

Short Communication

Two-Year Seroprevalence Surveys of SARS-CoV-2 Antibodies among Outpatients and Healthcare Workers in Ehime, Japan

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ABSTRACT: We conducted two-year seroprevalence surveys of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies among outpatients and healthcare workers (HCWs) at Ehime University Hospital. Data were collected for outpatients and HCWs in June 2020 (1st survey), December 2020 (2nd survey), July 2021 (3rd survey), and December 2021 (4th survey), focusing on demographics, occupation, and the seroprevalence of anti-SARS-CoV-2 antibodies. Blood samples were obtained from randomly selected outpatients who visited our hospital for medical care and HCWs undergoing regular medical checks with opt-out informed consent. SARS-CoV-2 antibody positivity was evaluated using two laboratory-based quantitative tests. The total number of participants enrolled was 6,369 (1st survey: 1,000 outpatients and 743 HCWs, 2nd survey: 1,000 outpatients and 407 HCWs, 3rd survey: 1,000 outpatients and 804 HCWs, 4th survey: 1,000 outpatients and 415 HCWs). The prevalence of SARS-CoV-2 antibodies among outpatients and HCWs was 0–0.1% and 0–0.124% during the research period, respectively, and changed little over time. These findings suggest that the magnitude of COVID-19 infection during the pandemic among outpatients and HCWs in this rural hospital might have been small.

The spread of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains an ongoing concern (1). Some reports have suggested that the risk of infection did not increase among healthcare workers (HCWs) in hospitals when adequate infection control measures were undertaken (2–4). However, a systematic review and meta-analysis has concluded that the seroprevalence of SARS-CoV-2 antibodies among HCWs was high, indicating an occupational health risk (5). SARS-CoV-2 infection of HCWs in hospitals is a concern for outpatients. However, information on the transmission of SARS-CoV-2 to outpatients and HCWs

is limited (6,7).

We have played a leading role in the treatment of patients with severe COVID-19 since the early phase of the pandemic in Ehime prefecture, Japan. Our hospital has converted an intensive care unit (ICU) ward to an infectious disease ward for severe COVID-19, accommodating 11 beds in a negative pressure airflow environment. Doctors and nurses from other departments have been transferred to this new infection ward in accordance with the number of patients being cared for at any one time. Patients with severe COVID-19 tend to have a high viral load, a long virus-shedding period, and prolonged viral shedding via multiple sources, including saliva, sputum, stools, urine, and blood (8). ICUs used for the treatment of severe COVID-19 are frequently contaminated with SARS-CoV-2 (9), thereby imposing a significant psychological burden on HCWs.

A COVID-19 survey by serologic assay has been a useful way to estimate the population of individuals who have been infected. Using this method, the Ministry of Health, Labour and Welfare of Japan reported the prevalence of positivity for anti-SARS-CoV-2 antibody

Received March 19, 2022. Accepted April 27, 2022.

J-STAGE Advance Publication May 31, 2022.

