*Epidemiology and Infection*

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# Seroprevalence of severe acute respiratory syndrome coronavirus 2 N antibodies between December 2021 and march 2023 in Japan

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## Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in 2019 in China and rapidly spread worldwide, leading to a pandemic. The threat of SARS-CoV-2 is subsiding as most people have acquired sufficient antibodies through vaccination and/or infection to prevent severe COVID-19. After the emergence of the omicron variants, the seroprevalence of antibodies against the N protein elicited by SARS-CoV-2 infection ranged from 44.4% to 80.2% in countries other than Japan. Here, we assessed the seroprevalence in Japan before and after the appearance of omicron variants. Serosurveillance of antibodies against N was conducted between December 2021 and March 2023 in Japan. In total, 7604 and 3354 residual serum or plasma samples were collected in the Tokyo metropolitan area and Sapporo, respectively. We found that the seroprevalence in representative regions of Japan increased approximately 3% to 23% after the emergence of the omicron variants. We also found higher seroprevalence among the young compared with the elderly. Our findings indicate that unlike other countries, most of the Japanese population has not been infected, raising the possibility of future SARS-CoV-2 epidemics in Japan.

## Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in 2019 in China and rapidly spread worldwide, leading to a pandemic. As of May 2023, 760 million cases including 6.9 million deaths have been reported to the World Health Organization (https://covid19.who.int/). The threat of SARS-CoV-2 is subsiding as most people have acquired sufficient antibodies through vaccination and/or infection to prevent severe COVID-19; more than 80% of the populations of major industrialized countries have antibodies against the S protein induced by vaccination and/or infection [1–5]. In contrast, the seroprevalence of antibodies against the N protein elicited by SARS-CoV-2 infection ranged from 44.4% to 80.2% in the United States, England, Spain, Slovenia, Switzerland, and Thailand between March and November 2022 after the emergence of the omicron variants [3, 4, 6–10]. The positive rate was higher among younger individuals than among the elderly [8].

In March 2022, cumulative confirmed cases in Japan were approximately 6.6 million, representing 5.5% of the Japanese population, and the seroprevalence of antibodies against the N protein was 3.5% [1]. This rate was significantly lower than that of the aforementioned countries. Subsequently, an epidemic continued in Japan, with cumulative confirmed cases as of May 2023 exceeding 33.8 million (28.2% of the Japanese population). Accordingly, we investigated the seroprevalence of antibodies against N between December 2021 and March 2023 in Japan.

## Short Paper

**Cite this article:** Yamayoshi S, Nagai E, Mitamura K, Hagihara M, Kobayashi R, Takahashi S, Shibata A, Uwamino Y, Hasegawa N, Iqbal A, Kamimaki I, Iwatsuki-Horimoto K, Nagamura-Inoue T and Kawaoka Y (2024). Seroprevalence of severe acute respiratory syndrome coronavirus 2 N antibodies between December 2021 and march 2023 in Japan. *Epidemiology and Infection*, **152**, e24, 1–4
https://doi.org/10.1017/S0950268824000141

