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Research article

# Live virus neutralizing antibodies against pre and post Omicron strains in food and retail workers in Québec, Canada

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ABSTRACT

*Background:* Measuring the ability of SARS-CoV-2 antibodies to neutralize live viruses remains an effective approach to quantify the level of protection of individuals. We assessed the neutralization activity against the ancestral SARS-CoV-2, Delta, Omicron BA.1, BA.2, BA.2.12.1, BA.4 and BA.5 strains, in 280 vaccinated restaurant/bar, grocery and hardware store workers in Québec, Canada.

*Methods:* Participants were recruited during the emergence of Omicron BA.1 variant. The neutralizing activity of participant sera was assessed by microneutralization assay.

*Results:* Serum neutralizing antibody (NtAb) titers of all participants against the ancestral SARS-CoV-2 strain were comparable with those against Delta variant (ranges of titers 10–2032 and 10–2560, respectively), however, their response was significantly reduced against Omicron BA.1, BA2, BA.2.12.1, BA.4 and BA.5 (10–1016, 10–1016, 10–320, 10–80 and 10–254, respectively). Individuals who received 2 doses of vaccine had significantly reduced NtAb titers against all SARS-CoV-2 strains compared to those infected and then vaccinated (≥1 dose), vaccinated (≥2 doses) and then infected, or those who received 3 doses of vaccine. Participants vaccinated with 2 or 3 doses of vaccine and then infected had the highest NtAb titers against all SARS-CoV-2 strains tested.

*Conclusion:* We assessed for the first time the NtAb response in food and retail workers. We found that vaccination prior to the emergence of Omicron BA.1 was associated with higher neutralizing activity against pre-Omicron variants, suggesting the importance of updating vaccines to increase antibody response against new SARS-CoV-2 variants. Vaccination followed by infection was associated with higher neutralizing activity against all SARS-CoV-2 strains tested.

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## 1. Introduction

Since the beginning of the Coronavirus Disease 2019 (COVID-19) pandemic, the global response has faced new challenges including the emergence of new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern (VOC). In late 2020, the B.1.617.2 (Delta) variant was identified in India [1] and spread widely throughout the world. In November 2021, Omicron (BA.1 sub-lineage of the B.1.1.529) variant emerged in South Africa [2] and rapidly spread around the world, becoming the dominant SARS-CoV-2 variant. Omicron BA.1 has acquired a large number of substitutions (>30), deletions and insertions in the spike protein and has been shown to escape protection conferred by vaccines and therapeutic monoclonal antibodies [3]. Since January 2022, several other Omicron sub-lineages have been detected such as BA.2, BA.2.12.1 (a variant of BA.2), BA.2.75, BQ.1.1, BA.4/5 and more recently XBB.1.5, XBB.1.16, EG.5 and JN.1 [4]. Therefore, the global dynamic landscape of SARS-CoV-2 sub-genomes has become increasingly complex causing waves of infection in people with variable immunity induced by both infection and/or vaccination and have shown greatest evasion against parental or bivalent BA.1 or BA.4/5 mRNA-booster vaccines, explaining the rapid spreading of these new sub-lineages [5].

Neutralizing antibodies are crucial for virus clearance and are a major determinant of protection from infection in humans [6] and macaques challenged with SARS-CoV-2 [7]. In contrast with influenza infections, where a hemagglutination inhibition titer of 1:40 is thought to provide 50 % protection from influenza infection [8], in the case of COVID-19, the role of NtAbs with regard to disease outcome remains undefined. It is also possible that neutralization is correlated with other immune responses such as T cell responses and B cell memory responses, which have also shown to contribute to protection [9]. Modeling studies have been used to estimate protective neutralization titer for COVID-19 [9], however, further studies and validations are needed.

Serological studies of SARS-CoV-2 antibodies have been conducted in several countries but have been focused on hospital and primary healthcare workers, blood donors, school children and staff and nursing homes [10–14]. Retail workers such as those working in grocery and hardware stores, restaurants and bars have been poorly or not studied.

In this study, we compared the neutralizing antibody levels in serum samples (n = 280) against the ancestral SARS-CoV-2, Delta, Omicron BA.1, BA.2, BA.2.12.1, BA.4 and BA.5 strains, from a unique population composed by four groups of non-hospitalized (vaccinated, vaccinated and infected or vice versa) retail workers, including grocery and hardware stores, restaurants and bar workers in Quebec, Canada.

## 2. Methods

### 2.1. Study participants

The 280 vaccinated participants were derived from a study which consisted of 304 food and retail workers who were recruited as part of a longitudinal study to assess the humoral and cellular responses to SARS-CoV-2 and its VOC in four groups of non-hospitalized retail workers [15]. Individuals were recruited in the Québec City area, specifically in the administrative regions of Capitale-Nationale and Chaudière-Appalaches, in Québec, Canada. The recruitment took place from October 2021 to May 2022, during the emergence of Omicron BA.1 variant, and individuals were classified into two groups: aged 18 to 59 and $\geq$ 60 years old. All participants provided information about vaccination and/or infection. The study was reviewed and approved by the CHU de Quebec-Université Laval Research Ethics Board (registration number 2021–5744). All experiments were performed in accordance with relevant guidelines and regulations. Adult volunteers were recruited at the *Centre Hospitalier Universitaire de Québec-Université Laval* (CHUL) in Quebec City. All participants provided informed written consent before enrolling. The study data is shared through the Maelstrom platform on a periodic basis as each visit is completed (https://www.maelstrom-research.org/study/cisacov).

