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ORIGINAL ARTICLE

Exposure to avian coronavirus vaccines is associated with increased levels of SARS-CoV-2-cross-reactive antibodies

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Abstract

Background: Although avian coronavirus infectious bronchitis virus (IBV) and SARS-CoV-2 belong to different genera of the *Coronaviridae* family, exposure to IBV may result in the development of cross-reactive antibodies to SARS-CoV-2 due to homologous epitopes. We aimed to investigate whether antibody responses to IBV cross-react with SARS-CoV-2 in poultry farm personnel who are occupationally exposed to aerosolized IBV vaccines.

Methods: We analyzed sera from poultry farm personnel, COVID-19 patients, and pre-pandemic controls. IgG levels against the SARS-CoV-2 antigens S1, RBD, S2, and N and peptides corresponding to the SARS-CoV-2 ORF3a, N, and S proteins as well as whole virus antigens of the four major S1-genotypes 4/91, IS/1494/06, M41, and D274 of IBV were investigated by in-house ELISAs. Moreover, live-virus neutralization test (VNT) was performed.

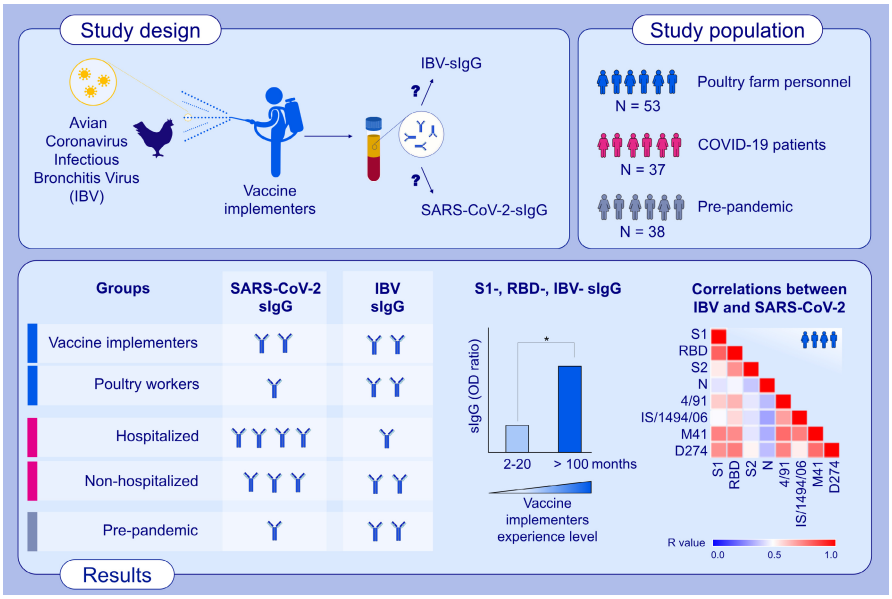
Results: A subgroup of poultry farm personnel showed elevated levels of specific IgG for all tested SARS-CoV-2 antigens compared with pre-pandemic controls. Moreover,

Ardicli and Carli contributed equally to the study.

poultry farm personnel, COVID-19 patients, and pre-pandemic controls showed specific IgG antibodies against IBV strains. These antibody titers were higher in long-term vaccine implementers. We observed a strong correlation between IBV-specific IgG and SARS-CoV-2 S1-, RBD-, S2-, and N-specific IgG in poultry farm personnel compared with pre-pandemic controls and COVID-19 patients. However, no neutralization was observed for these cross-reactive antibodies from poultry farm personnel using the VNT.

Conclusion: We report here for the first time the detection of cross-reactive IgG antibodies against SARS-CoV-2 antigens in humans exposed to IBV vaccines. These findings may be useful for further studies on the adaptive immunity against COVID-19.

KEYWORDS
COVID-19, cross-reactivity, IBV, neutralization, SARS-CoV-2



GRAPHICAL ABSTRACT
IBV- and SARS-CoV-2-specific IgG antibodies were measured in poultry farm personnel, COVID-19 patients and pre-pandemic controls. Elevated levels of IBV- and SARS-CoV-2-sIgG were detected in a subgroup of poultry farm personnel, in particular among vaccine implementers. Longer work experience in vaccine implementers was associated with higher levels of S1-, RBD-, 4/91-, IS/1494/06-, M41-, D274-sIgG. Significant positive correlations were observed between IBV-specific IgG and SARS-CoV-2-specific IgG in poultry farm personnel but not in COVID-19 patients or pre-pandemic controls.

1 | INTRODUCTION

The pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has persisted since December 2019,[1,2] despite the widespread use of rapidly developed vaccines.[3,4] Coronaviruses are classified into four genera (alpha-, beta-, gamma-, and delta-coronaviruses) in the *Coronaviridae* family. Alpha and beta coronaviruses infect mammals, whereas gamma and delta coronaviruses primarily infect birds, though some can infect mammals.[5] Certain coronaviruses are the causative agents of important epidemics or pandemics in animals and humans, whereas others cause infection in their hosts in the absence of any apparent clinical manifestation.[6] The oldest coronavirus pandemic reported in animals was caused by the avian coronavirus infectious bronchitis virus (AvCoV or IBV).[7] This IBV pandemic has persisted in chicken flocks since 1931 despite mass or flock-based vaccination of the chickens globally with mostly live attenuated IBV strains by aerosol application.[8]

Vaccine implementers are exposed to IBV vaccine strains conjunctivally as well as through their respiratory tract almost on a daily basis during the application of live attenuated strains of IBV vaccines in hatcheries and poultry houses. This intensive vaccine virus exposure continues throughout their working lives. Poultry workers are in close contact only via vaccinated poultry with the IBV vaccine,

but not with vaccine viruses directly. Therefore, their exposure to IBV vaccines seems to be significantly lower than that of vaccine implementers.

Like all coronaviruses, SARS-CoV-2 and IBV have a spherical pleomorphic electron-microscopic appearance, with an average size of 80 to 120 nm, similar genome organizations, and structural and non-structural proteins. IBV has seven genotypes and a number of recombinants based on the differences in nucleotide sequence of the S1 gene, which encodes the major immunological determinants.[9,10] Thus, amino acid alterations (<5%) in the S1 protein in field viruses may influence the vaccine effectiveness and cross-protection.[11,12] Hence, neutralizing antibodies against the S1 protein of each genotype by vaccination may not protect against infection with novel genotypes and recombinants. Clinical IBV cases persist unless vaccine strains homologous to the field IBV strains are used. On the surface of the envelope, S (spike), M (membrane), and E (envelope) proteins are found, while inside the envelope, the major protein present around the RNA of the virus is the nucleocapsid (N) protein.[5] Concerning the spike sequence, a highly conserved region between SARS-CoV-2 and IBV can be found between the amino acids 807 and 830 (Figure S8).

During the course of an infection, the first contact of a coronavirus with its host is established by binding of the S protein to its specific receptor(s), which are located on the mucous surfaces of the respiratory tract.[13,14] The S proteins of coronaviruses are formed by two subunits, S1 and S2. S1 contains a receptor binding domain (RBD) responsible for virus binding to its receptor and thus, determining the host and cell specificity of the virus.[15] S1 is the most frequent mutation-developing site of coronaviruses, while the S2 region is relatively constant and rarely develops mutations.[16,17] Besides the morphological and genomic similarities between coronaviruses infecting different animals and humans, there are large differences in the nucleotide and amino acid sequences of both structural and non-structural genes and proteins. However, there are also regions showing similarities. Despite their genetic and protein sequence differences, the topology and stereo-morphic structures of the antibody binding epitopes found in the S1 and N proteins of SARS-CoV-2 and IBV[18] may result in cross-reactivity of antibodies developed during an infection or vaccination in one species against the other. Coronavirus-specific IgG and IgA antibodies in serum and mucosal surfaces can function as neutralizing antibodies.[19,20] On the contrary, antibodies to other structural parts of the coronaviruses such as S2 and N can play an important role in antibody-dependent cell-mediated immunity, T-cell immunity, and in vivo viral neutralization although they do not directly affect RBD binding and thus epithelial cell infectivity.[21–23] Cross-reactive antibodies generated in response to exposure to coronaviruses other than SARS-CoV-2 may therefore provide a certain level of protection against SARS-CoV-2 infection and/or severe disease.[24] Conversely, non-neutralizing cross-reactive antibodies recognizing SARS-CoV-2 may also have detrimental effects if they facilitate antibody-dependent enhancement of viral entry into host cells.[25]

Although IBV and SARS-CoV-2 are in different genera of the *Coronaviridae* family, exposure to IBV may result in the development of cross-reactive antibodies to SARS-CoV-2. We hypothesized that poultry farm personnel, who are exposed to aerosolized IBV vaccines as a result of their occupation, will develop antibody responses to IBV, which may display cross-reactivity to SARS-CoV-2. Such cross-reactive antibodies may confer protection against SARS-CoV-2 infection and/or severe disease. The aim of the current study was to measure cross-reactive antibodies in poultry farm personnel and assess their neutralizing capacity on SARS-CoV-2 infection. Therefore, we measured serum IgG in three different cohorts: (1) poultry farm personnel, who had been previously frequently exposed to one of the IBV vaccine strains, (2) pre-pandemic controls, and (3) COVID-19 patients. IgG specific for the SARS-CoV-2 antigens S1, RBD, S2, and N antigen, including selected peptides from these antigens, were measured. In addition, we determined SARS-CoV-2 neutralization and IgG reactivity to the IBV S1 genotypes Massachusetts 41 (M41), Dutch 274 (D274), IS/1494/06 (Israel variant2), and 4/91.