Received: 04 October 2023
Revised: 01 January 2024
Accepted: 10 January 2024

**Keywords:**
COVID-19; Japan; SARS-CoV-2; seroprevalence; N protein

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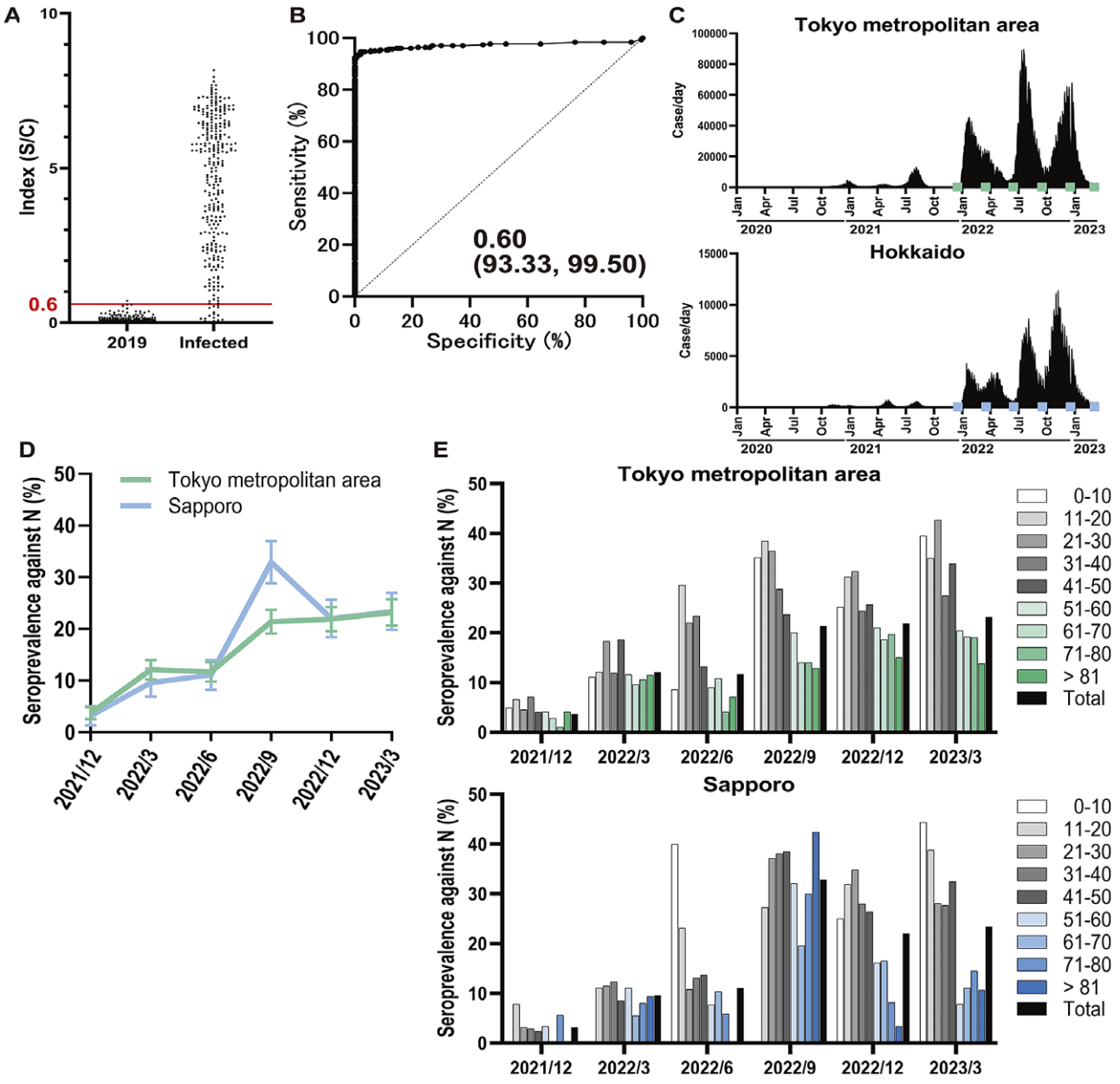


**Figure 1.** Seroprevalence of antibodies against N of SARS-CoV-2. (a) The results of the Abbott Alinity i SARS-CoV-2 anti-nucleocapsid protein IgG assay are shown for 300 convalescent sera and 200 residual serum samples collected in November 2019. The cut-off value of 0.60 was determined according to the ROC shown in (b). (b) The ROC was drawn to differentiate between infected and pre-pandemic samples. The cut-off value was determined by using Youden's index. The specificity and sensitivity of the test are shown in parentheses. (c) Epidemic curve of COVID-19 in the Tokyo metropolitan area and Hokkaido. The daily numbers of reported COVID-19 cases in the Tokyo metropolitan area and Hokkaido are shown. The light green and light blue lines indicate the period during which residual serum was collected. Total seroprevalence (d) and age-specific seroprevalence (e) of antibodies against N of SARS-CoV-2 in the Tokyo metropolitan area and Sapporo, Japan.