### 2.2. Sample collection and processing

Blood was collected in 6 mL tubes for serum, gently inverted, held at room temperature for 15–30 min and centrifuged at 1600×*g* for 15 min. Aliquots of 1 mL of serum were transferred in cryovials and frozen at −20 °C until used.

### 2.3. Cells and viruses

Virus stocks used in this study were propagated in African green monkey kidney E6 cell line (Vero American Type Culture Collection, ATCC® CRL-1586™) or Calu-3 cells (ATCC-HTB-55) in 2 % of Fetal Bovine Serum MEM and stored at −80 °C until use. Live microneutralization assays were done in Vero E6 (ancestral SARS-CoV-2 and Delta variant) or Vero cells overexpressing transmembrane protease serine 2 (TMPRSS2, JCRB), (Omicron BA.1, BA.2, BA.2.12.1, BA.4 and BA.5 variants) cell lines. SARS-CoV-2/Québec City/21697/2020 strain (ancestral Wuhan-1 like SARS-CoV-2), was isolated from a clinical sample in March 2020 in Quebec City, Canada. Delta (SARS-CoV-2 VOC B.1.617.2) and Omicron sub-lineages BA.1 (SARS-CoV-2 VOC B1.1.529) and BA.2, BA.2.12.1, BA.4 and BA.5 were obtained from the National Microbiology Laboratory (NML), Public Health Agency of Canada.

### 2.4. Live microneutralization assay

Microneutralization assays, the gold standard for evaluating virus NtAbs, were performed as previously described for influenza

virus [16,17] with some modifications. Briefly, sera from the participants were heat-inactivated (30 min at 56 °C), and serial two-fold dilutions were prepared from a 1:20 to 1:10,240 dilution of each sample. Equal volumes of serum and virus (100 $TCID_{50}$ (50 % tissue culture infectious dose) of each SARS-CoV-2 strain) were mixed and incubated for 60 min at room temperature. The residual infectivity of the virus-serum mixture was determined in Vero (ancestral and Delta variant) or Vero TMPRSS2, (Omicron BA.1 and BA.2, BA.2.12.1, BA.4 and BA.5 variants) cell lines using four wells for each dilution of serum. A virus back titration, positive controls (high, medium, and low titers) and negative controls were included in every experiment. Neutralizing antibody titer was defined as the reciprocal of the serum dilution that completely neutralized the infectivity of 100 $TCID_{50}$ of each SARS-CoV-2 strain as determined by the absence of cytopathic effect on Vero or Vero TMPRSS2 cells at day 4 as previously described [16–18]. These studies were performed in the Containment Level 3 (CL3) laboratory at the *CHU de Québec-Université Laval*.

### 2.5. Statistical analysis

Sera with undetectable (<20) antibody titers were assigned an antibody titer of 10 for purposes of mean titer calculations or statistical comparisons. Quantitative variables are described by their mean, standard deviation, and range. Titers of NtAbs were compared among 7 types of SARS-CoV-2 strains using an univariate Generalized Estimating Equations (GEE) linear regression mixed model to account the correlation due to multiple measurements taken from each participant. The Tukey-Kramer method was employed for adjustment for multiple comparisons. Statistical analyses were conducted using SAS Statistical Software v.9.4 (SAS Institute, Cary, NC, USA) with a two-sided significance level set at $p < 0.05$. For some graphs, comparisons between antibody titers against a specific strain of SARS-CoV-2 in different subgroups of participant were performed with Kruskal-Wallis one-way ANOVA followed by Dunn's multiple comparison test, using GraphPad Prism 9.0 (GraphPad Software, Inc, San Diego, CA).

## 3. Results

We included 280 vaccinated participants; 105 (37-%), 133 (48 %) and 42 (15 %) worked in grocery stores, restaurants/bars or in hardware stores, respectively (Table 1). The median age of the 280 vaccinated participants was 41 years old (range 18–74) and 158 (56 %) were females. Median body mass index was 26 $kg/m^2$ (range 17–50). Chronic diseases (e.g., hypertension, diabetes, asthma, chronic lung, heart, kidney or liver disease, cancer, chronic blood disorder, immunosuppression, chronic neurological disorder), were present in 117 (42 %) of the participants. Fifty-nine (21 %) of the participants were smokers or e-cigarette users (vaping) (Table 1).

Participants had been vaccinated with ≥ 1 dose of vaccine (messenger RNA (mRNA) vaccine BNT162b2 (Pfizer-BioNTech), mRNA-1273 (Moderna) or with viral vector vaccine ChAdOx1-S (AstraZeneca)), (Table 2). We analyzed participants regarding the number of vaccine doses received and if they were infected before or after vaccination and separated them in four groups: 1) infected and then vaccinated with 1–3 doses (n = 16), 2) vaccinated with 2 doses (n = 144), 3) vaccinated with 3 doses (n = 84), and 4) vaccinated with 2–3 doses and then infected (n = 36). We have considered participants in each group taking into consideration ≥7 days between last vaccination (or infection) and sample collection. The median delay between the last dose of vaccine or infection and the blood sample collection for group 1 was 315, 168 and 13 days for those who received one (n = 2), two (n = 11) or three doses of vaccine (n = 3), respectively; for group 2 it was 157 days (n = 144); for group 3 it was 48 days (n = 84) and for group 4 it was 44 and 23 days for those who received two doses (n = 28) and three doses of vaccine (n = 8), respectively, (Table 2). The neutralizing antibody titers after the most recent vaccine dose or infection and the sample collection for each group stratified in intervals between 7 and 30 days, 31–60 days, 61–90 days and over 90 days is presented in Supplementary Fig. 1.