2 | MATERIALS AND METHODS

2.1 | Human serum samples used in this study

A total of 53 serum samples of poultry farm personnel collected between August and September 2020 were used in this study. These samples were obtained from actively appointed personnel in four commercial poultry farms located in the Aegean and Marmara regions of Turkey. The samples were categorized into two groups. The first group ($n = 39$) consisted of vaccine implementers who administer IBV live attenuated vaccines through aerosol spraying in the poultry house. The second group ($n = 14$) comprises poultry workers, who collect the meat-type chickens before slaughter and raise the chickens in the poultry house. The mean age of poultry farm personnel was 31.53 ± 1.07 years. Of the 53 individuals, 51 were male. Concerning vaccine implementers (sample numbers from VI1 to VI39), mean work experience was 80.4 ± 14.4 months. The mean work experience for poultry workers (sample numbers from PW1 to PW14) was 81.1 ± 23.6 months (Table S1). In addition, sera from healthy voluntary donors ($n = 38$) obtained in the pre-pandemic era (i.e., before October 2019) were used as negative controls. RT-PCR-confirmed non-hospitalized ($n = 19$) (sample numbers from C1 to C19) and hospitalized COVID-19 patient samples ($n = 18$) (sample numbers from C20 to C37) were used as positive controls. Together, the COVID-19 patient cohort had a mean age of 42.86 ± 2.29 years and 35.14% of the patients were female. Demographics of poultry farm personnel, COVID-19 patients, and pre-pandemic controls are shown in Tables S1–S3, respectively. Following written informed consent, all participants provided blood specimens for analysis. The study protocol was approved by the Ethics Committees of the Bursa Uludag University of Turkey (2021–11/13) and the Ethics Committee of Zurich (2020–00898 and 2020–01322).

2.2 | ELISA and virus neutralization assay

The following experimental steps were applied in the analyses:

1. The serological analysis of SARS-CoV-2-specific IgG was performed by in-house ELISA. The following SARS-CoV-2 antigens were used: S1 (Acro Biosystems, Newark, DE, USA), RBD (ATUM, Newark, California, USA), S2 (Acro Biosystems, Newark, DE, USA), and N (Acro Biosystems, Newark, DE, USA). Detailed information about the selected antigens is shown in Table S4.
2. To determine the IBV-specific IgG response, the in-house ELISA procedure was performed using 4/91, IS/1494/06, M41, and D274 strains (Table S5).
3. For the measurement of IgG specific for SARS-CoV-2 peptides, peptide epitope ELISA was applied (As previously reported by Shrock et al[26]). SARS-CoV-2 peptides ORF3a (aa. 172–205), N (aa. 153–176), N (aa. 221–244), N (aa. 358–381), N (aa. 382–405), S (aa. 547–570), S (aa. 782–805), S (aa. 807–830), S (aa. 1138–1161), a rhinovirus A peptide (aa. 567–591), a human herpesvirus 4 peptide (aa. 398–422), and an HIV-1 peptide (aa. 967–991) (Biomatik, Ontario, Canada) were used. Detailed information about the selected peptides is listed in Table S6.
4. The amino acid sequence of each selected peptide of SARS-CoV-2 and the alignment of corresponding sequences in the endemic HCoVs (NL63, 229E, HKU1, and OC43) and IBV (4/91, M41, and D274), are shown in Figure S8A–H. Sequences were aligned by MUSCLE in JalView (http://www.jalview.org, version 2.11.1.4).
5. The presence of neutralizing antibodies against SARS-CoV-2 was assessed by live-virus neutralization assay.[27]

For further details, please refer to the Appendix S1.

2.3 | Statistical analysis

All statistical analyses were performed using GraphPad Prism (v.9.0; La Jolla, CA, USA). The Shapiro–Wilk test was performed to evaluate the normality of the data. Differences between the groups, including poultry farm personnel, positive, and negative controls, were determined using Kruskal–Wallis test with the Dunn's multiple comparisons. The alternative parametric test, one-way ANOVA, was implemented if data were normally distributed. In this case, Tukey's test was performed as a post hoc comparison. The correlation between antibodies was tested using Spearman correlation. Heatmaps were created using GraphPad Prism (v.9.0; La Jolla, CA, USA). Spearman rank correlation matrix generated by Morpheus matrix visualization software (https://software.broadinstitute.org/morpheus/). Two-tailed Mann–Whitney U test was used to compare differences between the groups (non-hospitalized vs. hospitalized COVID-19 patients and vaccine implementers vs. poultry workers) for IgG ratios. For all statistical analyses, a probability level of $p < .05$ was accepted as statistically significant.

3 | RESULTS

3.1 | SARS-CoV-2 antigen-specific IgG levels in poultry farm personnel, COVID-19 patients and pre-pandemic controls

We determined the levels of serum specific IgG for the SARS-CoV-2 antigens S1, RBD, S2, and N by ELISA. S1-specific IgG levels were significantly higher in COVID-19 patients compared with poultry farm personnel and pre-pandemic control samples (Figure 1A). RBD-specific IgG levels of COVID-19 patients were also higher than those of poultry farm personnel and pre-pandemic control samples (Figure 1B). Likewise, S2- and N-specific IgG levels were the highest in COVID-19 patients when compared with poultry farm personnel and pre-pandemic control samples (Figure 1C,D; Table S7). Hospitalized COVID-19 patients exhibited significantly higher S1-, RBD-, S2-, and N-specific IgG levels than non-hospitalized COVID-19 patients (Figure S1A–D). The heatmap (Figure 1E) illustrates that all of the COVID-19 patients had high levels of IgG against all four SARS-CoV-2 antigens. Of note, the levels of S1-, RBD-, and N-specific IgG were significantly higher in poultry farm personnel compared with the pre-pandemic control group ($p < .05$), while S2-specific IgG levels were not statistically different. A fraction of the poultry farm personnel (VI20, VI23, VI27, VI29, VI30, VI34, VI36, VI37, VI38, VI39, PW9) showed higher specific antibody levels than the pre-pandemic control. Ten out of these 11 poultry farm personnel were vaccine implementers (90.91%) and one was a poultry worker (9.09%) (Figure 1E). Only one sample (VI17) exhibited an IgG OD ratio of >0.6 for all SARS-CoV-2 antigens.

The OD ratios of S1-, RBD-, S2-, and N-specific IgG were significantly higher in COVID-19 patients than in poultry farm personnel and the pre-pandemic control group ($p < .0001$) (Figure 1A–D). There was no significant difference between vaccine implementers and poultry workers regarding S1-, RBD-, S2-, and N-specific IgG OD levels (Figure S1E–H).

The percentage of individuals that exhibited OD values of S1- and RBD-specific IgG above the threshold of 0.1, was higher among vaccine implementers (33.33% and 38.46%, respectively) than among poultry workers (21.43% and 14.29%, respectively). However, the percentages of poultry workers were predominant for S2- and N-specific IgG (71.43% and 78.57%, respectively), as shown in Figure 1F. The percentages of vaccine implementers with positive RBD-specific IgG were significantly higher than poultry workers ($p < .01$) whereas poultry workers with positive S2-specific IgG were significantly higher than vaccine implementers ($p < .01$). However, there were no significant differences for S1- and N- specific IgG.

