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Coronavirus Disease 2019 (COVID-19) Breakthrough Infection and Post-Vaccination Neutralizing Antibodies Among Healthcare Workers in a Referral Hospital in Tokyo: A Case-Control Matching Study

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Background. While increasing coverage of effective vaccines against coronavirus disease 2019 (COVID-19), emergent variants raise concerns about breakthrough infection. Data are limited, however, whether breakthrough infection during the epidemic of the variant is ascribed to insufficient vaccine-induced immunogenicity.

Methods. We describe incident COVID-19 in relation to the vaccination program among workers of a referral hospital in Tokyo. During the predominantly Delta epidemic, we followed 2415 fully vaccinated staff (BNT162b2) for breakthrough infection and selected 3 matched controls. We measured post-vaccination neutralizing antibodies against the wild-type, Alpha (B.1.1.7), and Delta (B.1.617.2) strains using live viruses and anti-spike antibodies using quantitative assays, and compared them using the generalized estimating equation model between the 2 groups.

Results. No COVID-19 cases occurred 1–2 months after the vaccination program during the fourth epidemic wave in Japan, dominated by the Alpha variant, while 22 cases emerged 2–4 months after the vaccination program during the fifth wave, dominated by the Delta variant. In the vaccinated cohort, all 17 cases of breakthrough infection were mild or asymptomatic and participants had returned to work early. There was no measurable difference between cases and controls in post-vaccination neutralizing antibody titers against the wild-type, Alpha, Delta, and anti-spike antibody titers, while neutralizing titers against the variants were considerably lower than those against the wild-type.

Conclusions. Post-vaccination neutralizing antibody titers were not decreased among patients with breakthrough infection relative to their controls under the Delta variant outbreak. The result points to the importance of infection-control measures in the post-vaccination era, irrespective of immunogenicity profile.

Keywords. COVID-19; breakthrough infection; neutralizing antibody; vaccination.

Clinical trials show that the mRNA-based vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are highly effective in lowering the risk of coronavirus disease 2019 (COVID-19) [1, 2]. In countries with high coverage of vaccination programs, however, the number of patients with COVID-19 has started to grow, which has been attributed to the emergence of variants of concern, especially the Delta (B.1.617.2) variant [3, 4], and the waning of vaccine efficacy over time [5, 6].

Epidemiological evidence is scarce regarding the role of vaccine-induced immunogenicity against variants of concern. In a case-control study among vaccinated healthcare workers, patients with breakthrough infection during the epidemic of the Alpha (B.1.1.7) variant had significantly lower peri-infection neutralizing antibody titers than controls [7]. A similar result was reported from a Vietnamese study [8] on breakthrough infection during the epidemic of the Delta variant, which is more resistant to the current vaccines than the Alpha variant [9].

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In Japan, the vaccination program started in mid-February 2021, initially among healthcare workers using the BNT162b2 mRNA vaccine (Pfizer-BioNTech). After that, Japan was hit by the 2 large waves of the COVID-19 epidemic: the fourth wave (April and May 2021), dominated by the Alpha variant, and the fifth wave (from July to September), dominated by the Delta variant [10]. During the fifth wave, we observed a surge of breakthrough infection among the staff of a large referral hospital in Tokyo, Japan, where post-vaccination serum was available. This prompted us to examine whether the breakthrough infection was ascribed to a failure of vaccination to build immunity or waning antibody immunity compounded by the emergence of variants of concern by comparing post-vaccination neutralizing antibodies between breakthrough infection cases and their matched controls during the large epidemic of the Delta variant.

METHODS

Study Setting and Population

The National Center for Global Health and Medicine, Japan (NCGM), comprising 2 hospitals and affiliated facilities, is a medical research center for specific areas, including infectious disease. As their mission, the NCGM has played a major role in the care and research of COVID-19 since the early stage of the epidemic [11] and has accepted many patients with severe COVID-19. During the in-house vaccination program using COVID-19 mRNA-LNP BNT162b2 (Pfizer-BioNTech) from March through June 2021, more than 90% of the NCGM staff received the 2-dose vaccine.

In the NCGM, a repeat serological study was launched in July 2020 to monitor the spread of SARS-CoV-2 infection among the staff during the course of the COVID-19 epidemic. As of October 2021, we have completed 3 surveys; in each of which we measured anti–SARS-CoV-2 nucleocapsid (all surveys) and spike (from the second survey onward) protein antibodies, stored serum samples at −80°C, and collected COVID-19–related information (vaccination, occupational infection risk, infection-prevention practices, etc) via a questionnaire. The results of the first and second surveys have been reported elsewhere [12, 13]. Participants of the third survey, which was conducted in June 2021 after the in-house vaccination program, formed the basis of the present case-control analysis. Of 3072 workers invited to the third survey, 2779 (90%) participated. Written informed consent was obtained from all participants, and the study procedure was approved by the NCGM Ethics Committee (approval number: NCGM-G-003598).