**Table 1**
Demographic description and clinical characteristics of study participants.

| | All participants | | Female | | Male | |
|---|---|---|---|---|---|---|
| Participant, n (%) | 280 | (100) | 158 | (56) | 122 | (44) |
| Grocery store | 105 | (37) | 52 | (50) | 53 | (50) |
| Restaurant/bar | 133 | (48) | 82 | (62) | 51 | (38) |
| Hardware store | 42 | (15) | 24 | (57) | 18 | (43) |
| **Age (years), median (range)** | 41 | (18–74) | 42 | (18–70) | 41 | (18–74) |
| Grocery store | 45 | (18–74) | 45 | (18–70) | 47 | (18–74) |
| Restaurant/bar | 35 | (18–69) | 38 | (18–67) | 32 | (18–69) |
| Hardware store | 53 | (18–72) | 51 | (18–64) | 58 | (18–72) |
| **Body Mass Index, median (range)** | 26 | (17–50) | 26 | (17–50) | 27 | (18–47) |
| Grocery store | 28 | (17–39) | 28 | (17–39) | 28 | (18–37) |
| Restaurant/bar | 25 | (18–50) | 24 | (18–50) | 26 | (18–47) |
| Hardware store | 25 | (20–44) | 26 | (20–44) | 25 | (21–38) |
| **Chronic disease, n (%)** | 117 | (42) | 67 | (57) | 50 | (43) |
| Grocery store | 51 | (44) | 25 | (49) | 26 | (51) |
| Restaurant/bar | 41 | (35) | 26 | (63) | 15 | (37) |
| Hardware store | 25 | (21) | 16 | (64) | 9 | (36) |
| **Smoking/Vaping, n (%)** | 59 | (21) | 32 | (54) | 27 | (46) |
| Grocery store | 17 | (29) | 9 | (53) | 8 | (47) |
| Restaurant/bar | 35 | (63) | 19 | (51) | 18 | (49) |
| Hardware store | 5 | (8) | 4 | (80) | 1 | (20) |

**Table 2**
Median delay between last vaccine dose or infection and sample collection for groups 1 to 4.

| | Median delay between last vaccine dose or infection and sample collection (days) | | | | | |
|---|---|---|---|---|---|---|
| | **1 dose Delay (n)** | | **2 doses Delay (n)** | | **3 doses Delay (n)** | |
| **GROUP 1 (N=16)** | 315 | (2) | 168 | (11) | 13 | (3) |
| Infected, vaccinated 1–3 doses | | | | | | |
| Pfizer (1–3 doses) | 336 | (1) | 126 | (9) | 13 | (1) |
| Moderna (2–3 doses) | – | | 179 | (2) | 9 | (1) |
| Mix Pfizer/Moderna | – | | – | | 15 | (1) |
| AstraZeneca (1 dose) | 294 | (1) | – | | – | |
| **GROUP 2 (N=144)** | – | | 157 | (144) | – | |
| Vaccinated 2 doses | | | | | | |
| Pfizer (2 doses) | – | | 149 | (85) | – | |
| Moderna (2 doses) | – | | 156 | (41) | – | |
| Mix Pfizer/Moderna | – | | 98 | (2) | – | |
| AstraZeneca/Pfizer or Moderna | – | | 184 | (16) | – | |
| **GROUP 3 (N=84)** | – | | – | | 48 | (84) |
| Vaccinated 3 doses | | | | | | |
| Pfizer (3 doses) | – | | – | | 24 | (17) |
| Moderna (3 doses) | – | | – | | 60 | (16) |
| Mix Pfizer/Moderna | – | | – | | 39 | (27) |
| Mix AstraZeneca/Pfizer or Moderna | – | | – | | 49 | (24) |
| **GROUP 4 (N=36)** | – | | 44 | (28) | 23 | (8) |
| Vaccinated 2–3 doses, infected | | | | | | |
| Pfizer (2–3 doses) | – | | 19 | (18) | 16 | (5) |
| Moderna (2 doses) | – | | 50 | (7) | – | |
| Mix Pfizer/Moderna | – | | – | | 54 | (2) |
| AstraZeneca/Pfizer or Moderna | – | | 46 | (3) | 30 | (1) |