## Methods

### *Ethical considerations*

The study protocol was reviewed and approved by the institutional review board of the Institute of Medical Science, the University of Tokyo. The protocol was also reviewed and approved by each research institute and healthcare facility involved. The study participants gave informed consent during their healthcare facility visits for their data and residual samples to be used anonymously for clinical research.

### *Validation samples*

Convalescent sera from patients with laboratory-confirmed COVID-19, which were collected previously [2], were used as positive controls, and residual serum samples collected in Nov 2019 in Japan were used as negative controls.

### *Samples*

The study subjects were patients who visited Sapporo Medical University Hospital, Japanese Red Cross Ashikaga Hospital, Keio

https://doi.org/10.1017/S0950268824000141 Published online by Cambridge University Press

**Table 1.** Number of samples collected in the Tokyo metropolitan area and Sapporo.

| | Tokyo metropolitan area | | | | | | Sapporo | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Month/year | | | | | | Month/year | | | | | |
| Age | 12/2021 | 3/2022 | 6/2022 | 9/2022 | 12/2022 | 3/2023 | 12/2021 | 3/2022 | 6/2022 | 9/2022 | 12/2022 | 3/2023 |
| 0–10 | 183 | 162 | 93 | 162 | 143 | 43 | 4 | 3 | 5 | 3 | 8 | 9 |
| 11–20 | 61 | 66 | 54 | 39 | 67 | 20 | 51 | 36 | 39 | 33 | 47 | 80 |
| 21–30 | 67 | 93 | 82 | 74 | 74 | 75 | 62 | 78 | 65 | 70 | 63 | 89 |
| 31–40 | 113 | 134 | 128 | 118 | 123 | 102 | 105 | 114 | 107 | 105 | 100 | 94 |
| 41–50 | 100 | 102 | 114 | 93 | 105 | 106 | 85 | 94 | 95 | 78 | 91 | 77 |
| 51–60 | 147 | 146 | 166 | 175 | 157 | 157 | 58 | 54 | 65 | 53 | 56 | 51 |
| 61–70 | 211 | 177 | 194 | 200 | 183 | 182 | 74 | 73 | 68 | 77 | 79 | 90 |
| 71–80 | 300 | 284 | 243 | 250 | 249 | 273 | 89 | 87 | 85 | 80 | 73 | 76 |
| >81 | 148 | 156 | 170 | 186 | 180 | 174 | 26 | 32 | 28 | 33 | 29 | 28 |
| Total | 1,330 | 1,320 | 1,244 | 1,297 | 1,281 | 1,132 | 554 | 571 | 557 | 532 | 546 | 594 |

University Hospital, National Hospital Organization Saitama Hospital, or Eiju General Hospital, Japan, in December 2021, March 2022, June 2022, September 2022, December 2022, and March 2023. Sapporo Medical University Hospital is located in Hokkaido Prefecture, whereas all of the other healthcare facilities are located in the Tokyo metropolitan area. Residual serum or plasma samples were collected in the Tokyo metropolitan area (n = 7604) and Sapporo (n = 3354), respectively. The samples collected in December 2021 and March 2022 were also used in a previously study [2].

Residual serum or plasma samples collected for medical examination were analyzed. The reason for the healthcare facility visit was not considered for inclusion in this study. Because the samples were collected anonymously, it is possible that some of them were from multiple visits by the same patients, but we could not identify or exclude them.

### *Measurement of antibodies against N*

We used the Abbott Alinity i SARS-CoV-2 anti-nucleocapsid protein IgG kit (Abbott), which is a semiquantitative chemiluminescent microparticle assay. Before measurement, all samples were incubated for more than 45 min at 56 °C.

### *Statistical analysis*

A receiver operating characteristic (ROC) curve was plotted using GraphPad Prism 9.0.3 to determine a threshold for whether the index (S/C) values of the serum samples were negative or positive according to Youden's index.