Identification of COVID-19 Cases

We identified COVID-19 cases among the study participants against the COVID-19 patient records kept by the NCGM Hospital Infection Prevention and Control Unit, which provided information on date of diagnosis, diagnostic procedure, possible route of infection (close contact), symptoms, hospitalization, and return to work for all cases, and virus strain and cycle threshold (Ct) values for those who were diagnosed at the NCGM. For COVID-19 cases among nonparticipants, the above data were anonymized and submitted to the study committee after the opt-out procedure (approval number: NCGM-S-004382).

Case-Control Selection

We conducted a case-control study nested in a subcohort of vaccinated participants who donated serum (Figure 1). Of 2456 participants who completed the 2 vaccinations before the third survey, we sequentially excluded those with a history of polymerase chain reaction (PCR)–confirmed COVID-19 (n = 14) and those with positive results on a SARS-CoV-2 nucleocapsid protein antibody test at any of the 3 surveys (n = 27), leaving 2415 participants. Of these, we identified 17 cases of breakthrough infection, which were defined as cases diagnosed at least 14 days after the second dose of vaccine by 6 September 2021. For each case, we randomly selected 3 uninfected controls from the subcohort while matching worksite, sex, age, the interval between the second vaccination and blood sampling, and the propensity score, which was created based on body mass index, occupational exposure risk of SARS-CoV-2, and several infection-prevention/risk behaviors. The details of the case-control matching algorithm are described in Supplementary Text 1.

Neutralizing Antibody Testing

The neutralizing activity of serum of cases and selected controls was determined by quantifying the serum-mediated suppression of the cytopathic effect (CPE) of each SARS-CoV-2 strain in VeroE6$_{TMPRSS2}$ cells [14]. The obtained routes of the cells and each virus are described in Supplementary Text 2. Each serum sample was 4-fold serially diluted in culture medium. The diluted sera were incubated with 50% tissue culture infectious dose ($TCID_{50}$) of virus at 37°C for 20 minutes (final serum dilution range of 1:20 to 1:4000), after which the serum-virus mixtures were inoculated with VeroE6$_{TMPRSS2}$ cells (1.0×10^4/well) in 96-well plates. The SARS-CoV-2 strains used in these assays are as follows: a Wuhan, wild-type strain (SARS-CoV-$2_{05\text{-}2N}$, PANGO lineage B) [14], an Alpha variant (SARS-CoV-2_{QHN001}), and a Delta variant (SARS-CoV-2_{1734}). After culturing the cells for 3 days, the levels of CPE observed in SARS-CoV-2–exposed cells were determined using the WST-8 assay with the use of the Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). The serum dilution that gave 50% inhibition of CPE was defined as the 50% neutralization titer (NT_{50}). Each serum sample was tested in duplicate, and the average value was used for analysis. The laboratory technicians were blinded to the case-control status.