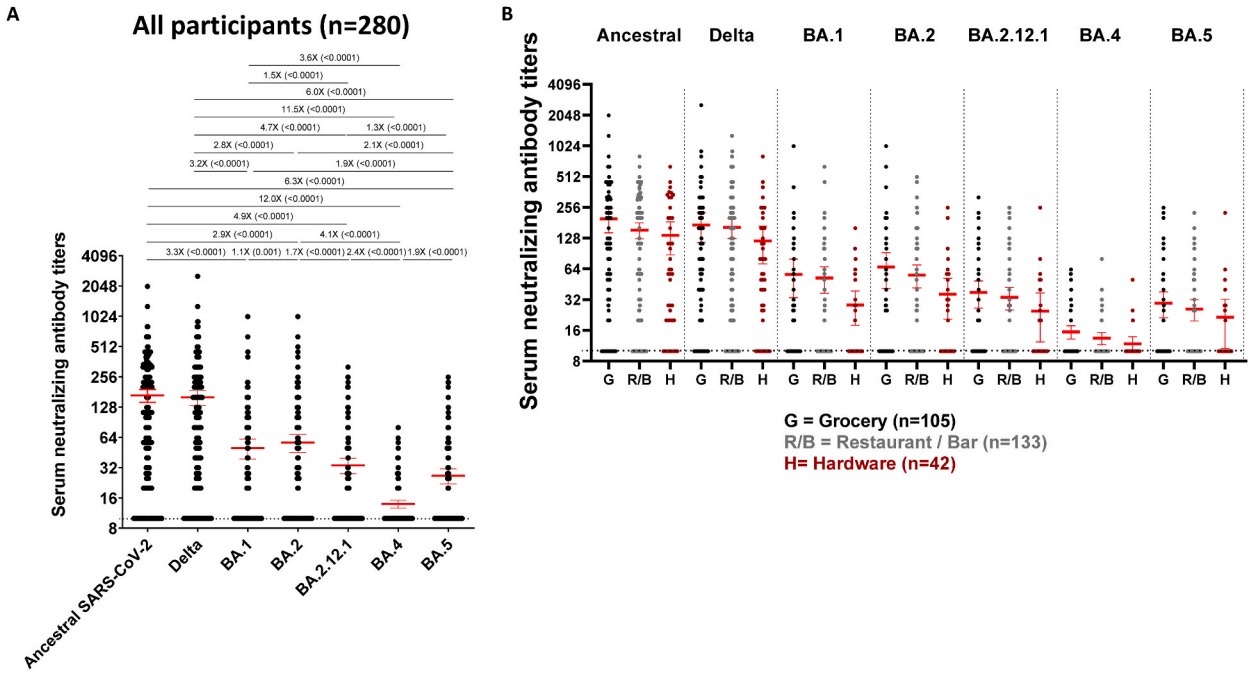


**Fig. 1.** Comparison of live neutralizing antibody titers in participants after homologous or heterologous vaccination, infection followed by vaccination or vice-versa. Food and retail workers received two or three doses of mRNA vaccines (Pfizer, Moderna or AstraZeneca) or a combination of them. **(A)** Serum neutralizing antibody of all participants against ancestral SARS-CoV-2, Delta, BA.1, BA.2, BA.2.12.1, BA.4 and BA.5 strains. Each circle represents a single participant. Bars identify mean NtAbs titers of the group with 95 % confidence intervals (CI). The horizontal dashed line indicates the limit of detection for the neutralization assay (neutralizing titer of 10). The samples that did not neutralize SARS-CoV-2 at 1:20 serum dilution was given a neutralizing titer of 10 for graphic representation and statistical analysis. The fold-change of the mean NtAbs titer and significant p-value ($p \leq 0.05$) are denoted on the line. Significance was assessed using GEE linear regression mixed model with Tukey-Kramer adjusted p-values. **(B)** Serum neutralizing antibody of participants from grocery stores (105), restaurants/bar (133) and hardware stores (42) against ancestral SARS-CoV-2, Delta, BA.1, BA.2, BA.2.12.1, BA.4 and BA.5 strains. Significance was assessed with Kruskal-Wallis one-way ANOVA followed by Dunn's multiple comparison test.