### *Other data sources*

The website (https://covid19.mhlw.go.jp/public/opendata/newly_confirmed_cases_daily.csv) provided the daily number of reported human COVID-19 cases from 16 January 2020 to 31 March 2023. COVID-19 confirmation before May 2020 and after relied on quantitative reverse transcription polymerase chain reaction (RT-qPCR) alone and RT-qPCR and rapid antigen tests, respectively, approved for clinical use.

## Results and discussion

The Abbott Alinity i SARS-CoV-2 anti-nucleocapsid protein IgG kit was used to detect antibodies against the N protein of SARS-CoV-2 in human sera. We analyzed 200 negative serum samples and 300 COVID-19 convalescent samples to establish a cut-off value and to evaluate the specificity and sensitivity of the kit. We found that the kit appropriately classified the positive and negative samples (Figure 1a). Based on the ROC curves (Figure 1b), Youden's index determined that the cut-off value was 0.60 and the specificity and sensitivity were 93.33% and 99.50%, respectively. Using this kit, we then examined 10,958 residual serum or plasma samples collected between December 2021 and March 2023 (Figure 1c) in the Tokyo metropolitan area and Sapporo (Table 1). The seroprevalence in the Tokyo metropolitan area was 3.7%, 12.1%, 11.7%, 21.4%, 21.9%, and 23.2%, whereas that in Sapporo was 3.2%, 9.6%, 11.1%, 32.9%, 22.0%, and 23.4% in December 2021, March 2022, June 2022, September 2022, December 2022, and March 2023, respectively (Figure 1d). We also found higher seroprevalence among the young compared with the elderly (Figure 1e), as previously reported [8].

Our results showed that the seroprevalence in the Sapporo and Tokyo areas before the emergence of the omicron variant was only approximately 3%, despite the pandemic of D614G, alpha, and delta variants of SARS-CoV-2 in Japan lasting nearly 2 years. However, the seroprevalence progressively increased to approximately 23% after the emergence of the omicron variants. The seroprevalence of antibodies against the N protein in Japan was reported to be 3.5% in February to March 2022 [1] and cumulative confirmed cases as of May 2023 exceeded 33.8 million (28.2% of the Japanese population). Unlike other countries where seroprevalence rates exceeded 50% [3, 4, 6–10], most of the Japanese population had not been infected at that time, suggesting the possibility of future SARS-CoV-2 epidemics in Japan.

In September 2022, the seroprevalence was higher in Sapporo than in Tokyo, whereas it was similar between the two areas at other

https://doi.org/10.1017/S0950268824000141 Published online by Cambridge University Press

time points. The higher seroprevalence among individuals aged 70 years and older in Sapporo accounted for the difference in September 2022. Further investigation is required to determine the reason for this high prevalence among the elderly.

**Acknowledgements.** We thank Chiaki Kawana, Konatsu Hagihara, and Wakana Sato for assistance with experiments and Noriaki Harada, Izuru Iwase, Junya Shigenaga, Kazumi Yanagi, Taro Kaneda, Ryoko Karakama, Yuka Suzuki, Syoma Ookaji, Ayano Aketa, Sayaka Sato, Misako Sugaya, Misa Hayasaka, Yoko Kira, Yuki Fujiwara, Akimi Kaku, Masako Moteki, Moe Yasuda, and the clinical laboratory staff at Keio University Hospital for their efforts with the organization and collection of blood samples. We also thank Susan Watson for editing the manuscript.

**Author contribution.** S.Y. and Y.K. designed the study, analyzed the data, and wrote the manuscript. E.N., K. M., M.H., R.K., S.T., A.S., Y.U., N.H., A.I., I.K., K.I.-H., and T.N.-I. collected data and samples. All authors reviewed and approved the manuscript.

**Funding statement.** This work was supported by the Japan Agency for Medical Research and Development (AMED) (JP23wm0125002, JP21fk0108552, and JP223fa627001) and the National Institutes of Allergy and Infectious Diseases funded Center for Research on Influenza Pathogenesis (CRIP; HHSN272201400008C).

**Competing interest.** The authors declare no conflicts of interest.

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https://doi.org/10.1017/S0950268824000141 Published online by Cambridge University Press