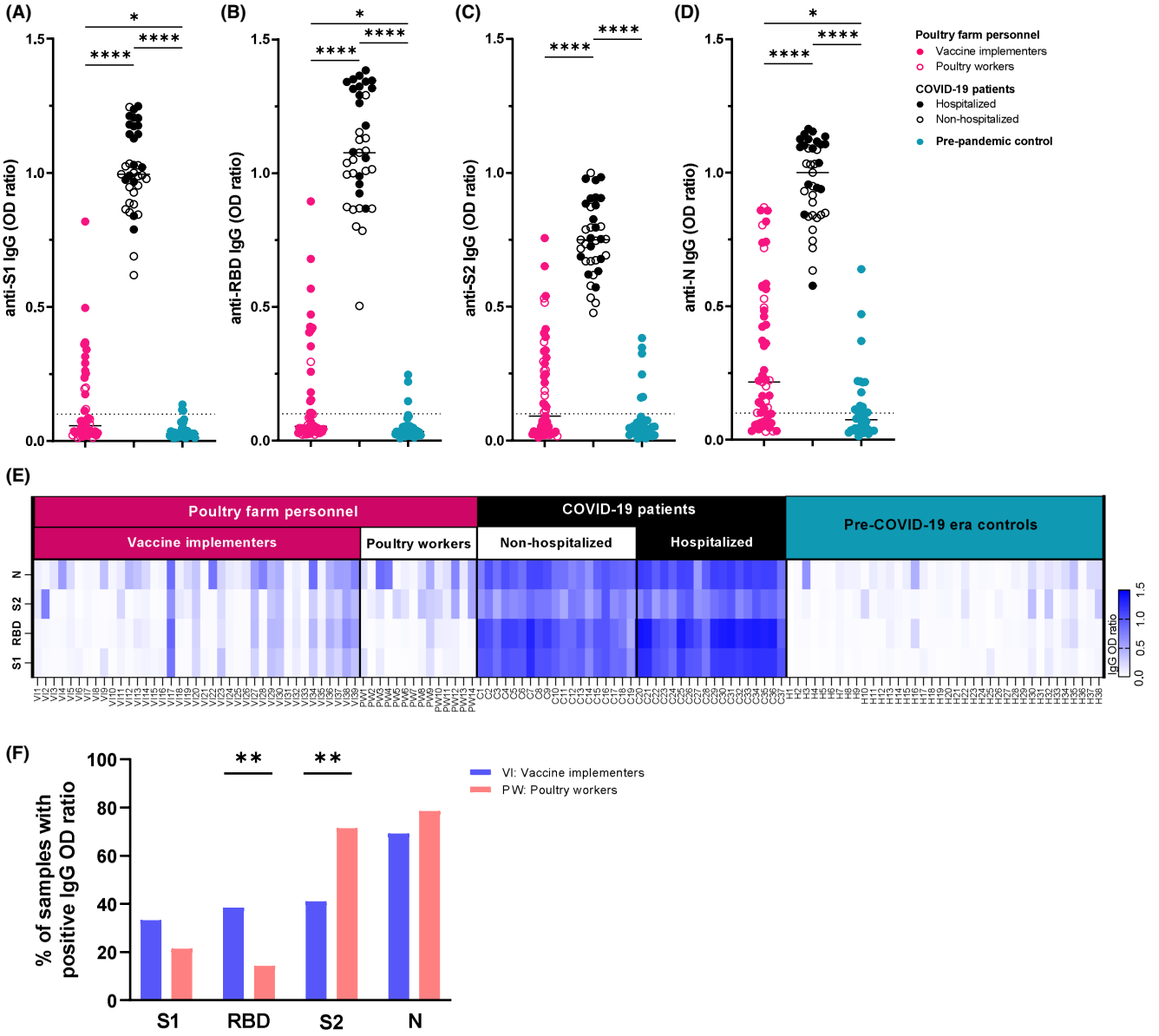


FIGURE 1 SARS-CoV-2 antigen-specific IgG levels in poultry farm personnel, COVID-19 patients, and pre-pandemic controls. (A) anti-S1 IgG, (B) anti-RBD IgG, (C) anti-S2 IgG, and (D) anti-N IgG OD ratios in sera from vaccine implementers ($n = 39$, pink circles closed) and poultry workers ($n = 14$, pink circles open), non-hospitalized COVID-19 patients ($n = 19$, black circles open), hospitalized COVID-19 patients ($n = 18$, black circles closed), and pre-pandemic control samples ($n = 38$, turquoise circles). Dashed lines indicate the threshold level at 0.1. Statistical analyses were performed using the Kruskal–Wallis test. *$p < .05$, ****$p < .0001$. Horizontal bar represents mean values. (E) Heatmap of IgG antibody levels in sera from vaccine implementers (VI1-VI39) and poultry workers (PW1-PW14), non-hospitalized COVID-19 patients (C1-C19), hospitalized COVID-19 patients (C20-C37), and pre-pandemic control samples (H1-H38). The color key indicates IgG OD ratios, with blue shades indicating elevated ratios while white shades indicating lower ratios. (F) Frequency of vaccine implementers and poultry workers with >0.1 IgG OD ratios for S1, RBD, S2, and N. Statistical analysis was performed using the Fisher's exact test. **$p < .01$

3.2 | Avian coronavirus infectious bronchitis virus-specific IgG levels in poultry farm personnel, COVID-19 patients, and pre-pandemic controls

We next analyzed the levels of IgG antibodies specific for the IBV genotypes 4/91, IS/1494/06, M41, and D274. As shown in Figure 2B–D, significant differences were observed for IBV-specific IgG in poultry farm personnel, COVID-19 patients, and pre-pandemic controls. In this context, IS/1494/06-specific IgG levels were significantly higher in pre-pandemic control samples than in COVID-19 patients ($p < .05$) (Figure 2B). In addition, the levels of M41-specific IgG in the pre-pandemic control group were significantly higher than in COVID-19 patients ($p < .05$) (Figure 2C). The D274-specific IgG OD ratios in poultry farm personnel were significantly higher than in COVID-19 patients ($p < .001$) (Figure 2D; Table S7). However, we did not observe significant differences in the 4/91-specific IgG levels, between

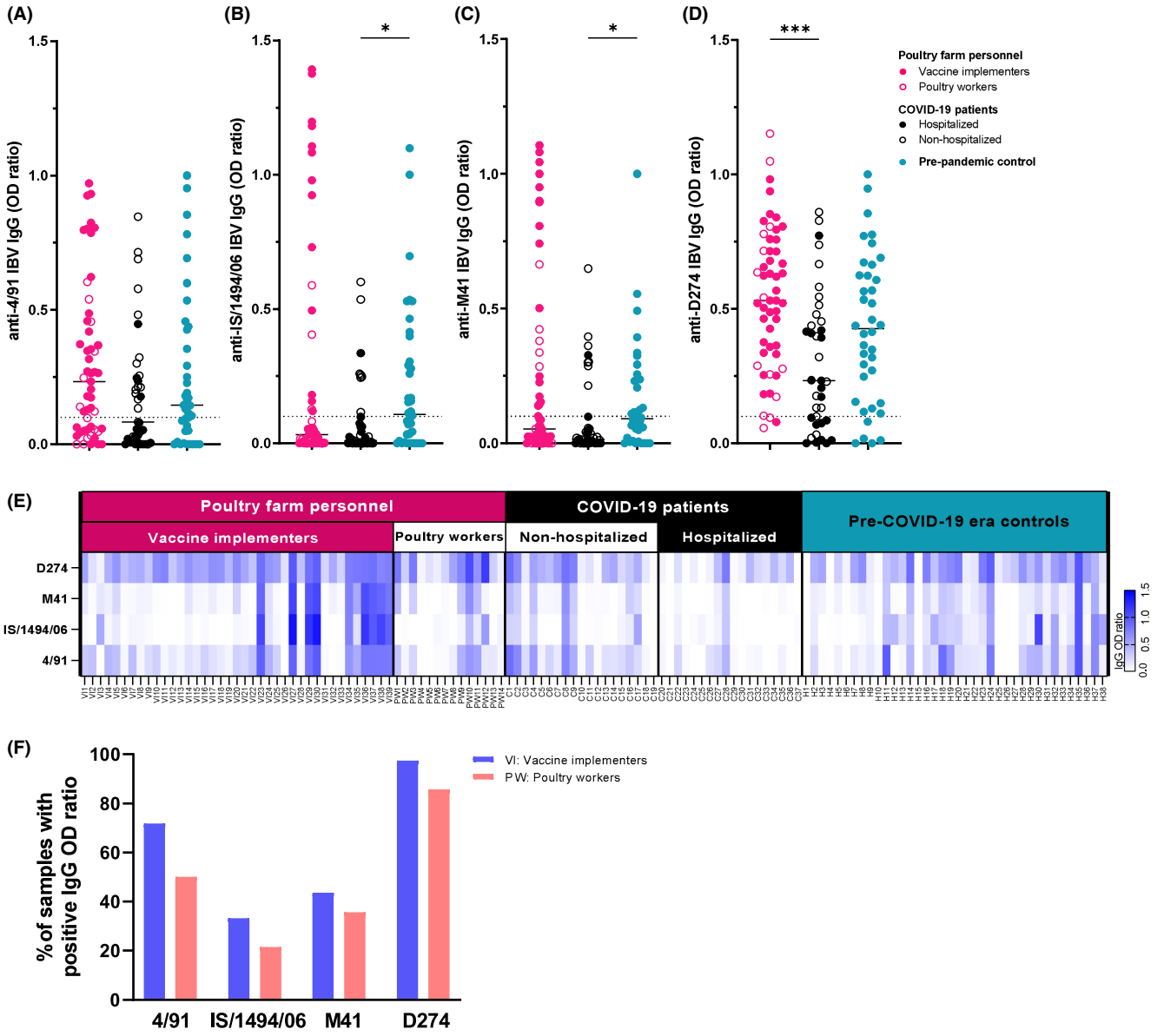


FIGURE 2 IBV-specific IgG levels in poultry farm personnel, COVID-19 patients, and pre-pandemic controls. (A) anti-4/91 IgG, (B) anti-IS/1494/06 IgG, (C) anti-M41 IgG, and (D) anti-D274 IgG OD ratios in sera from vaccine implementers ($n = 39$, pink circles closed) and poultry workers ($n = 14$, pink circles open), non-hospitalized COVID-19 patients ($n = 19$, black circles open), hospitalized COVID-19 patients ($n = 18$, black circles closed), and pre-pandemic control samples ($n = 38$, turquoise circles). Dashed lines indicate the threshold level at 0.1. Statistical analyses were performed using the Kruskal–Wallis test. $^{*}p < .05$, $^{***}p < .001$. Horizontal bar represents mean values. (E) Heatmap of IgG antibody levels in sera from vaccine implementers (VI1-VI39) and poultry workers (PW1-PW14), non-hospitalized COVID-19 patients (C1-C19), hospitalized COVID-19 patients (C20-C37), and pre-pandemic control samples (H1-H38). The color key indicates IgG OD ratios, with blue shades indicating elevated ratios while white shades indicating lower ratios. (F) Frequency of vaccine implementers and poultry workers with >0.1 IgG OD ratios for 4/91, IS/1494/06, M41, and D274