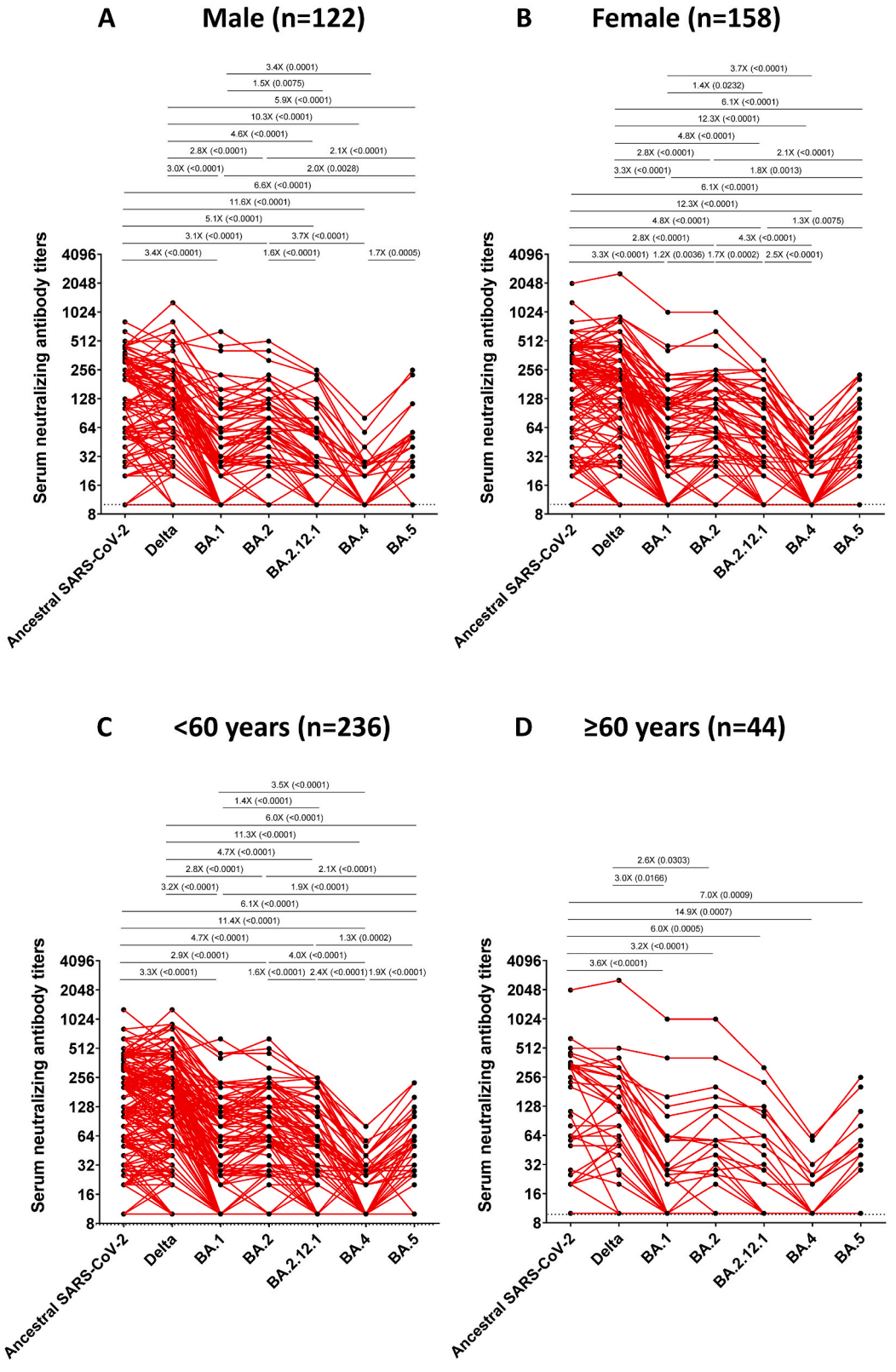


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**Fig. 2.** Neutralizing antibody titers in **(A)** males (n = 122), **(B)** females (n = 158), **(C)** < 60 years (n = 236, median 37 (range 18–59) and **(D)** ≥60 years (n = 44, median 64 (60–73) against ancestral SARS-CoV-2, Delta, BA.1, BA.2, BA.2.12.1, BA.4 and BA.5 strains. Each circle represents a single participant. Bars identify mean NtAbs titers of the group with 95 % CI. The horizontal dashed line indicates the limit of detection for the neutralization assay (neutralizing titer of 10). The samples that did not neutralize SARS-CoV-2 at 1:20 serum dilution was given a neutralizing titer of 10 for graphic representation and statistical analysis. The fold-change of the mean NtAbs titer and significant p-value ($p \leq 0.05$) are denoted on the line. Significance was assessed using GEE linear regression mixed model with Tukey-Kramer adjusted p-values.

By the time of Omicron BA.1 emergence, 82 % (86/105), 77 % (103/133) and 81 % (34/42) of grocery workers, restaurant/bar workers and hardware workers, respectively, were seropositive for ancestral SARS-CoV-2 and 80 % (84/105), 77 % (103/133) and 79 % (33/42) were seropositive for the Delta variant. However, only 46 % (48/105), 44 % (59/103) and 38 % (16/42) had cross-NtAbs against BA.1 variant.

Serum neutralizing antibody titers of all participants (n = 280) against the ancestral SARS-CoV-2 strain was comparable with the response against Delta variant, with a mean titer of 167 (range 10–2032) and 160 (10–2560), respectively (Fig. 1A). However, live NtAb response was reduced against the other variants tested, where the titers of NtAbs against BA.1, BA2, BA.2.12.1, BA.4 and BA.5 were 50 (range 10–1016), 57 (10–1016), 34 (10–320), 14 (10–80) and 27 (10–254), respectively. Thus, compared with the NtAbs titer of the ancestral SARS-CoV-2 strain, the NtAbs titers of the BA.1, BA2, BA.2.12.1, BA.4 and BA.5 were reduced by 3.3-, 2.9-, 4.9-, 12.0-, and 6.3-fold, respectively, ($p < 0.0001$, Fig. 1A). The titers of NtAbs of BA.1 were 1.5-, 3.6-, and 1.9-fold higher compared to BA.2.12.1, BA.4 and BA.5, respectively, ($p < 0.0001$, Fig. 1A). No statistical difference was observed in the NtAbs titers of each group of workers against each of the SARS-CoV-2 strain tested (Fig. 1B). When we compared the titers of NtAbs against all SARS-CoV-2 strains of each of the groups (grocery and hardware stores and restaurant/bars), we observed a similar pattern than the whole cohort (Supplementary Fig. 2).

We examined additional factors that may contribute to the NtAb response to vaccination or vaccination/infection (or vice-versa) in our food and retail workers cohort (all groups together) including sex and age, against all SARS-CoV-2 strains. We found a similar pattern in the humoral response in males (n = 122) and females (n = 158) between ancestral SARS-CoV-2 and the other variants. The NtAbs titers against ancestral, Delta, BA.1, BA2, BA.2.12.1, BA.4 and BA.5 strains were 153, 136, 46, 49, 30, 13 and 23, respectively, for males (Fig. 2A) and 178, 178, 54, 63, 37, 14, and 29, respectively, for females (Fig. 2B). A similar pattern was observed between the age groups of <60 years (n = 236, median 37 (range 18–59)) and ≥60 years (n = 44, median 64 (range 60–73)) when we compared the NtAbs titers for the ancestral SARS-CoV-2 (NtAbs titer of 159 and 211 (ranges 10–1280 and 10–2032, respectively)) to BA.1, BA2, BA.2.12.1, BA.4 and BA.5 strains ($p < 0.0001$) (Fig. 2C and D), with a 3.3-, 2.9-, 4.7-, 11.4- and 6.1-fold reduction among participants of <60 years of age (Fig. 2C) and a 3.6-, 3.2-, 6.0-, 14.9- and 7.0-fold reduction among participants of ≥60 years old (Fig. 2D). For the age groups of <60 years, the Delta variant had the same pattern as the ancestral SARS-CoV-2 ($p < 0.0001$) (Fig. 2C). However, no significant difference was observed between the titer of NtAbs for Delta, BA.2.12.1, BA.4 and BA.5 strains for the age groups of ≥60 years (Fig. 2D). No statistically significant differences were found in the NtAbs titers against any SARS-CoV-2 strain in relation to the presence of chronic diseases and to smoking or vaping (Supplementary Fig. 3). Interestingly, we found significantly higher titers of NtAbs against all strains except Delta in females with BMI ≥30 (Supplementary Fig. 3C).