DOI: 10.7883/yoken.JJID.2022.155

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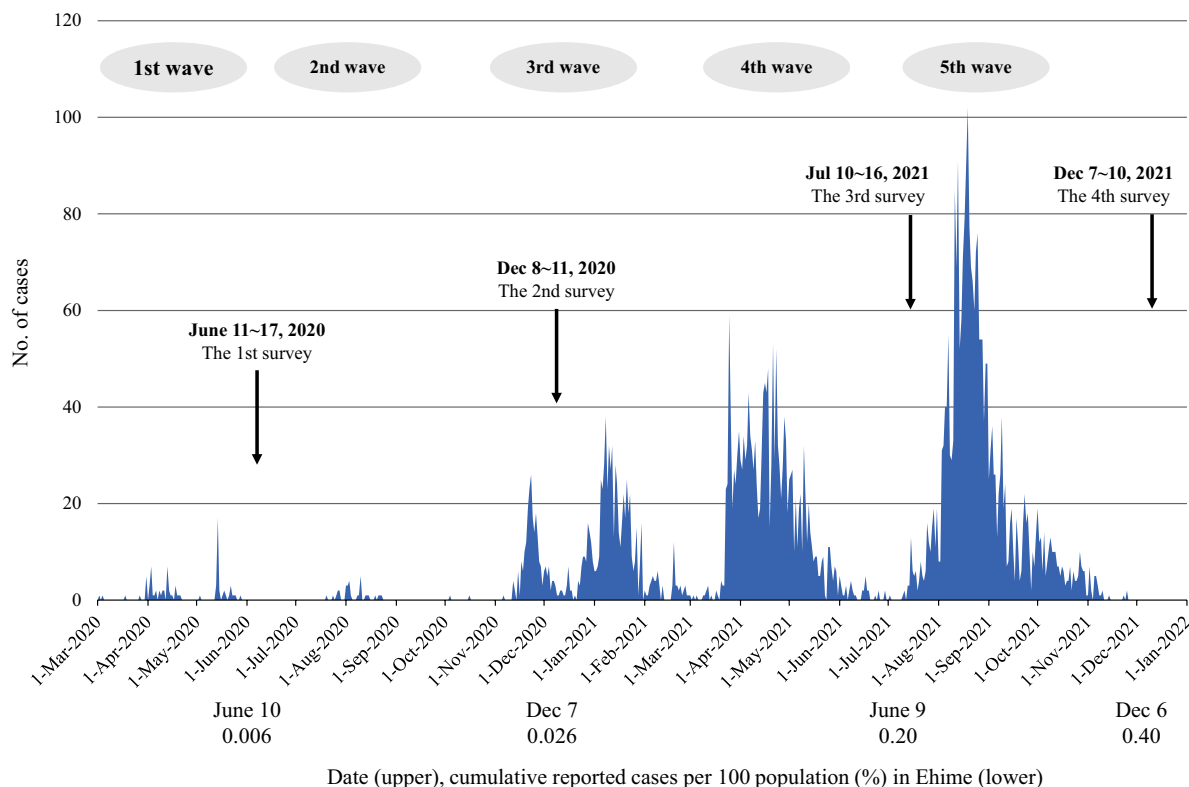


Fig. 1. (Color online) Transition in the number of patients diagnosed with COVID-19 in Ehime, Japan. Data for the number of patients diagnosed with COVID-19 in Ehime was obtained from the website (13). The cumulative reported cases per 100 population were calculated as the number of diagnosed COVID-19 cases per date/population in Ehime.

in Tokyo, Osaka, Aichi, Fukuoka, and Miyagi in June and December, 2020 (10). We adopted the same two SARS-CoV-2 antibodies used for this survey to acquire further epidemiological data for Japan.

All outpatients and HCWs were automatically enrolled in this study via the internet and intranet at our hospital, and no individuals opted out. We enrolled a total of 6,369 participants (4,000 outpatients and 2,369 HCWs) by an opt-out consent in June 2020 (1st survey), December 2020 (2nd survey), July 2021 (3rd survey), and December 2021 (4th survey) at Ehime University Hospital. We collected data on demographics, occupation, and the seroprevalence of anti-SARS-CoV-2 antibodies. We randomly selected a total of 4,000 outpatients (1,000 in each survey) who visited our hospital for medical care and blood sampling according to the order of visit. We also included 2,369 HCWs (1st survey, 743; 2nd survey, 407; 3rd survey, 804; and 4th survey, 415) who underwent blood sampling as part of regular medical checks. All samples were anonymized, as no personal identifiers were used. The study was approved by the institutional review board of Ehime University Hospital (approval number: 2005002).

Samples were stored at 4°C until ready for use, and measurements were performed within 2 weeks using electrochemiluminescence immunoassay ([ECLIA], Elecsys® Anti-SARS-CoV-2 assay; Roche Diagnostics International Ltd, Rotkreuz, Switzerland) as a first screening test, and the chemiluminescence immunoassay ([CLIA], Architect® SARS-CoV-2 IgG assay; Abbott, Abbott Park, IL, USA) against the first

positive samples to confirm the first results. The Roche and Abbott assays detect antibodies reactive against the SARS-CoV-2 nucleocapsid protein. A cutoff index (COI) of > 1.0 for the Roche assay is considered positive, whereas an index of > 1.4 for the Abbott assay indicates a positive result (11,12). In the present study, SARS-CoV-2 antibody was considered to be positive when both assays yielded a positive result. Samples with COI > 0.2 in the Roche assay were tested with the Abbott assay as a confirmation in this study.