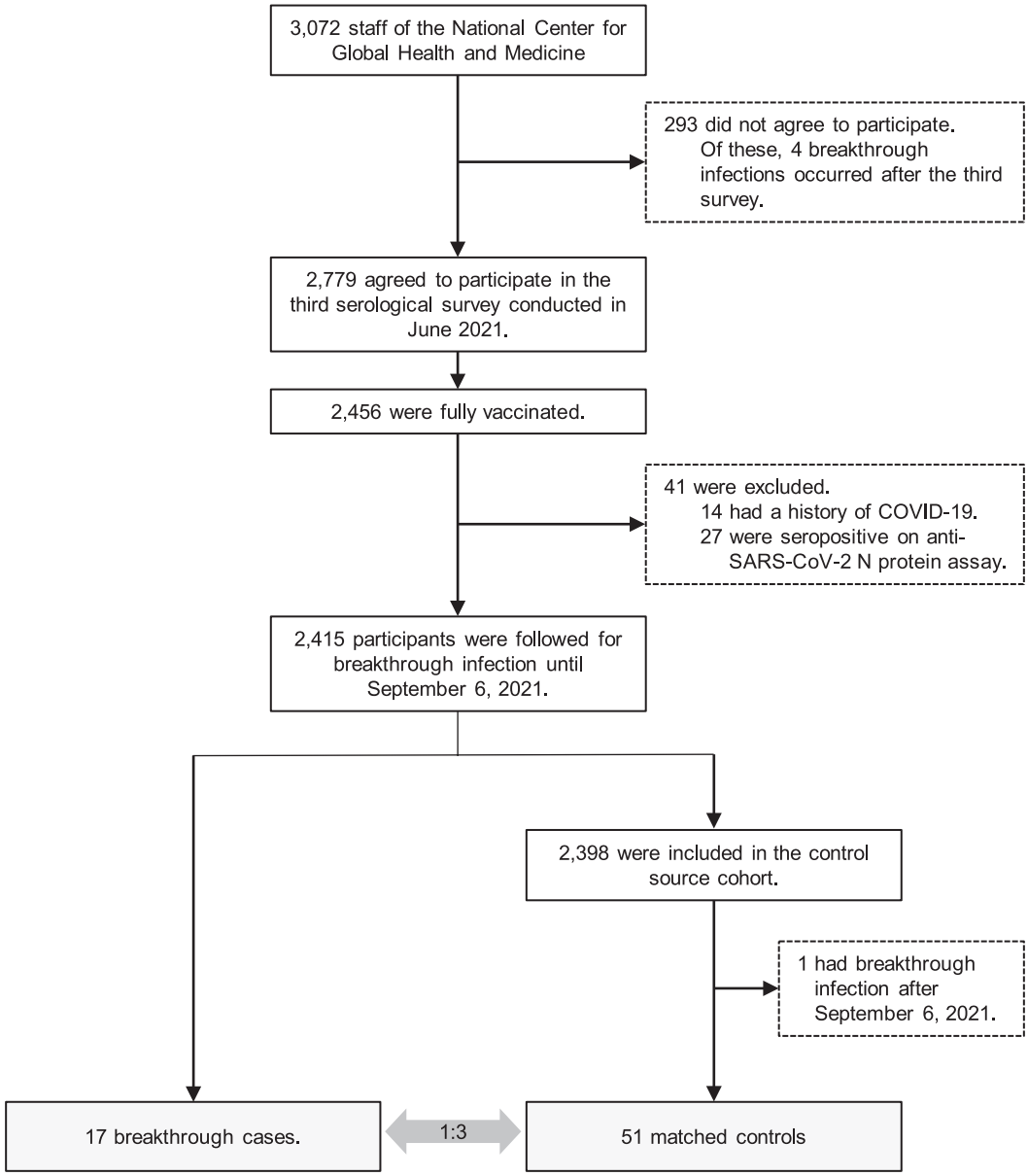

Figure 1. Flowchart for the nested case-control study. Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

SARS-CoV-2 Antibody Testing

We assessed anti–SARS-CoV-2 antibodies for all participants of the third survey and retrieved those data for the case-control subsets. We quantitatively measured antibodies against the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein by using the AdviseDx SARS-CoV-2 IgG II assay (Abbott) (immunoglobulin [Ig] G [IgG]) and Elecsys Anti-SARS-CoV-2 S RUO (Roche) (predominantly IgG, also IgA and IgM). The sensitivity and specificity were reported as 97.6% and 100%, respectively, for the Abbott assay [15], and 97.9% and 99.9%, respectively, for the Roche assay [16]. We also qualitatively measured antibodies against SARS-CoV-2 nucleocapsid protein using the SARS-CoV-2 IgG assay (Abbott) and Elecsys Anti-SARS-CoV-2 RUO (Roche), and used these data to exclude those with possible infection in the past. The sensitivity and specificity were 100% and 99.9%, respectively, for the Abbott assay [17], and 99.5% and 99.8%, respectively, for the Roche assay [18].

Statistical Analysis

We compared the characteristics between cases and matched controls using Mann–Whitney *U* test or Fisher's exact test. To examine the differences in antibody-mediated immune response after the second vaccination between cases and controls, we compared the log-transformed titers of neutralizing (wild-type, Alpha, and Delta) and anti-spike antibodies between the 2 groups using a generalized estimating equation (GEE) with the group assignment (case or control) and robust

variance estimator. Then, we back-transformed and presented these values in geometric mean titers (GMTs) with 95% confidence intervals (CIs). To compare the interindividual differences in neutralizing antibody titers against the wild-type, Alpha, and Delta strains, we used the Wilcoxon signed-rank test with adjustment using the Bonferroni method for multiple tests. Statistical analysis was performed using Stata version 17.0 (StataCorp LLC), and graphics were made by GraphPad Prism 9 (GraphPad, Inc). All P values were 2-sided and $P < .05$ was considered statistically significant.

RESULTS

Description of COVID-19 Incidence

Before the in-house vaccination program, 17 cases of COVID-19 (5.9 per 1000 persons) occurred among NCGM staff between November 2020 and February 2021 during the third wave of the epidemic in Japan, dominated by the Japan-specific B.1.1.214 variant (Figure 2). During the fourth wave between April and May 2021 (within 2 months after the vaccination program in the Toyama ward [80% of NCGM staff]), no cases were identified among staff in that ward. During the fifth epidemic wave between July and September 2021, when Delta was the dominant strain, 23 cases were identified (7.5 per 1000 persons). Of 23 cases, 22 (96%) were fully vaccinated. The risk of infection during the 2 months in the fifth wave (after the in-house vaccination program) was only slightly higher than that observed during the third wave (December 2020 to January 2021; before the program; 5.2 per 1000 persons), while the number of cases in Tokyo during the fifth wave (n = 133 989) was 3.5 times higher than that during the third wave (n = 38 492).