The titers of NtAbs against the ancestral SARS-CoV-2 strain were statistically lower for group 2 (2 doses of vaccine, NtAbs titer = 63) than for other groups: group 1 (infected, vaccinated with ≥1 dose, NtAbs titer = 206, $p < 0.0001$), group 3 (vaccinated with 3 doses, NtAbs titer = 299, $p < 0.0001$), group 4 (vaccinated with ≥2 dose and infected, NtAbs titer = 259, $p < 0.0001$) (Fig. 3A). A similar profile was seen among the 4 groups against Delta variant. The titers of NtAbs were lower in all groups against the other SARS-CoV-2 Omicron variants (BA.1, BA.2, BA.2.12.1, BA.4 and BA.5). Of note, the NtAbs titers of participants who were vaccinated with 2 or 3 doses of vaccine and then infected (group 4) had consistently higher NtAb titers against all variant tested, although a statistical significance was not always observed (Fig. 3A). A similar trend was found for the other SARS-CoV-2 variants. Fig. 3B shows the NtAb titers against each of the SARS-CoV-2 strains tested for participants of the 4 groups, each subgroup representing the type of vaccine regimen they have received. No significant differences were observed in the NtAbs titers of the different subgroups against the ancestral SARS-CoV-2, except for those individuals who were vaccinated with 2 doses of Moderna (NtAbs titer = 80) versus those who received AstraZeneca/Pfizer or AstraZeneca/Moderna (NtAbs titer = 27), where a 3.0-fold reduction was observed ($p = 0.0057$). Regarding the Delta variant, the combination of 2 doses of Pfizer ($p = 0.0313$) or Moderna ($p = 0.0026$) or 3 doses of Pfizer ($p = 0.0218$) showed better response than a mixed schedule containing the AstraZeneca vaccine. For BA.1 and BA.2 variants, 3 doses of mixed vaccine containing Pfizer and Moderna seemed more effective than those combined with AstraZeneca vaccine ($p = 0.0224$ and 0.0314, respectively). No significant differences were found when analyzing against BA.2.12.1, BA.4 and BA.5 (Fig. 3B).

## 4. Discussion

One of the important roles of vaccination is to generate broad, long-lasting immunity that will contribute to protect individuals from future infections or at least from severe clinical outcomes if infected. Since the emergence of the Omicron lineage in late 2021, several Omicron variants continue to evolve into new subvariants which are increasingly resistant to monovalent and bivalent vaccination [19–22].

During the first two years of the COVID-19 pandemic (i.e from March 2020 to February 2022), the provincial government of Québec in Canada has used a plethora of measures and restrictions on its territory in an attempt to reduce the infection rate. These measures included house confinement, suspension of activities deemed non-essential, etc. These measures varied in time and length,