poultry farm personnel, COVID-19 patients, and pre-pandemic control samples (Figure 2A). In the non-hospitalized and hospitalized COVID-19 patients' comparison, we found that 4/91-, M41-, and D274-specific IgG OD ratios were significantly higher in non-hospitalized cases ($p < .01$) (Figure S2A, C, D). However, IS/1494/06-specific IgG OD ratios did not differ significantly in non-hospitalized and hospitalized COVID-19 patients (Figure S2B). There were no significant differences between vaccine implementers and poultry workers in IBV-specific IgG levels (Figure S2E–H). Here it should be noted that the percentage of individuals showing IBV-specific IgG values > 0.1 was slightly higher in vaccine implementers than in poultry workers (Figure 2F).

Among 39 vaccine implementers, 36 (92.31%), 28 (71.79%), 34 (87.18%), and 39 (100%) individuals had >0 IgG ratios for 4/91, IS/1494/06, M41, and D274, respectively. The frequency of individuals with the >0 IgG ratios was 12 (85.71%), 9 (64.29%), 8 (57.14), and 14 (100%) for 4/91, IS/1494/06, M41, and D274, respectively, in a total of 14 poultry workers (Figure 2E).

3.3 | Specific antibody responses to SARS-CoV-2 peptide epitopes with homology to endemic HCoVs and avian coronavirus infectious bronchitis virus

After observing cross-reactivity between SARS-CoV-2 and IBVs, we selected peptides with varying degrees of a homology between SARS-CoV-2 and endemic HCoVs and IBV. We investigated the IgG levels against a selection of SARS-CoV-2 peptides; ORF3a (aa. 172–205), N (aa. 153–176), N (aa. 221–244), N (aa. 358–381), N (aa. 382–405), S (aa. 547–570), S (aa. 782–805), S (aa. 807–830), and S (aa. 1138–1161). As shown in Figure S8G, the most homologous region is S (aa. 807–830) in the comparison between IBV and SARS-CoV-2. Rhinovirus A (aa. 567–591) and a human herpesvirus 4 (aa. 398–422) peptides were included as positive controls and an HIV-1 (aa. 967–991) peptide was included as a negative control. IgG levels against ORF3a (aa. 172–205) were higher in COVID-19 patients than in poultry farm personnel and pre-pandemic control samples (Figure 3A). Similarly, IgG against the regions corresponding to residues 153 to 176, 221 to 244, 358 to 381, and 382 to 405 of the SARS-CoV-2 N protein was higher in COVID-19 patients than in poultry farm personnel and pre-pandemic control samples (Figure 3B–E). According to the S (aa. 547–570), S (aa. 782–805), S (aa. 807–830), and S (aa. 1138–1161) regions, the IgG OD ratios in COVID-19 patients were significantly higher than in poultry farm personnel and pre-pandemic control samples (Figure 3F–I and M; Table S7).

IgG OD ratios against all peptides, except ORF3a (aa. 172–205), were significantly higher in COVID-19 patient samples than in poultry farm personnel and pre-pandemic control samples. In pre-pandemic control group, the IgG OD ratios against ORF3a (aa. 172–205) and N (aa. 221–244) regions were statistically higher than poultry farm personnel.

IgG levels specific for the positive control rhinovirus A (aa. 567–591) and human herpesvirus 4 (aa. 398–422) peptides were significantly higher in pre-pandemic control samples and poultry farm personnel than in COVID-19 patients (Figure 3J,K). In contrast, HIV-1 (aa. 967–991)-specific IgG levels were significantly higher in COVID-19 patients than in poultry farm personnel and pre-pandemic control samples (Figure 3L).

IgG levels specific for the SARS-CoV-2 peptides N (aa 153–176), N (aa 221–244), N (aa 358–381), N (aa 382–405), S (aa 547–570), S (aa 782–805), and S (aa 1138–1161) were significantly higher in hospitalized COVID-19 patients than in non-hospitalized COVID-19 patients (Figure S3A). In contrast, IgG specific for rhinovirus A (aa. 567–591) and human herpesvirus 4 (aa. 398–422) peptides, was higher in non-hospitalized than in hospitalized COVID-19 patients (Figure S3B). IgG specific for the S (aa 1138–1161) peptide was significantly higher in vaccine implementers than in poultry workers (Figure S4A). However, there were no significant differences between vaccine implementers and poultry workers in the evaluation of IgG levels specific to rhinovirus A (aa. 567–591), human herpesvirus 4 (aa. 398–422), and HIV-1 (aa. 967–991) peptides (Figure S4B).

3.4 | Long-term employment as poultry farm personnel is associated with higher levels of SARS-CoV-2 S1-, RBD-, and avian coronavirus infectious bronchitis virus-specific IgG

Next, we wanted to determine whether poultry farm personnel with more work experience had higher levels of SARS-CoV-2- and IBV-specific IgG. Vaccine implementers and poultry workers were categorized into three groups (2–20 months, 21–100 months, >100 months). The levels of IgG specific for S1, RBD, 4/91, IS/1494/06, M41, and D274 were significantly higher in the vaccine implementers with >100 months' experience than in those with 2–20 months' experience (Figure 4A). In the poultry workers, only anti-4/91 IgG levels were higher in the group with >100 months' experience than in the group with 2–20 months' experience (Figure 4B). The comparison between vaccine implementers and poultry workers did not show a significant difference.

3.5 | Correlation among SARS-CoV-2-, avian coronavirus infectious bronchitis virus-, SARS-CoV-2 peptide-, rhinovirus-, human herpesvirus 4-specific IgG in poultry farm personnel, COVID-19 patients, and pre-pandemic controls

A positive correlation between IBV-specific IgG and SARS-CoV-2-specific IgG may be indicative of the presence of cross-reactive antibodies. The strongest positive correlations between SARS-CoV-2- and IBV-specific IgG were observed in poultry farm personnel (Figure 5A, marked by green square), while there were no significant positive correlations between SARS-CoV-2 antigen-specific IgG and IBV-specific IgG in COVID-19 patients. Only one negative correlation ($r = -0.3456$; $p < .05$) was found between N-specific IgG and 4/91-specific IgG in COVID-19 patients (Figure 5B, marked by green square). We observed moderate positive correlations in the pre-pandemic control group between IS/1494/06- and S1-specific IgG, 4/91- and RBD-specific IgG, IS/1494/06- and RBD-specific IgG, M41- and RBD-specific IgG, D274- and RBD-specific IgG, IS/1494/06- and S2-specific IgG, 4/91- and N-specific IgG, IS/1494/06- and N- specific IgG, and M41- and N-specific IgG (Figure 5C, marked by green square). The individual graphs of Spearman correlation analysis for each SARS-CoV-2 and IBV antigens are shown in Figure S5A–C. Only the significant correlations are shown.