Characteristics of Breakthrough Infection in a Case-Control Analysis

Of 17 cases of breakthrough infection in the case-control analysis, 47% were men, the median age was 29 (interquartile range [IQR]: 25–44) years, and the median interval between the second COVID-19 vaccination and breakthrough infection was 111 (IQR: 98–123) days. The major occupations were nurses (53%), allied healthcare professionals (24%), and doctors (18%), and more than half (59%) were at low occupational risk of SARS-CoV-2 infection. The majority (88–100%) showed

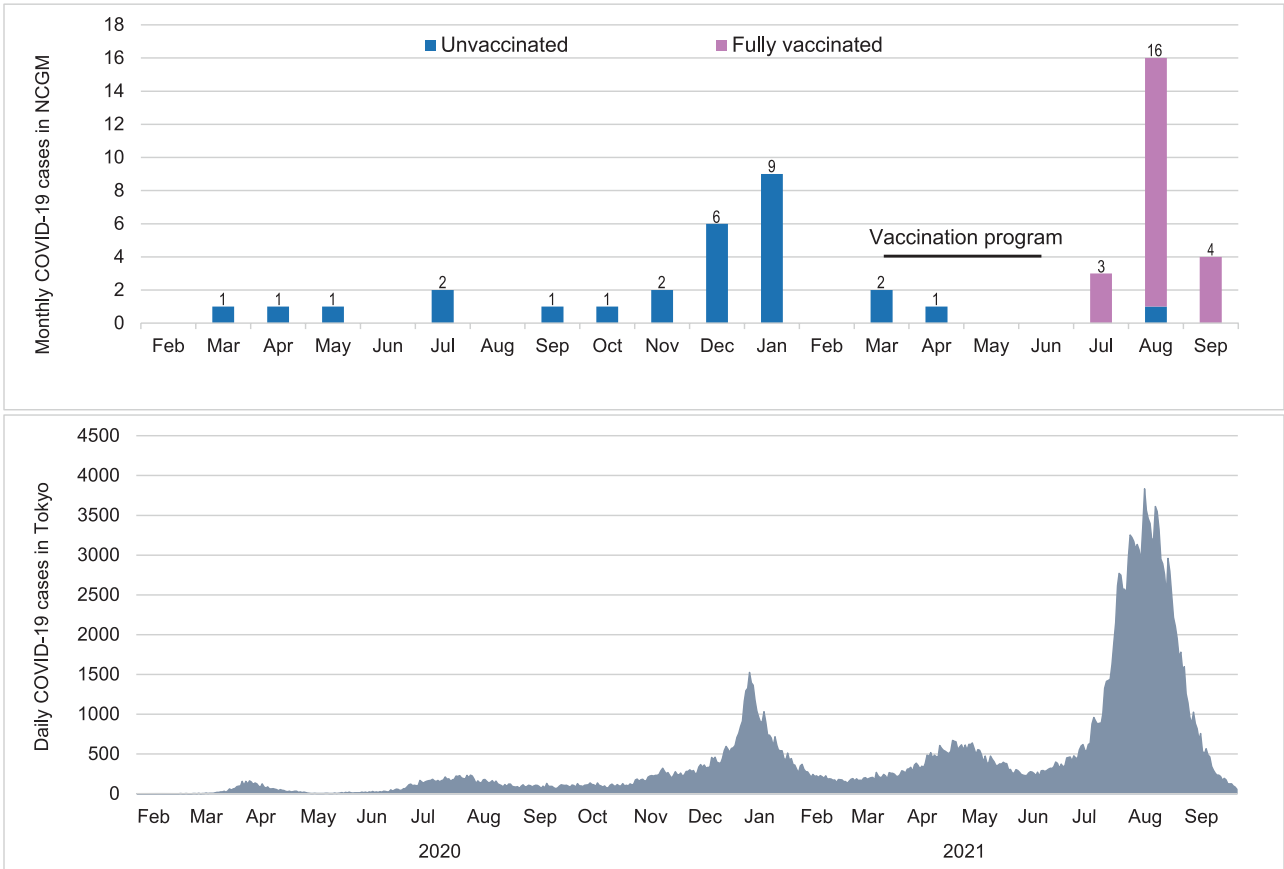

Figure 2. The number of confirmed COVID-19 cases at the NCGM and Tokyo between February 2020 and September 2021. The upper panel indicates the monthly confirmed COVID-19 cases among the NCGM workers. The lower panel indicates the daily confirmed COVID-19 cases in Tokyo, Japan, where the NCGM is located. In the NCGM, the vaccination program was conducted in March–April 2021 in the Toyama ward (wherein 80% of NCGM staff work) and April–June 2021 in the Kohnodai ward (20% of NCGM staff). Abbreviations: COVID-19, coronavirus disease 2019; NCGM, National Center for Global Health.

good adherence to infection-prevention practices. With regard to risky behavior related to leisure time, 18% reported having spent 30 minutes or more in crowded places, close-contact settings, and confined and enclosed spaces (the 3Cs) without a mask, and 12% reported having dined in a group of 5 or more people for more than 1 hour. There was no significant difference in these figures between cases and controls (Table 1).