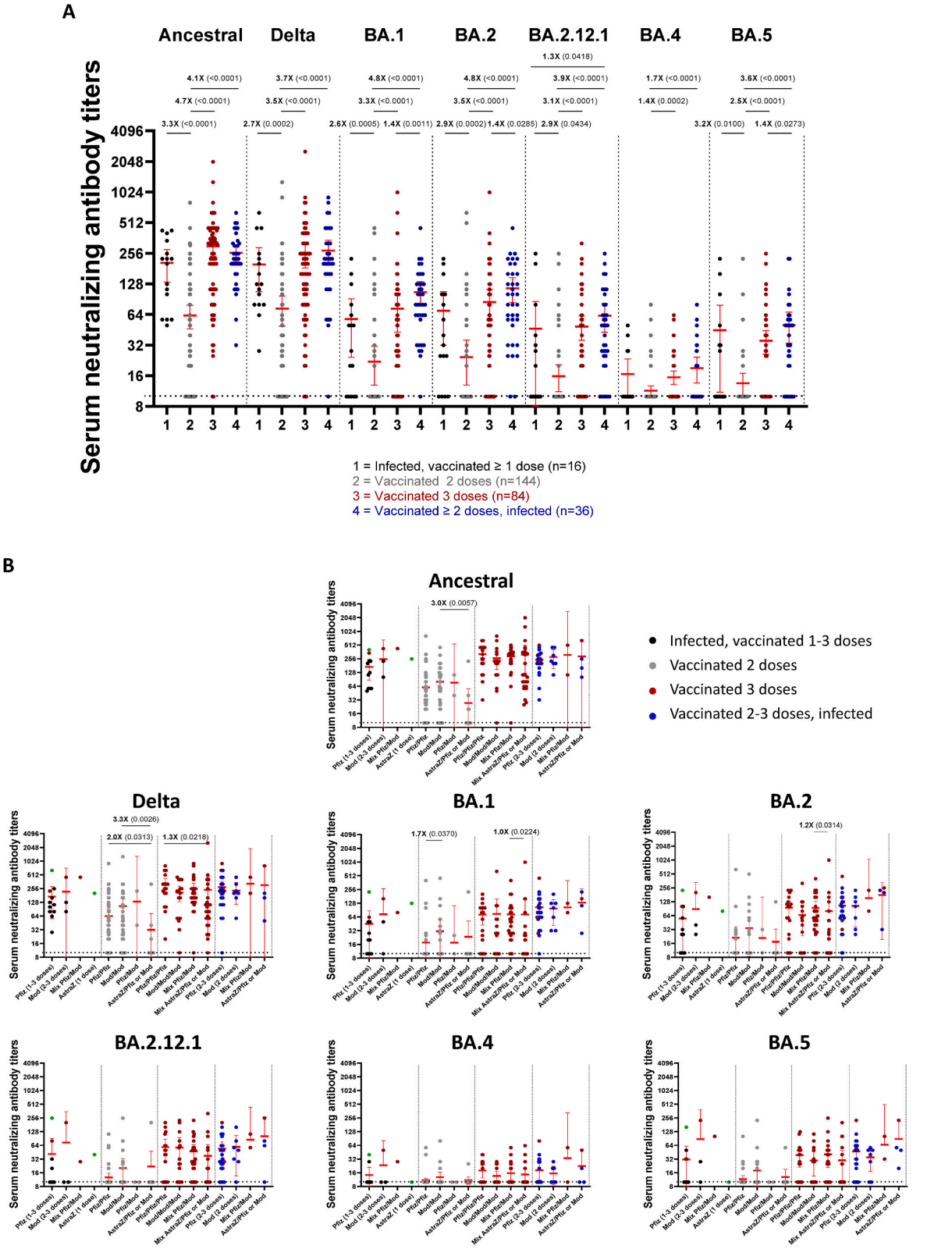


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**Fig. 3.** Neutralizing antibody titers against ancestral SARS-CoV-2, Delta, BA.1, BA.2, BA.2.12.1, BA.4 and BA.5 strains in all volunteers regarding the number of vaccine doses received and if they were infected before or after vaccinations. **(A)** Group 1 (n = 16); infected, Pfizer (n = 11), Moderna (n = 3), Pfizer/Moderna (n = 1) or AstraZeneca (n = 1)); Groupe 2 (n = 144); 2 doses of Pfizer (n = 85), Pfizer/Moderna or vice versa (n = 2), Moderna/Moderna (n = 41) and AstraZeneca and Pfizer or Moderna (n = 16); Group 3 (n = 84); 3 doses of Pfizer (n = 17), 3 doses of Moderna (n = 16), Moderna/Moderna/Pfizer (n = 5), Pfizer/Pfizer/Moderna (n = 19), Pfizer/Moderna/Pfizer or Moderna (n = 3), AstraZeneca/AstraZeneca/Pfizer or Moderna (n = 4) or AstraZeneca/Pfizer or Moderna/Moderna (n = 20); Group 4 (n = 36): participants received at least 2 doses of vaccine (Pfizer/Pfizer (n = 25), Moderna/Moderna (n = 7), AstraZeneca/AstraZeneca or AstraZeneca/Moderna (n = 4)) and then were infected. **(B)** Neutralizing antibody titers for each participant against ancestral SARS-CoV-2, Delta, BA.1, BA.2, BA.2.12.1, BA.4 and BA.5 strains regarding type of vaccine regimen. Each circle represents a single participant. In the first group, the green and red points represent participants vaccinated with 1 and 3 doses and then infected, respectively. Bars identify mean NtAbs titers of the group with 95 % CI. The horizontal dashed line indicates the limit of detection for the neutralization assay (neutralizing titer of 10). The samples that did not neutralize SARS-CoV-2 at 1:20 serum dilution was given a neutralizing titer of 10 for graphic representation and statistical analysis. The fold-change of the mean NtAbs titer and significant p-value ($p \leq 0.05$) are denoted on the line. Significance was assessed with Kruskal-Wallis one-way ANOVA followed by Dunn's multiple comparison test.

based on infection rate within sub-regions of the province. During this period, grocery stores were considered essential institutions in support of food and general supplies, and remained open most of the time. Public health measures (e.g., mask wearing, social distancing, etc.) were enforced and generally well-respected. Hardware stores and restaurants were instead intermittently opened and closed by health authorities over the same period, and were at higher risk of SARS-CoV-2 transmission due to the intrinsically social nature of these businesses and difficulty to enforce public health measures. In December 2020, vaccines were approved in Canada and priority was given to essential workers as a measure to protect themselves, their families, co-workers, and their community, while ensuring the constant availability of food/hardware supplies.

Many studies have been performed to analyze the antibody response of health-care workers, school children and staff and nursing homes in Canada and elsewhere [10–12,19,21–23], however, no serological studies in a cohort of food/retail workers have been conducted in Canada.

Antibody measurements are a crucial aspect of estimating the level of herd immunity in communities. Importantly, NtAbs are important for virus clearance and their titers have been demonstrated to be correlated with vaccine efficacy [9,24,25]. Indeed, several studies have shown that high levels of NtAbs are associated with protection from symptomatic SARS-CoV-2 infection after vaccination [9,26,27], however, all the studies have different approaches to estimate the relationship between NtAb titers and vaccine efficacy. A recent study performed by Khoury et al. [24] has analyzed four phase 3 clinical studies evaluating the performance of COVID-19 vaccines against the ancestral SARS-CoV-2 strain at the beginning of the vaccine campaign and before the emergence of the VOCs. Each study [9,26–28] reported on a significant relationship between neutralizing titers and vaccine efficacy using different neutralization methodologies. Interestingly, Khoury et al. found that when centering the data on the geometric mean titer elicited by the vaccine, the four studies converged on a common correlating prediction between neutralization capacity and protection against infection [24], supporting the use of NtAb titers to predict the efficacy of new vaccines or vaccine efficacy against new VOCs.

In this study we compared the neutralizing antibody levels against the ancestral SARS-CoV-2, Delta, Omicron BA.1, BA.2, BA.2.12.1, BA.4 and BA.5 strains in 280 serum samples collected from vaccinated food and retail workers including those from grocery and hardware stores, restaurants and bars recruited in the Québec City area during the emergence of Omicron BA.1 variant (October 2021 to May 2022). By the time of the sample collection, all participants had received at minimum one dose of either mRNA vaccines (BNT162b2, Pfizer-BioNTech); mRNA-1273, Moderna) or viral vector vaccine (ChAdOx1-S, AstraZeneca).