There were no significant correlations between SARS-CoV-2 peptide-specific IgG and IBV-specific IgG in poultry farm personnel (Figure 5A, marked by yellow rectangle). We observed negative correlations between several SARS-CoV-2 peptide-specific IgG and IBV-specific IgG in COVID-19 patients and pre-pandemic control group (Figure 5B,C, marked by yellow rectangle). The only significant positive correlation in poultry farm personnel between SARS-CoV-2 antigen- and SARS-CoV-2 peptide-specific IgG was between N- and

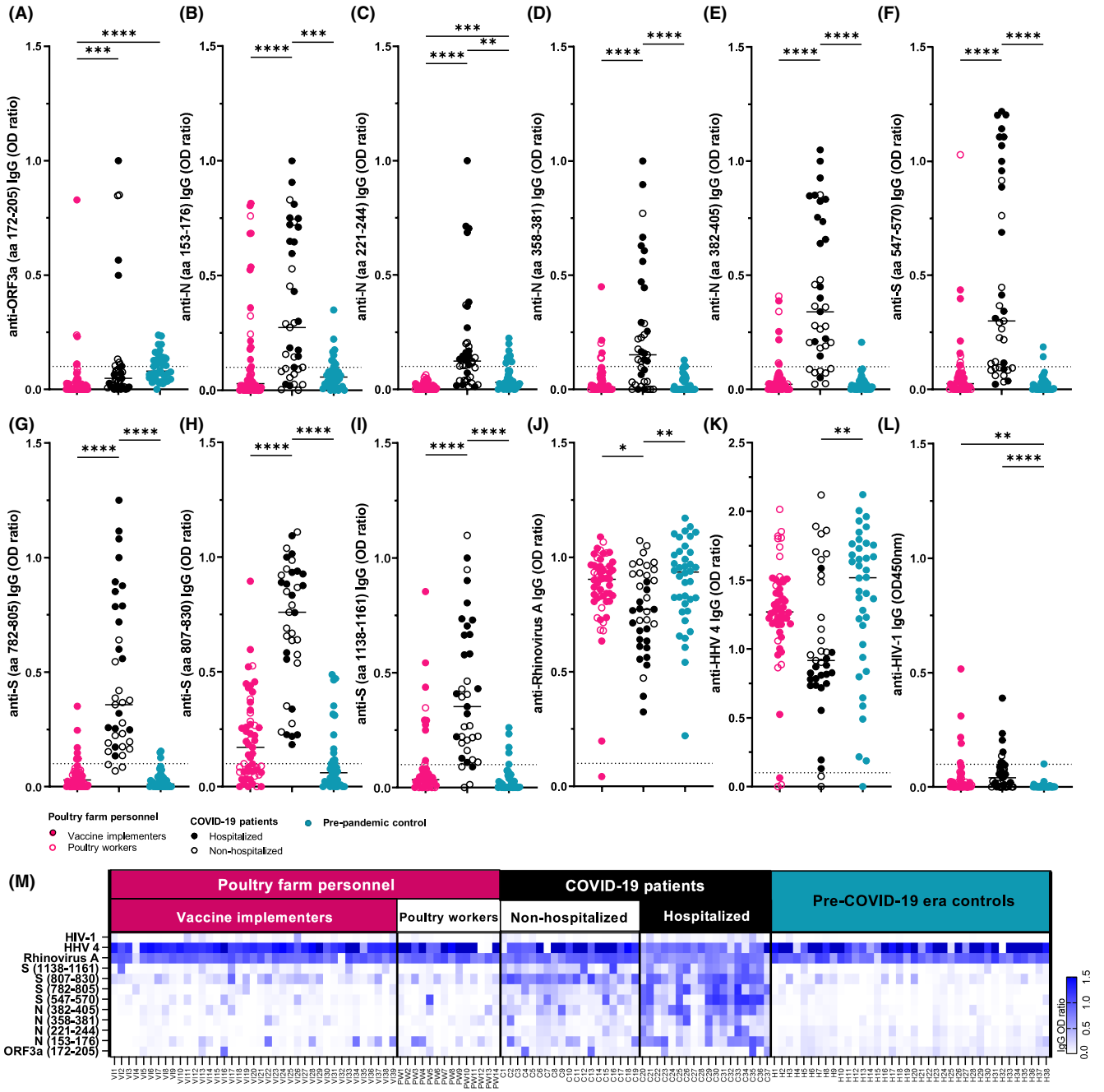


FIGURE 3 SARS-CoV-2 peptide-specific IgG levels in poultry farm personnel, COVID-19 patients, and pre-pandemic controls. (A) anti-ORF3a (aa 172–205) IgG, (B) anti-N (aa 153–176) IgG, (C) anti-N (aa 221–244) IgG, (D) anti-N (aa 358–381) IgG, (E) anti-N (aa 382–405) IgG, (F) anti-S (aa 547–570) IgG, (G) anti-S (aa 782–805) IgG, (H) anti-S (aa 807–830) IgG, (I) anti-S (aa 1138–1161) IgG, (J) anti-Rhinovirus A (aa 567–591) IgG, (K) anti-HHV 4 (aa 398–422) IgG, and (L) anti-HIV-1 (aa 967–991) IgG OD levels in sera from vaccine implementers ($n = 39$, pink circles closed) and poultry workers ($n = 14$, pink circles open), non-hospitalized COVID-19 patients ($n = 19$, black circles open), hospitalized COVID-19 patients ($n = 18$, black circles closed), and pre-pandemic control samples ($n = 38$, turquoise circles). Dashed lines indicate the threshold level at 0.1. Statistical analyses were performed using the Kruskal–Wallis test. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, $^{****}p < .0001$. Horizontal bar represents mean values. (M) Heatmap of IgG antibody levels in sera from vaccine implementers (VI1-VI39) and poultry workers (PW1-PW14), non-hospitalized COVID-19 patients (C1-C19), hospitalized COVID-19 patients (C20-C37), and pre-pandemic control samples (H1-H38). The color key indicates IgG OD ratios, with blue shades indicating elevated ratios while white shades indicating lower ratios.

N (153–176)-specific IgG (Figure 5A, marked by red rectangle). In contrast, COVID-19 patients showed significant positive correlations between all SARS-CoV-2 antigen- and SARS-CoV-2 peptide-specific IgG except for ORF3a (172–205)- and S (807–830)-specific IgG and N (153–176)- and S2-specific IgG not (Figure 5B, marked by red rectangle).

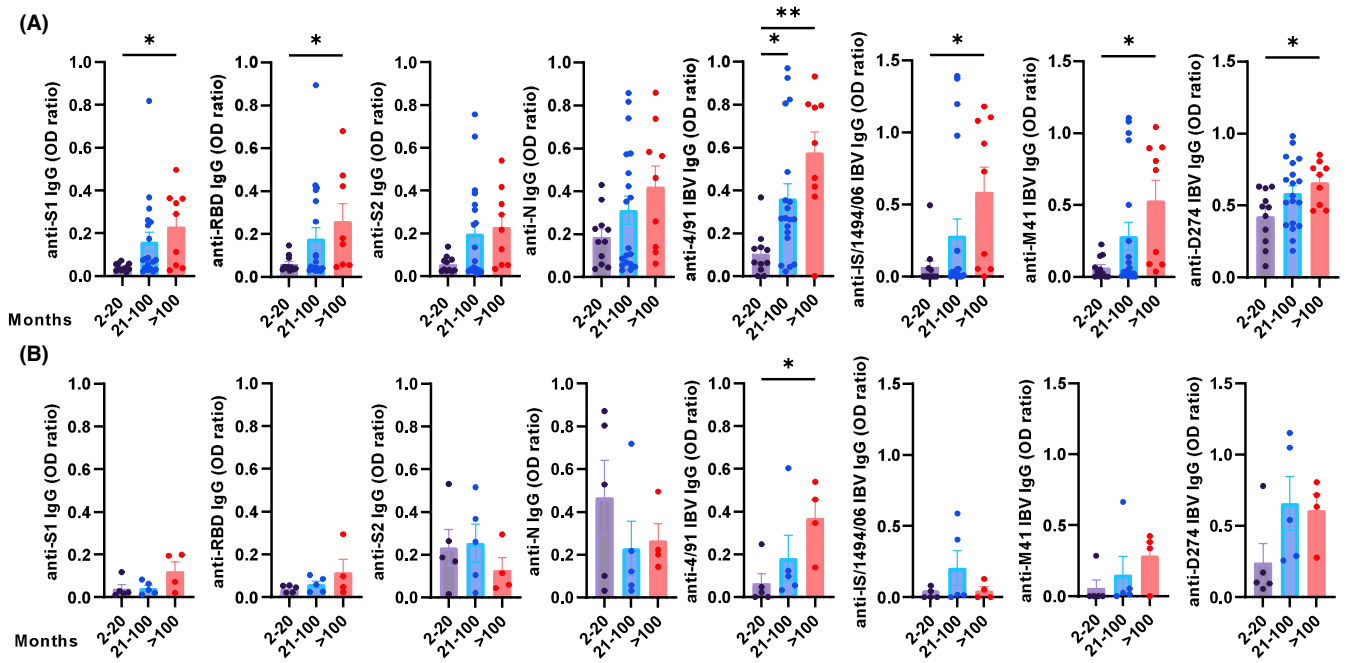


FIGURE 4 Differences in IgG OD ratios based on the work experience of vaccine implementers and poultry workers. Statistical analyses were performed using the Kruskal–Wallis test (ANOVA if parametric distribution). $^{*}p < .05$, $^{**}p < .01$. Individuals were categorized into three groups as follows: (A) vaccine implementers (2–20 months, $n = 11$, purple circles; 21–100 months, $n = 19$, blue circles; >100 months, $n = 9$, pale red circles) and (B) poultry workers (2–20 months, $n = 5$, purple circles; 21–100 months, $n = 5$, blue circles; >100 months, $n = 4$, pale red circles)

Spearman correlation results of IgG OD ratios of vaccine implementers and poultry workers are presented in Table S8 and were compared via their r values. There were remarkable differences in the level of correlations between SARS-CoV-2 antigen- and IBV-specific IgG OD ratios in 4/91 (S2 and N), IS/1494/06 (S1), D274 (S2). In this context, vaccine implementers had higher r values than poultry workers in Spearman correlation analysis (Figure S6A,B; Table S8).

