmann P, et al. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. N Engl J Med **2020**; 382:970–1.
11. Tong Z-D, Tang A, Li K-F, et al. Potential presymptomatic transmission of SARS-CoV-2, Zhejiang Province, China, 2020. Emerg Infect Dis **2020**; 26:1052–4.
12. Goyal SM, Chander Y, Yezli S, Otter JA. Evaluating the virucidal efficacy of hydrogen peroxide vapour. J Hosp Infect **2014**; 86:255–9.
13. Chia PY, Coleman KK, Tan YK, et al. Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients. Nat Commun **2020**; 11:2800.
14. Guo ZD, Wang ZY, Zhang SF, et al. Aerosol and surface distribution of severe acute respiratory syndrome coronavirus 2 in hospital wards, Wuhan, China, 2020. Emerg Infect Dis **2020**; 26:1583–91.
15. Siegel JD, Rhinehart E, Jackson M, Chiarello L, Health Care Infection Control Practices Advisory Committee. 2007 Guideline for isolation precautions: preventing transmission of infectious agents in health care settings. Am J Infect Control **2007**; 35(10 suppl 2):S65–164.