When we analyzed each of the participating groups of workers (grocery stores, restaurants/bars or hardware stores), no statistical difference was observed in the NtAbs titers of the groups against any of the SARS-CoV-2 strains tested. Overall, when considering all participants, we found no statistical difference in the NtAb titers against the ancestral SARS-CoV-2 strain and the Delta variant. In general, the NtAb response was reduced against the other variants tested, where the NtAbs titers against BA.1, BA2, BA.2.12.1, BA.4 and BA.5 were reduced by 3.3-, 2.9-, 4.9-, 12.0-, and 6.3-fold compared to the ancestral SARS-CoV-2, respectively. This decreasing pattern of NtAb response is in line with studies reported in other populations such as healthcare workers, healthy vaccinated adults and adolescents and immunocompromised patients [29–35]. No statistically significant difference in the titer of NtAbs was observed between male/female participants, in males and females with chronic disease, smoking or vaping or among those volunteers with all three characteristics. However, for the age groups ≥60 years, using the GEE analysis, the titers of NtAbs against Delta were statistically similar to BA.2.12.1, BA.4 and BA.5 variants. Interestingly, our study found a significant difference in the NtAbs titers against all SARS-CoV-2 strains tested except Delta in obese females (BMI ≥30; n = 48/158). Of note, according to the vaccine safety and efficacy information for Pfizer, Moderna, and Johnson & Johnson formulations showed similar efficacy in individuals with or without obesity [36]. However, a systematic review [37] of published studies on the safety and efficacy of COVID-19 vaccine in people who were overweight or obese reported that in 9/12 studies a reduced response with increased BMI was observed. Of note, contradictory results may be due to different measure of obesity (e.g. central obesity or BMI), vaccination, comorbidities, the use of antibody titer kits or assays, etc. Therefore, more research needs to be done to assess the impact of obesity on immunogenicity of SARS-CoV-2 vaccines.

The seroprevalence of ancestral SARS-CoV-2 antibodies in the 280 participants during the period of October 2021 to May 2022 (emergence of the first Omicron sub-lineage (BA.1) was 80 %. Cross-variant neutralization capacity was found in 99 %, 55 %, 58 %, 46 %, 21 % and 39 % of the participants against the Delta, BA.1, BA.2, BA.2.12.1, BA.4 and BA.5, respectively. Similar to other studies in different groups of individuals [19,29–35,38], our data show that uninfected food and retail workers from Québec who received 2 doses of vaccine had significantly lower NtAb titers against the ancestral SARS-CoV-2 and all variants than those who were infected and then vaccinated (≥1 dose), vaccinated (≥2 doses) and then infected, or those who received 3 doses of vaccine. As previously

reported, hybrid immunity led to better humoral response against SARS-CoV-2 variants than vaccination alone, as participants who were infected after two or three doses of vaccine had higher NtAbs levels against BA.1, BA.2 and BA.5 variants, compared with other groups, suggesting a substantial degree of cross-reactive natural immunity. Therefore, workers with hybrid immunity acquired during Omicron BA.1 emergence might be better protected against reinfection with subsequent Omicron variants such as BA.2, BA.4/5 etc.

The strength of this study lies in the evaluation of the neutralizing antibodies against currently and post-circulating SARS-CoV-2 strains in a unique cohort of food/retail workers. However, our study has several limitations. An important limitation is that the interval between the last vaccination/infection and sample collection was variable; for example, for participants who received 2 or 3 doses of vaccine, the delay was 157 and 48 days (median), respectively, amplifying the potential difference in the antibody response. Importantly, our conclusions concerning the differences between the 4 groups apply even when comparing NtAbs titers for similar intervals. We were also unable to confirm the specific SARS-CoV-2 strain that caused the infection among participants. The use of different vaccine platforms finally highlights a potential limitation of our study as the immunogenicity of each vaccine type differs. Reassuringly, however, almost all participants (279/280) received at least one mRNA vaccine.

In summary, we assessed for the first time the neutralizing antibody response of a unique population of food and retail workers. Overall, we found that vaccination was associated with higher neutralizing activity against pre-Omicron variants and that vaccination followed by infection was associated with higher neutralizing activity against Omicron sub-lineages, in line with the humoral response in other populations (health-care workers, healthy vaccinated adults and adolescents as well as immunocompromised patients). Interestingly, we did not observe any significant difference in the NtAb response in terms of sex, or male/female with chronic diseases, smokers or vaping or among those volunteers with all three characteristics. Additional public health measures may be warranted to increase antibody response against new SARS-CoV-2 variants such as updated vaccines for the population.

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## Ethical approval

This study was approved by the « Comité d'éthique de la recherche du CHU de Québec-Université Laval (registration number 2021–5744). A unique, anonymized identifier was assigned to each participant and used to store the data and the samples.

## CRediT authorship contribution statement

**Henintsoa Rabezanahary:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Formal analysis, Conceptualization. **Caroline Gilbert:** Writing – review & editing, Funding acquisition, Conceptualization. **Kim Santerre:** Writing – review & editing, Software. **Martina Scarrone:** Writing – review & editing, Investigation. **Megan Gilbert:** Writing – review & editing, Investigation. **Mathieu Thériault:** Writing – review & editing, Software. **Nicholas Brousseau:** Writing – review & editing, Formal analysis. **Jean-François Masson:** Writing – review & editing, Funding acquisition, Formal analysis. **Joelle N. Pelletier:** Writing – review & editing, Funding acquisition, Formal analysis. **Denis Boudreau:** Writing – review & editing, Funding acquisition, Formal analysis. **Sylvie Trottier:** Writing – review & editing, Funding acquisition, Formal analysis, Conceptualization. **Mariana Baz:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31026.

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