All breakthrough cases occurred independently at different times and in different departments, and there was no evidence of the clustering of infection in the hospital. The suspected sources of breakthrough infection were contact with patients with COVID-19 in the household (35%), those in the community (24%), and unknown (41%), whereas nosocomial infection was not suspected in any breakthrough infections. Data on the type of SARS-CoV-2 strains were available for only 5 cases, all of those were the Delta variant. All patients, except for 1 patient (asymptomatic), experienced only mild symptoms—fever (65%), sore throat (35%), cough (24%), nasal discharge (18%), and malaise (18%)—and they returned to work without hospitalization or special medical care (Table 1). The detailed characteristics of each breakthrough infection case are described in Supplementary Table 1.

Neutralizing and Anti-Spike Antibodies

The post-vaccination neutralizing antibody titers against the wild-type, Alpha, and Delta (median: 62 days between the second vaccination and blood sampling) were not statistically different between breakthrough cases and their matched controls (Table 2, Figure 3A–C). The GEE predicted that neutralizing antibody GMTs (NT_{50}) against the wild-type virus was 405 (95% CI, 327–501) for cases and 408 (95% CI, 320–520) for controls, and the predicted case-to-control ratio was .99 (95% CI, .74–1.34). Those against the Alpha variant were 116 (95% CI, 80–169) for cases and 122 (95% CI, 96–155) for controls, with a ratio of .95 (95% CI, .71–1.28). Those against the Delta variant were 123 (95% CI, 85–177) for cases and 135 (95% CI, 108–170) for controls, and the ratio was .91 (95% CI, .61–1.34).

The predicted GMTs of post-vaccination anti-spike antibody titers were comparable among cases and controls (Table 2, Figure 3D and 3E). The predicted GMT of post-vaccination anti-spike antibody on the Abbott assay (arbitrary units [AU]/mL) was 5129 (95% CI, 3881–6779) for cases and 6274 (95% CI, 5017–7847) for controls, for a ratio of .82 (95% CI, .65–1.02). The predicted GMT on the Roche assay (U/mL) was 1144 (95% CI, 802–1632) for cases and 1208 (95% CI, 1053–1385) for controls, and the ratio was .95 (95% CI, .70–1.27).

The neutralization titers against the Alpha and Delta variants were much lower than those against the wild-type virus (Figure 4). Among cases, the GMTs (95% CI) of neutralizing antibody were 116 (77–175), 123 (83–182), and 404 (321–508) for the Alpha and Delta variants and the wild-type virus, respectively ($P < .01$ for each variant vs wild-type). Similar results were obtained for controls.

DISCUSSION

Among the staff of a large referral hospital in Tokyo, a series of breakthrough infections were documented during the largest COVID-19 epidemic wave, dominated by the Delta variant. In a case-control study nested within the cohort of vaccinated staff, post-vaccination neutralizing antibody titers did not materially differ between breakthrough infection cases and their matched controls.

In the NCGM, the number of patients with COVID-19 during the fifth wave (n = 23, 2 to 5 months after the in-house vaccination program) was slightly higher than that during the third wave (n = 17, before the vaccination program) despite 3.5 times the number of cases recorded during the former period than the latter period in Tokyo as a whole [19]. The vaccine effectiveness against Delta variant infection after 4 months was 53% in the United States [4]. and 35.1% in Qatar [5]. It is clear that a vaccination program alone cannot eliminate the risk of infection by the Delta variant. Still, we could reasonably infer that the program has contributed to the sizable reduction in the number of patients with COVID-19 among the staff during the largest wave.

We confirmed that the breakthrough cases occurred independently at different times and in different departments, showing no evidence of clustered infection in the hospital. This finding is consistent with our previous reports from the first and second serological surveys in the NCGM before the vaccination program [12, 13], suggesting that infection among the staff had occurred mainly outside the hospital (household, community, etc). The NCGM has adopted comprehensive measures against nosocomial infection since the early phase of the epidemic. The current data confirm the significant role of these measures to protect healthcare workers against infections with the Delta variant.

Contrary to our prior expectation, we observed no measurable difference in the level of both neutralizing and anti-spike antibodies between cases with breakthrough infection and their controls. This finding contradicts data from Israel [7] and Vietnam [8], both showing significantly lower neutralizing antibody titers among those with breakthrough infection than controls. While the reason for the discrepant findings is not clear, it could be ascribed to the difference in the timing of blood sampling for antibody testing (peri-infection [7, 8] or pre-infection [present study]), the predominant variants (Alpha [7] and Delta [8] [present study]), measurement of neutralizing antibody (use of 3 predominant types of live virus [present study] or use of unspecific surrogate virus [7, 8]), and the case-control matching strategy.