The S1-, RBD-, and N-specific IgG negatively correlated with both rhinovirus- and human herpesvirus 4-specific IgG in COVID-19 patients. There were no significant correlations between SARS-CoV-2- and rhinovirus-, SARS-CoV-2-, and human herpesvirus 4-specific IgG in poultry farm personnel and pre-pandemic control group (Figure 5A–C).

3.6 | Virus neutralization assay results

To determine whether serum samples in which SARS-CoV-2 antigen-specific IgG was detected were able to neutralize SARS-CoV-2, we performed a live-virus neutralization assay. We selected samples from four categories, which were determined based on the corresponding ELISA results: I. Positive for SARS-CoV-2- and IBV-specific IgG (comprised of 10 vaccine implementers and one poultry worker); II. Positive for SARS-CoV-2-specific IgG (comprised of four COVID-19 patients and one vaccine implementer); III. Negative for SARS-CoV-2- and IBV-specific IgG (comprised of one vaccine implementer, two poultry workers, and one pre-pandemic control); IV. Negative for SARS-CoV-2-specific IgG and positive for IBV-specific IgG (comprised of two vaccine implementers). The IgG ratios of samples from each group are presented in Figure S7A. Only samples from group II (antibody-positive group against SARS-CoV-2) were able to neutralize in vitro SARS-CoV-2-mediated killing of Vero E6 cells. The geometric mean of neutralization titers was 1:16, 1:35.78, 1:80, 1:16, and 1:80 for VI17, C12, C13, C14, and C16, respectively (Table 1). Representative examples of positive, negative, and poultry farm personnel serum samples are shown in Figure S7B.

4 | DISCUSSION

In this study, we assessed the cross-reactivity of IgG antibodies with SARS-CoV-2 and IBV in poultry farm personnel, pre-pandemic controls, and COVID-19 patients. The poultry farm personnel consisted of vaccine implementers and poultry workers, who had been exposed to IBV vaccines or worked in the poultry houses in which IBV vaccinated chickens are held. Virus neutralization assay was performed on some of the poultry farm personnel sera that had cross-reactive antibodies to SARS-CoV-2 and IBV. Moreover, we investigated the antibody responses against peptides expressed in both SARS-CoV-2 and IBV.

A subset of poultry farm personnel showed elevated levels of IgG specific for all SARS-CoV-2 antigens compared than pre-pandemic controls (Figure 1A–D). This may be the result of prior exposure to IBV via aerosol vaccination, which aims to elicit the development of IBV-specific antibodies in chicken. We found that a significant

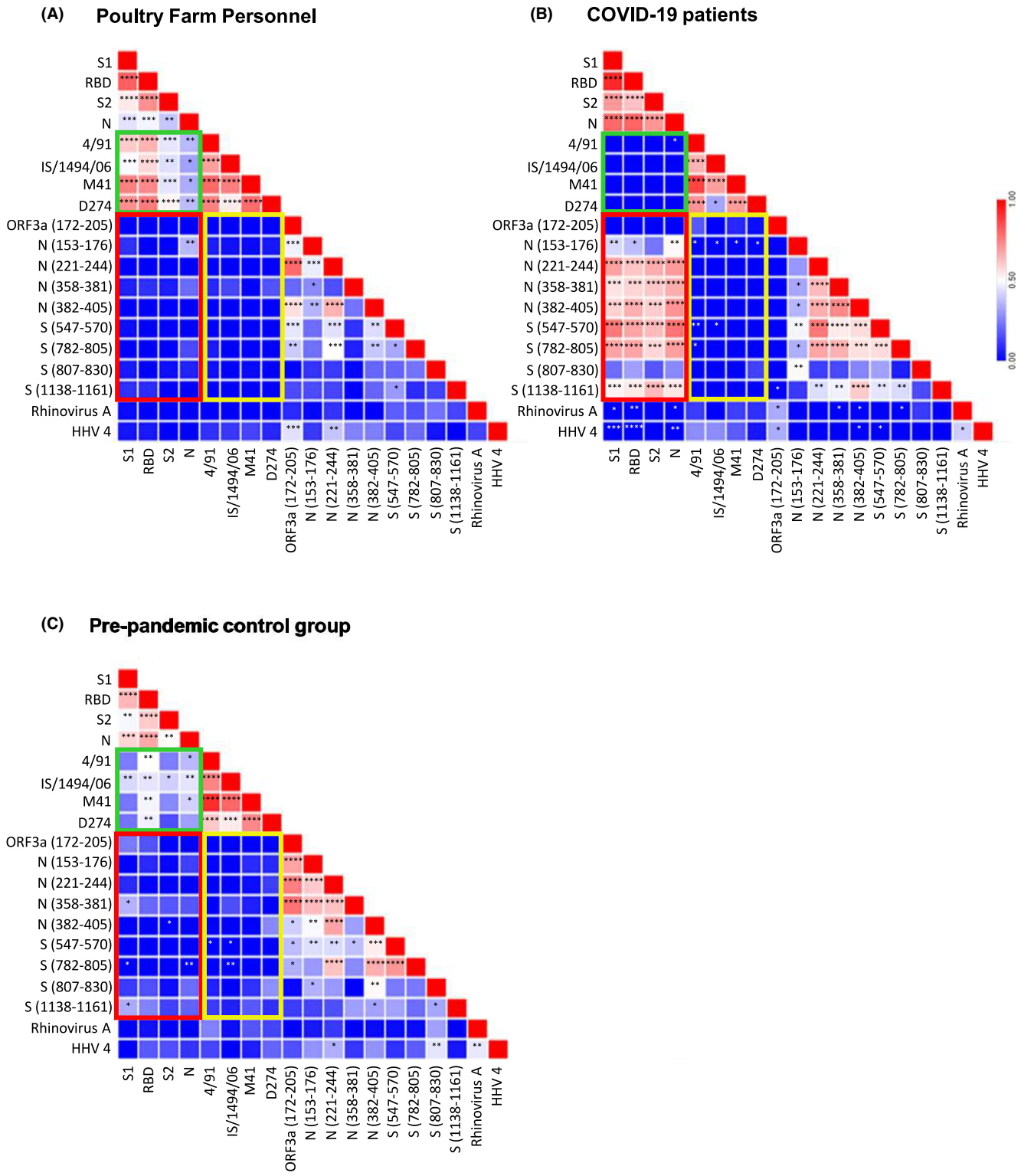


FIGURE 5 Heatmap with Spearman correlation analysis. Correlation analysis of between SARS-CoV-2-, IBV-, SARS-CoV-2 peptide-, Rhinovirus-, and human herpesvirus 4-specific IgG levels in (A) poultry farm personnel, (B) COVID-19 patients, and (C) pre-pandemic controls. White color asterisks indicate negative correlation. *$p < .05$, **$p < .01$, ***$p < .001$, ****$p < .0001$

number of vaccine implementers and one poultry worker developed IBV specific antibodies that cross-react with all four SARS-CoV-2 antigens (S1, RBD, S2, and N) that were analyzed. Due to the partial sequence homology of HCoVs with SARS-CoV-2, exposure to HCoVs may induce cross-reactive immune responses to SARS-CoV-2 proteins. Similarly, Tso et al[28] reported that prior exposure to HCoVs (particularly HCoV-NL63 and HCoV-229E) can also be a source of cross-reactive antibodies against SARS-CoV-2 in pre-COVID-19

TABLE 1 Results of the virus neutralization test

Group	Sample	Replicate 1	Replicate 2	Neutralizing titer (GMT)	VNT result
I	VI20	0	0	<16	N
	VI23	0	0	<16	N
	VI27	0	0	<16	N
	VI29	0	0	<16	N
	VI30	0	0	<16	N
	VI34	0	0	<16	N
	VI36	0	0	<16	N
	VI37	0	0	<16	N
	VI38	0	0	<16	N
	VI39	0	0	<16	N
	PW9	0	0	<16	N
II	VI17	16	16	16	P
	C12	80	16	35.78	P
	C13	80	80	80	P
	C14	16	16	16	P
	C16	80	80	80	P
III	VI33	0	0	<16	N
	PW5	0	0	<16	N
	PW13	0	0	<16	N
	H10	0	0	<16	N
IV	VI5	0	0	<16	N
	VI35	0	0	<16	N

Note: Groups: I. positive for SARS-CoV-2- and IBV-specific IgG; II. positive for SARS-CoV-2-specific IgG; III. negative for SARS-CoV-2- and IBV-specific IgG; and IV. negative for SARS-CoV-2-specific IgG and positive for IBV-specific IgG.