Fig. 1 shows the period of each survey with the transition in the number of patients diagnosed with COVID-19 in Ehime (13). The 1st, 2nd, 3rd, and 4th surveys were conducted after the 1st wave, in the middle of the 3rd wave, after the 4th wave, and after the 5th wave of the COVID-19 pandemic in Japan. The cumulative reported cases per 100 population of COVID-19 in Ehime was 0.006% in the 1st survey, 0.026% in the 2nd, 0.20% in the 3rd, and 0.40% in the 4th.

The characteristics and seroprevalence of SARS-CoV-2 antibodies in the participants are summarized in Tables 1 and 2. One thousand outpatients were enrolled in each survey, and the median age of patients in the 1st, 2nd, 3rd, and 4th surveys did not change significantly. Only one person in the 1st survey was positive for SARS-CoV-2 antibodies, giving a seroprevalence of 0.1% (95% confidence interval [CI]: 0.018–0.56). Of the 1,965 staff in our hospital, 743 (37.8%), 403 (20.5%), 804 (40.9%), and 415 (21.1%) HCWs were enrolled in the 1st, 2nd, 3rd, and 4th surveys, respectively.

COVID-19 Survey in Ehime, Japan

Table 1. The characteristics of the participants

Category	1st survey <i>n</i> (%)	2nd survey <i>n</i> (%)	3rd survey <i>n</i> (%)	4th survey <i>n</i> (%)
Outpatient	1000	1000	1000	1000
Female	519 (51.9)	519 (51.9)	543 (54.3)	539 (53.9)
Median age (range)	64 (0–96)	65(0–97)	64 (0–94)	64 (0–94)
Visited departments				
Internal medicine	557 (55.7)	543 (54.3)	573 (57.3)	572 (57.2)
Surgery	149 (14.9)	192 (19.2)	154 (15.4)	216 (21.6)
Pediatrics	64 (6.4)	38 (3.8)	48 (4.8)	29 (2.9)
Obstetrics and gynecology .	52 (5.2)	68 (6.8)	59 (5.9)	54 (5.4)
Others	195 (19.5)	159 (15.9)	166 (16.6)	129 (12.9)
HCW	743	407	804	415
Female	509 (68.5)	273 (67.1)	575 (71.5)	276 (66.5)
Median age (range)	43 (20–66)	46 (23–65)	44 (20–67)	46 (24–66)
Occupation				
Nurse	341 (45.9)	255 (62.7)	359 (44.7)	249 (60.0)
Doctor	183 (24.6)	100 (24.6)	185 (23.0)	115 (27.7)
Medical technologist	78 (10.5)	44 (10.8)	77 (9.6)	38 (9.2)
Medical assistant	75 (10.1)		103 (12.8)	
Pharmacist	22 (3.0)		15 (1.9)	2 (0.5)
Other profession not involved in patient	44 (5.9)	8 (2.0)	65 (8.1)	11 (2.7)
HCWs engaged in COVID-19 ward	36 (4.8)	18 (4.4)	24 (3.0)	22 (5.3)

n, number; HCW, healthcare worker.