Table 1. Characteristics of Participants in the Case-Control Study

Characteristics	Cases (n = 17)	Controls (n = 51)	*P*
Men, n (%)	8 (47)	24 (47)	1.00
Age, years	29 (25–44)	30 (25–44)	.68
Interval between second vaccination and blood sampling, days	63 (43–69)	62 (40–69)	.76
Interval between blood sampling and breakthrough infection, days	55 (45–64)	…	…
Interval between second vaccination and breakthrough infection, days	111 (98–123)	…	…
Body mass index, kg/m^2	21 (20–22)	20 (19–22)	.29
Job category, n (%)			.70
Doctors	3 (18)	10 (20)	
Nurses	9 (53)	22 (43)	
Allied health professionals	4 (24)	8 (16)	
Administrative staff	0	5 (10)	
Others	1 (6)	6 (12)	
Occupational SARS-CoV-2 exposure risk,[a] n (%)			.32
Low	10 (59)	23 (45)	
Moderate	6 (35)	17 (33)	
High	1 (6)	11 (22)	
Adherence to infection-prevention practices,[b] n (%)			
Avoiding 3Cs[c]	16 (94)	49 (96)	1.00
Keeping social distance	15 (88)	45 (88)	1.00
Wearing a mask	17 (100)	51 (100)	…
Practicing cough etiquette	17 (100)	51 (100)	…
Not touching eyes, nose, and mouth.	16 (94)	46 (90)	1.00
Washing or sanitizing hands	17 (100)	51 (100)	…
Use of public transportation for commuting to work, n (%)			.69
None or <1 time/week	8 (47)	27 (53)	
1–4 times/week	2 (12)	9 (18)	
≥5 times/week	7 (41)	15 (29)	
Spending ≥30 minutes in the 3Cs without mask, n (%)			.14
None	14 (82)	33 (65)	
1–5 times	2 (12)	17 (33)	
≥10 times	1(6)	1 (2)	
Dinner in a group of ≥5 people for >1 hour, n (%)			.72
None	15 (88)	42 (82)	
1–5 times	1 (6)	7 (14)	
≥6 times	1 (6)	2 (4)	
Suspected sources of infection, n (%)			
Household	6 (35)	…	…
Community	4 (24)	…	…
Unknown	7 (41)	…	…
Type of SARS-CoV-2 strain, n (%)			
Delta	5 (29)	…	…
Unknown (unmeasured)	12 (71)	…	…
Symptoms, n (%)			
Fever	11 (65)	…	…
Sore throat	6 (35)	…	…
Cough	4 (24)	…	…
Nasal discharge	3 (18)	…	…
Malaise	3 (18)	…	…
Asymptomatic	1 (6)	…	…
Returned to work, n (%)	17 (100)	…	…

Data are presented as median (interquartile range) for continuous measures and n (%) for categorical measures.

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; 3Cs, crowded places, close-contact settings, and confined and enclosed spaces.

[a]Occupational SARS-CoV-2 exposure risk was categorized as follows: low (those who were not engaged in COVID-19–related work), moderate (those who were engaged in COVID-19–related work without heavy exposure to the virus), and high (those who were heavily exposed to SARS-CoV-2).

[b]In each question related to infection-prevention practice, participants' responses were categorized using a 4-point Likert scale: "always," "often," "seldom," and "not at all," with the first 2 response options defined as good adherence to infection-prevention practice. These *P* values represent the results of comparison between always or often and seldom or not at all.

Table 2. Comparison of Post-Vaccination Antibody Titers Between Cases and Controls

Variables	Cases (n = 17)	Controls (n = 51)	Ratio of Cases to Controls	*P*
Neutralizing antibody (wild-type strain), NT_{50}				
Observed GMT (95% CI)	404 (321–508)	422 (349–511)	…	…
Predicted GMT by GEE model (95% CI)	405 (327–501)	408 (320–520)	.99 (.74–1.34)	.96
Neutralizing antibody (Alpha variant), NT_{50}				
Observed GMT (95% CI)	116 (77–175)	122 (101–147)	…	…
Predicted GMT by GEE model (95% CI)	116 (80–169)	122 (96–155)	.95 (.71–1.28)	.76
Neutralizing antibody (Delta variant), NT_{50}				
Observed GMT (95% CI)	123 (83–182)	135 (108–169)	…	…
Predicted GMT by GEE model (95% CI)	123 (85–177)	135 (108–170)	.91 (.61–1.34)	.63
Anti-spike antibody (Abbott), AU/mL				
Observed GMT (95% CI)	5129 (3794–6935)	6275 (5212–7553)	…	…
Predicted GMT by GEE model (95% CI)	5129 (3881–6779)	6274 (5017–7847)	.82 (.65–1.02)	.07
Anti-spike antibody (Roche) ,U/mL				
Observed GMT (95% CI)	1144 (779–1680)	1208 (1050–1389)	…	…
Predicted GMT by GEE model (95% CI)	1144 (802–1632)	1208 (1053–1385)	.95 (.70–1.27)	.72