Abbreviations: GMT, geometric mean titer; P, positive; N, negative.

pandemic plasma samples. These researchers demonstrated that the SARS-CoV-2 N and S proteins were the predominant sources of the cross-reactivity, which is consistent with the results found in the current study. The sequence homology between SARS-CoV-2 and IBV (4/91, M41, and D274) is 27–29% for S and 30% for N.[29] The level of sequence homology between SARS-CoV-2 and other β-CoVs (HCoV-OC43 and HCoV-HKU1) is 32–33% for S and 34% for N, while the homology between SARS-CoV-2 and α-CoVs (HCoV-229E and HCoV-NL63) is 28–30% for S and 28–29% for N.[30] Although sequence conservation is lower for more common HCoVs, their high prevalence may lead to widespread antibodies with cross-reactivity to SARS-CoV-2.[31] Therefore, IBV exposure has the potential to induce SARS-CoV-2 cross-reactive antibodies. The protein homology between IBV genotypes and endemic HCoVs (OC43, HKU1, 229E, and NL63) is ~31%–45%, ~32%–36%, and ~27%–32% for ORF1ab, S, and N proteins, respectively. Although the similarity is not remarkably high within S and N proteins, sequence identities of the putative epitopes for B cells within S and N proteins may lead to higher phylogenetic relatedness.[32] However this needs further investigation.

4/91-, IS/1494/06-, M41-, and D274-specific IgG could be detected in the majority of poultry farm personnel, COVID-19 patients, and pre-pandemic controls. None of the control and COVID-19 individuals in this study are likely to have encountered IBV or IBV vaccines. Therefore, the presence of IBV-specific IgG in these individuals is indicative of a cross-reactivity that may be associated with exposure to endemic HCoVs. Of note, the IBV-specific IgG levels were significantly lower in COVID-19 patients than the poultry farm personnel and pre-pandemic controls (Figure 2B–D). In addition, non-hospitalized COVID-19 patients showed significantly higher levels of 4/91-, M41-, and D274-specific IgG than hospitalized patients (Figure S2A, C, D). As shown in Table S3, hospitalized patients exhibited differences in comorbidities compared with non-hospitalized individuals. SARS-CoV-2 severity is associated with various factors including sex, age, and comorbidities.[19] In our study, hospitalized patients were characterized by high levels of SARS-CoV-2-specific IgG but low IBV-specific IgG. It cannot be excluded that some of the comorbidities in the hospitalized COVID-19 patients influence the levels and cross-reactivity of antibodies against SARS-CoV-2. IgG against IBV could not be measured in the same COVID-19 patients before infection because samples of this time-point were not available. Therefore, it remains unclear whether SARS-CoV-2 infection resulted in a reduction of IBV-reactive IgG levels in COVID-19 patients or that these levels were already reduced prior to infection. Similarly, we observed reduced IgG levels

against rhinovirus A and human herpesvirus 4 in COVID-19 patients (Figure 3J, K). This reduction was most pronounced in hospitalized COVID-19 patients (Figure S3B). These observations are in line with what has been reported in the context of measles virus infection. As suggested by Mina et al.[33] measles virus infection is associated with a reduced population immunity against other infections, resulting from measles-induced immune amnesia. Measles virus can infect memory T-, B-, and plasma-cells[34] and measles virus infection is associated with a broad reduction in circulating antibodies against pathogens unrelated to measles.[35] Recent findings indicate that SARS-CoV-2 can directly infect T cells in an ACE2-independent manner that is consistent with the previously reported mechanism of SARS-CoV-2-induced lymphopenia.[36] Hence, reduced IgG levels against IBV, rhinovirus A, and human herpesvirus 4 observed in hospitalized COVID-19 patients may be caused by a mechanism similar to measles-induced immune amnesia affecting systemic immune memory. It should be noted that hospitalized COVID-19 patients were older than non-hospitalized patients (Table S2). However, this age difference does not explain the above-mentioned differences in IBV-, rhinovirus A-, and human herpesvirus 4-specific IgG, as elderly patients typically do not have significantly reduced antibody levels against common infectious agents.[37]

Importantly, we noticed a strong correlation between IBV-specific IgG and SARS-CoV-2 S1-, RBD-, S2-, and N-specific IgG in poultry farm personnel compared with the pre-pandemic control group and COVID-19 patients. This finding indicates that these cross-reactive antibodies may be triggered by exposure to IBV in poultry farm personnel. Theoretically, if these cross-reactive antibodies would be the result of HCoV exposure, we would have observed similar correlation results in the pre-pandemic samples. But the present results did not confirm this interpretation.

Several differences were observed between poultry farm personnel and COVID-19 patients in the correlation patterns for IBV- and SARS-CoV-2-specific IgG titers (Figure 5A, B). It can be postulated that IBV-specific antibodies in poultry farm personnel are likely the result of direct exposure to IBV leading to cross-reactivity. It is obvious that SARS-CoV-2 exposure leads to the development of SARS-CoV-2 specific antibodies in COVID-19 patients. In these patients, the strong correlation between SARS-CoV-2 peptide-specific IgG and SARS-CoV-2 antigen-specific IgG can be partially explained by exposure to SARS-CoV-2. In pre-pandemic controls, who have no IBV and SARS-CoV-2 exposure history, the moderate positive correlations shown in Figure 5C, may be the result of prior exposure to HCoVs.

COVID-19 patients had higher levels of SARS-CoV-2 S1-, RBD-, S2-, and N-specific IgG than pre-pandemic controls and poultry farm personnel. In addition, we found that SARS-CoV-2 antigen-specific IgG levels in COVID-19 patients correlated with disease severity. Hospitalized COVID-19 patients showed higher levels of S1-, RBD-, S2-, and N-specific IgG than non-hospitalized COVID-19 patients. These findings corroborate results published by other research groups.[27] Bruni et al.[38] reported that non-hospitalized patients had lower S ectodomain-, RBD-, and N-specific IgG titers and blood pro-inflammatory cytokine profiles than patients in intensive care units. Similarly, Chen et al.[39] demonstrated that severe COVID-19 patients mounted the highest S1-, RBD-, and S2-specific IgG titers compared with moderate, mild, and asymptomatic patients. In addition, Kowitdamrong et al.[40] found that the levels of S1-specific IgA and IgG were higher in severe COVID-19 patients. Similar observations have been reported in many other studies.[30,41,42] However, the molecular mechanism underlying this association has not yet been elucidated in detail.[40] One of the possible explanations is that increased levels of IgG may be related to the high viral loads. Moreover, increased inflammatory signals, antigen presentation, and stimulatory signals for humoral responses may play a role in this process.[43]

Next, we measured IgG levels against nine SARS-CoV-2 peptides that were defined as highly indicative of SARS-CoV-2 exposure history by Shrock et al.[26] in all three groups. We detected elevated levels of antibody responses to SARS-CoV-2 peptides in COVID-19 patients compared with poultry farm personnel and pre-pandemic control samples. IgG levels against SARS-CoV-2 peptides [N (aa. 153–176), N (aa. 221–244), N (aa. 358–381), N (aa. 382–405), S (aa. 547–570), S (aa. 782–805), and S (aa. 1138–1161)] of hospitalized COVID-19 patients were significantly higher than non-hospitalized COVID-19 patients. This is in line with the findings published by Shrock et al.[26] indicating that hospitalized COVID-19 patients developed stronger and broader antibody responses to SARS-CoV-2 S and N proteins than non-hospitalized patients. The most homologous region among the studied SARS-CoV-2 peptides and IBV is S (aa. 807–830) (Figure S8G). This immunodominant Coronavirus peptide domain of spike has been identified as a recognizable region for immune responses. Shrock et al.[26] showed antibody responses to SARS-CoV-2 S (aa. 807–830) in 79.9% of COVID-19 patients and to the corresponding peptides from HCoV-OC43 and HCoV-229E of 20% in the pre-COVID-19 individuals. Loyal et al.[44] achieved remarkable results demonstrating the functional role of pre-existing SARS-CoV-2- and HCoV-reactive $CD4^+$ T cells. The SARS-CoV-2 S (816–830) region is recognized by $CD4^+$ T cells in 20% of healthy individuals, 50–60% of SARS-CoV-2 convalescents, and 97% of BNT162b2-vaccinated individuals. Another indicative peptide sequence for SARS-CoV-2 recognition is S (aa. 1138-1161). In this respect, Shrock et al.[26] reported that both SARS-CoV-2 and HCoV-OC43 peptides corresponding to this peptide are recognized much more frequently by COVID-19 patients compared with pre-COVID-19 controls. Notably, we also observed elevated levels of IgG specific for S (aa. 1138–1161) in poultry farm personnel (Figure 3I). Cross-reactive responses to S (aa. 807–830) and S (aa. 1138–1161) were more frequently detected in poultry farm personnel than in pre–COVID-19 era controls. Moreover, some poultry farm personnel had high IgG levels against SARS-CoV-2 N (aa. 153–176). It is important to note that, all poultry farm personnel declared that they did not have COVID-19 symptoms. It can however not be excluded that elevated IgG specific for SARS-CoV-2 antigens may be caused by subclinical infection with SARS-CoV-2.