Table 2. The seroprevalence of SARS-CoV-2 antibodies in the participants

Category	1st survey <i>n</i> (%)	2nd survey <i>n</i> (%)	3rd survey <i>n</i> (%)	4th survey <i>n</i> (%)
Outpatient	1000	1000	1000	1000
Positive for both assays, <i>n</i> (%), 95% CI	1 (0.1), 0.018–0.56	0	0	0
The titer of SARS-CoV-2 antibodies				
COI > 1.0 (Roche assay)	1 (0.1)	2 (0.2)	0 (0)	2 (0.2)
Index > 1.4 (Abbott assay)	1/1	0/2	0	0/2
COI > 0.2, ≤ 1.0 (Roche assay)	5 (0.5)	9 (0.9)	12 (1.2)	17 (1.7)
Index > 1.4 (Abbott assay)	0/5	0/9	0/12	0/17
HCW	743	407	804	415
Positive for both assays, <i>n</i> (%), 95% CI	0	0	1 (0.124), 0.022–0.70	0
The titer of SARS-CoV-2 antibodies				
COI > 1.0 (Roche assay)	3 (0.40)	3 (0.74)	5 (0.62)	3 (0.72)
Index > 1.4 (Abbott assay)	0/3	0/3	1/5	0/3
COI > 0.2, ≤ 1.0 (Roche assay)	5 (0.67)	4 (0.98)	4 (0.98)	8 (1.93)
Index > 1.4 (Abbott assay)	0/5	0/4	0/4	0/8

n, number; CI, confidence interval; COI, cutoff index.

The median age of the HCWs did not change among the surveys. The major occupations of the HCWs were nurses, doctors, and medical technologists. The proportion of HCWs engaged in the COVID-19 ward during this study was 3.0%–5.3%. Only one person who had not worked in the COVID-19 ward in the third survey was positive for SARS-CoV-2 antibodies, giving a seroprevalence of 0.124% (95% CI: 0.022–0.70).

We have confirmed that the positive samples

determined by the Roche assays have positive antibody titers in the Abbott assays. However, responses for Roche assays slowly rose and started to decline from 12 weeks, but remained above the positive threshold. Conversely, antibodies were detected earliest with the Abbott assay but declined rapidly (14). Thus, for all samples showing a COI of 0.2–1.0 in the Roche assay, we also measured the antibody titer by the Abbott assay, indicating that the results were negative.

Of the 57 infected patients who had been treated up to the 4th survey, 51 (89.5%) had severe COVID-19, necessitating ventilator management. Among the 49 patients with severe COVID-19, 22 (44.9%) had a viral load beyond the upper limit of abnormal in the quantitative SARS-CoV-2 antigen test ([CLIA], Lumipulse® SARS-CoV-2 Ag; Fujirebio, Tokyo, Japan) using nasopharyngeal swabs upon admission to our hospital (15). Thus, some HCWs in our hospital who had intimate contact with severe COVID-19 cases tended to have high viral loads. HCWs use personal protective equipment (PPE) according to the World Health Organization (WHO) guidelines on the prevention of SARS-CoV-2 infection when dealing with patients who had confirmed or suspected COVID-19 (16). This study found no evidence of clustering of seropositive outpatients and HCWs in our hospital over time, suggesting that there was no increased risk of patient-to-HCW, HCW-to-HCW, or HCW-to-outpatient COVID-19 transmission. In our hospital, the Pfizer-BioNTech COVID-19 vaccine was administered to HCWs between March and May, 2021. Of the 804 HCWs who participated in the 3rd survey, 761 (94.7%) received the vaccine. Further, as COVID-19 vaccinations for the general elderly population in Ehime began on April 12, 2021, this might have affected the results of 3rd and 4th surveys.

This study had some limitations. First, the number of HCWs was limited because those receiving regular medical checks without blood sampling were excluded. Second, pediatric and very elderly outpatients might have had an immature immune response, indicating lower antibody levels due to inadequate antibody response, which might not have represented the exact seroprevalence during the study period. Finally, we were unable to examine the number of HCWs and outpatients who had had contact with confirmed or suspected COVID-19 cases because this study employed an opt-out approach. However, the opt-out strategy potentially provided a less-biased picture. Therefore, we consider that this study has provided realistic data on COVID-19 epidemiology.

In conclusion, this study has provided evidence that the magnitude of COVID-19 infection among outpatients and HCWs in a hospital serving a rural area might have been small and changed only slightly over time.

Acknowledgments We thank Kazutaka Nishimura at the Ehime General Health Care Association for providing the samples from HCWs at Ehime University Hospital. We thank the staff engaged in COVID-19-related work at Ehime University Hospital and Risako Yamashita at the Clinical Therapeutic Trial Center, Ehime University Hospital, for the support with the research plan. The work was supported by a donation from Sumitomo Mitsui Trust Bank.

Conflict of interest None to declare.

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