Data are shown as the observed GMTs of antibodies in cases and matched controls and the predicted GMT using a GEE with the group assignment (case or control) used as the predictor. Three controls matched to each case were randomly selected from the study cohort using the following matching variables: worksite, sex, interval between the second-dose vaccination and blood sampling, age, and the propensity score, which was created based on body mass index, occupational exposure risk of SARS-CoV-2, use of public transportation, the frequency of spending 30 minutes or more in the 3Cs without a mask, the frequency of having dined in a group of 5 or more people for more than 1 hour, and the adherence to 6 types of infection-prevention behaviors (avoiding 3Cs; keeping social distance; wearing a mask; practicing cough etiquette; not touching eyes, nose, and mouth; and washing or sanitizing hands).

Abbreviations: AU, arbitrary units; CI, confidence interval; GEE, generalized estimating equation; GMT, geometric mean titer; NT_{50}, 50% neutralization titer; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; 3Cs, crowded places, close-contact settings, and confined and enclosed spaces.

Neutralizing capacity against the Alpha and Delta variants in the sera of the vaccinated participants was much lower than that against the wild-type strain, irrespective of breakthrough infection. This finding agrees with a previous report [20] and is consistent with the decreased vaccine effect observed during the epidemic, predominantly related to the variants of concern [3–5]. More than 90% of NCGM-Toyama ward staff had completed the second dose of vaccine

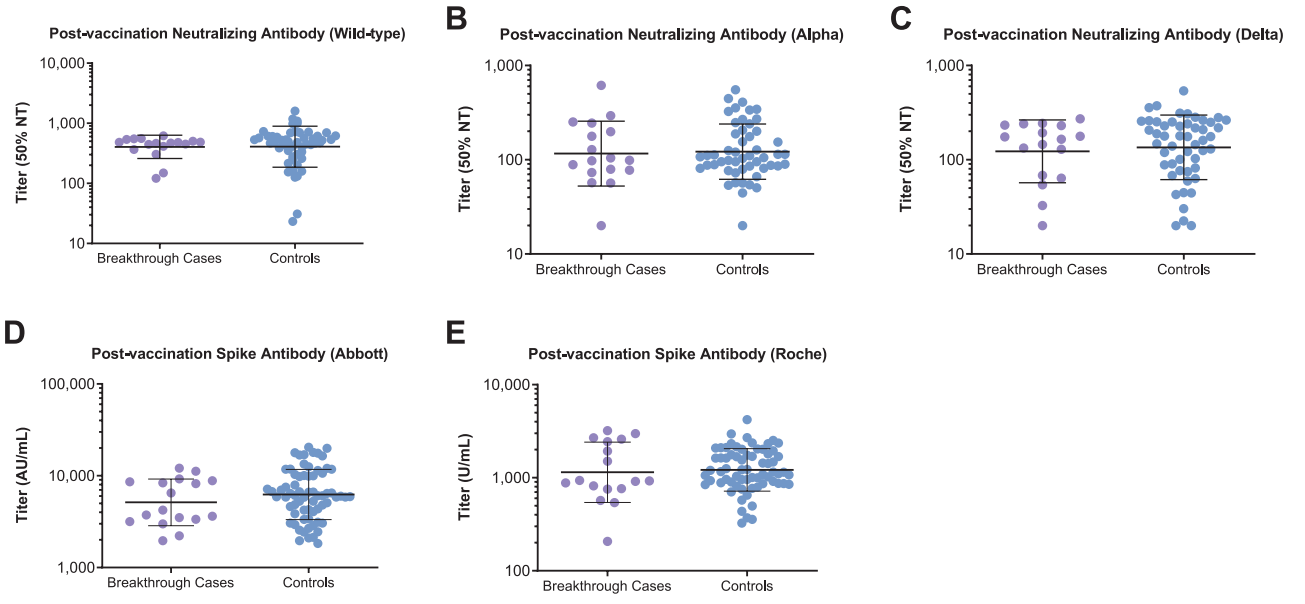

Figure 3. Post-vaccination neutralizing and anti-spike antibody titers among cases and controls. Among the 17 fully vaccinated healthcare workers who had breakthrough infection with SARS-CoV-2 and the 51 matched controls, shown are post-vaccination neutralizing antibody titers against the wild-type strain (*A*), the Alpha variant (*B*), and the Delta variant (*C*) during the pre-infection period (median of 62 days since the second vaccination). Also shown is the comparison of post-vaccination anti-spike antibody titers measured by the Abbott reagent (*D*) and those by the Roche reagent (*E*) in the 2 groups. Each case of breakthrough infection was matched with 3 controls according to worksite, sex, age, the interval between the second vaccination and blood sampling, and propensity score, estimated by body mass index, occupational exposure risk of SARS-CoV-2, and adherence to several infection-prevention/risky behaviors. In each panel, the horizontal bars indicate the geometric mean titers, and the I-shaped bars indicate geometric standard deviations. Abbreviations: AU, arbitrary units; COVID-19, coronavirus disease 2019; NT, neutralizing titer; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

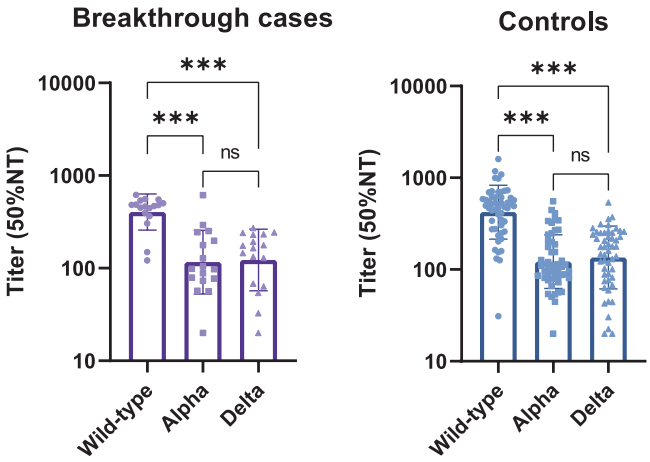

Figure 4. Neutralization of Delta (B.1.617.2) and Alpha (B.1.1.7) live viruses by serum from breakthrough cases (n = 17) or controls (n = 51) in comparison with the wild-type virus (Wuhan strain). Shown are the 50% neutralizing titer (50%NT), the serum dilution required for 50% virus inhibition, expressed as GMTs with geometric standard deviations (I-shaped bars). The purple bars indicate breakthrough infections (n = 17) and the blue bars indicate the matched controls (n = 51). ***$P < .001$ by the Wilcoxon matched-pairs signed-rank test. Abbreviations: GMT, geometric mean titer; ns, not significant.

by mid-April 2021. Soon after, the fourth epidemic wave of COVID-19 occurred in Japan, dominated by Alpha; however, no vaccinated staff acquired COVID-19, confirming the high vaccine effect against infection of the Alpha variant within 2 months of vaccination [4, 5]. Breakthrough infection in the NCGM from July to August 2021 could be attributed to the subsequent waning of neutralizing capacity and the largest epidemic wave due to the highly vaccine-resistant Delta variant [9, 20].

We found that all patients with COVID-19 who completed the 2-dose vaccination experienced no or only mild symptoms and had successfully returned to work, a finding compatible with previous reports [5, 7, 8, 20]. These data support that vaccination contributes to the maintenance of hospital function by shortening sick leave due to COVID-19 of healthcare workers and facilitating their earlier return to work.

The present study has several strengths. Both cases and controls were derived from a well-defined cohort. We rigorously matched each case and control using the propensity score estimated by several factors potentially associated with infection risk (including the occupational risk of infection and prevention practices). The blood samples for antibody assays were obtained before the infection. We measured neutralizing antibody titers against the wild-type strain and 2 major variants using live viruses.

We also acknowledge limitations of the study. We measured antibody levels using samples obtained 5 to 10 weeks (median, 8 weeks) before the infection, which might have decreased at the time of infection due to the waning of antibodies over time. Nevertheless, we found no evidence of a difference between cases and controls in the association of the duration of time between vaccination and blood sampling with neutralizing antibody titers (Supplementary Figure 1), faster waning of antibodies among cases than among controls. Despite the robust study design and rigorous matching strategy, we cannot exclude the possibility of bias due to unmeasured factors, including contact with the infected patient prior to breakthrough infection (not available for controls). All 4 cases of breakthrough infection among nonparticipants were employees of NCGM contracting companies (cleaning and restaurant), and they were older than patients with COVID-19 included in the case-control analysis (median age: 53 vs 29 years), reflecting the characteristics of nonparticipants. Data on virus type were available for only 5 cases (all Delta variants). However, the remaining cases of breakthrough infection were most likely due to the Delta variant, which accounted for more than 90% of sequenced COVID-19 samples in Japan during the fifth epidemic wave [10].

Conclusions

In conclusion, post-vaccination neutralizing antibodies against the wild-type, Alpha, and Delta variants did not materially differ between healthcare workers who experienced breakthrough infection and their matched controls under the Delta variant outbreak. The result points to the importance of continued adherence to infection-control measures in the post-vaccination variant-circulating era, irrespective of the level of immunogenicity.

Supplementary Data

Supplementary materials are available at *Clinical 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

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Potential conflicts of interest. All authors: No reported conflicts of interest. 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.

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