Hospitalized COVID-19 patients showed a modest increase in HIV-1-specific IgG levels compared with non-hospitalized patients.

HIV-1 (aa 967–991) shares 28% sequence homology with SARS-CoV-2.[29] Slightly increased levels of HIV-1-specific IgG in hospitalized COVID-19 patients may be explained by this sequence homology.

Repeated antigen exposure may stimulate antibody responses, and the elevated levels of S1-, RBD-, and higher amounts of IBV-specific IgG observed in our study in long-term vaccine implementers support this association. Vaccine implementers with >100 months' experience showed significantly increased levels of S1-, RBD-, 4/91-, IS/1494/06-, M41-, and D274-specific IgG compared with vaccine implementers with 2–20 months of experience. This suggests that long-term exposure to IBV leads to higher levels of SARS-CoV-2 S1- and RBD-cross-reactive IgG. Kosikova et al.[45] reported that repeated influenza exposure imprinted not only increased antibody quantity but also improved quality as shown by higher affinity antibody development. However, it should be emphasized that the relatively low number of subjects that could be included in our analysis precludes definitive conclusions regarding the relation between duration of exposure to IBV and SARS-CoV-2 S1- and RBD-specific IgG levels.

In the present study, we found cross-reactive antibodies between IBV and SARS-CoV-2, but there was no virus neutralization in the poultry farm personnel (samples selected based on antibody titers against SARS-CoV-2 and IBV) while this was the case in COVID-19 patients (Figure S7). This could be due to (1) low affinity for the RBD compared with COVID-19 patients, (2) low concentrations of antibody against SARS-CoV-2, or (3) the antibodies do not bind to the part of RBD that is responsible for binding to ACE2. Although no neutralization was observed in the selected poultry farm personnel as mentioned above, one sample from the vaccine implementer group (VI17) was a notable exception. This sample showed low IBV-specific IgG titers and high IgG titers specific for SARS-CoV-2 antigens and was found to have SARS-CoV-2 neutralizing capacity. A possible explanation may be that this individual had a prior subclinical SARS-CoV-2 infection that was not diagnosed.

Besides direct neutralization of viral infection, antibodies may protect against infection or severe disease through alternative mechanisms including recruitment of complement or Fc receptor-mediated effector functions such as complement-dependent cytotoxicity, antibody-dependent cellular phagocytosis, and antibody-dependent cellular cytotoxicity.[46] Unlike direct neutralization, which we measured in the current study, such mechanisms are not easily quantified using in vitro assays.

Thorough assessment of the clinical implications of this study would require a much larger study cohort with a follow-up analysis in which the incidence of SARS-CoV-2 infections as well as the course of disease are assessed and correlated with the presence of cross-reactive antibodies. Although this would be highly interesting, it is beyond the scope of this study. Moreover, as a result of the extensive rollout of vaccination programs for SARS-CoV-2, the relative contribution of prior exposure to IBV vaccines to protection against infection and/or severe disease in our study population may be obscured by effects of the SARS-CoV-2 vaccination. It should be noted that the contribution of antibodies directed against endemic HCoVs to the humoral response against SARS-CoV-2 remains incompletely understood. On one hand, cross-reactive and pre-existing HCoV antibodies may contribute to protection against SARS-CoV-2. On the other hand, pre-existing immune memory in response to exposure to HCoVs may repress the generation of SARS-CoV-2-specific IgG by expanding cross-reactive antibodies that do not neutralize SARS-CoV-2.[47] Our study provides preliminary but important results for the clinical implications through cross-reactivity of IBV-IgGs.

A limitation of this study was the lack of COVID-19 testing in the poultry farm personnel before the sampling began. Samples were taken from individuals that had shown no symptoms for COVID-19 or any infection. It should be noted that asymptomatic infection is a general problem in serologic studies for all viral infections. Potential false-negative results from RT-PCR COVID-19 tests and the difficulties in regular testing of individuals in the acute pandemic period make the evaluation for asymptomatic infection more troublesome. Nevertheless, our results show that the pattern of anti-SARS-CoV-2 IgG levels in poultry farm personnel is different than the symptomatic COVID-19 infection. It is generally expected that although the infection is asymptomatic the pattern of antibody response does not differ from the symptomatic infection. Here, we conducted a comprehensive analysis of IgG cross-reactivity between SARS-CoV-2 and IBV. The most conspicuous finding of this study is that a subset of poultry farm personnel, particularly long-time exposed vaccine implementers, showed elevated levels of IgG specific for all IBV and SARS-CoV-2 antigens that were analyzed. Furthermore, there was a strong correlation between IBV-specific IgG and SARS-CoV-2 antigen-specific IgG in these individuals. However, these cross-reactive antibodies did not have neutralizing capacity in the SARS-CoV-2 neutralization assay that we employed. It is important to note that, conducting a similar cohort study may not be possible in the future, because of the extensive SARS-CoV-2 vaccine applications, particularly for farm personnel. In conclusion, our data demonstrate that exposure to IBV may cause SARS-CoV-2-cross-reactive IgG.

AUTHOR CONTRIBUTIONS

K.T.C., M.A., C.A.A., and W.V. designed the study. O.A., P.S., B.R., and D.M. carried out the ELISA experiments of the study. O.A., K.T.C., P.S., M.A., C.A.A., and W.V. contributed to the data analysis and interpretations of the results. H.R.J., B.W., and A.E. performed live-virus neutralization assay. A.D., A.C., S.H., and M.C.B. provided COVID-19 patients' samples. C.C. and O.B. contributed to the ELISA procedure development, and intellectually reviewed the manuscript. All authors intellectually reviewed the manuscript.

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CONFLICT OF INTEREST

M. Akdis has received research grants from Swiss National Science Foundation, Bern; research grant from the Stanford University; Leading House for the Latin American Region, Seed Money Grant. She is in the Scientific Advisory Board member of Stanford University-Sean Parker Asthma Allergy Center, CA; Advisory Board member of LEO Foundation Skin Immunology Research Center, Kopenhagen; and Scientific Co-Chair of World Allergy Congress (WAC) Istanbul, 2022. A. Eggel is a co-founder and consultant of Excellergy, Inc. and ATANIS Biotech AG; has received research grants from Swiss National Science Foundation, VELUX Stiftung, Fondation ACTERIA, Swiss Lung Association, the National Institutes of Health, Innosuisse, Bühlmann Laboratories AG and Novartis; has consulted for Bühlmann Laboratories AG, Novartis and GSK. C. A. Akdis has received research grants from the Swiss National Science Foundation, European Union (EU CURE, EU Syn-Air-G), European Union, Novartis Research Institutes, (Basel, Switzerland), Stanford University (Redwood City, Calif), and SciBase (Stockholm, Sweden); is the Co-Chair for EAACI Guidelines on Environmental Science in Allergic diseases and Asthma; is on the Advisory Boards of Sanofi/Regeneron, Stanford University Sean Parker Asthma Allergy Center, Novartis, GlaxoSmithKline, Bristol-Myers Squibb (London) and SciBase; and is the Editor-in-Chief of Allergy. W. van de Veen has received research grants from Promedica Stiftung, EoE stiftung, European Society of Clinical Microbiology and Infectious Diseases, Novartis Freenovation; has consulted for Mabylon AG. All other authors declare that they have no relevant conflicts of interest.

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REFERENCES

1. Zhang J-J, Dong X, Cao Y-Y, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy*. 2020;75(7):1730-1741.
2. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020;382:727-733.
3. Hein S, Herrlein ML, Mhedhbi I, et al. Analysis of BNT162b2-and CVnCoV-elicited sera and of convalescent sera toward SARS-CoV-2 viruses. *Allergy*. 2022;77(7):2080-2089.
4. Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA vaccine against SARS-CoV-2—preliminary report. *N Engl J Med*. 2020;383:1920-1931.
5. Jackwood MW, de Wit S. Infectious bronchitis. In: Swayne DE, Boulianne M, Logue CM, McDougald LR, Nair V, Suarez DL, eds. *Diseases of Poultry*. 14th ed. John Wiley & Sons, Inc.; 2020:167-188.
6. Saif LJ, Wang Q, Vlasova AN, Jung K, Xiao S. Coronaviruses. In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, Zhang J, eds. *Diseases of Swine*. 11th ed. John Wiley & Sons, Inc.; 2019:488-523.
7. Fabricant J. The early history of infectious bronchitis. *Avian Dis*. 1998;42(4):648-650.
8. Tucciarone CM, Franzo G, Bianco A, et al. Infectious bronchitis virus gel vaccination: evaluation of mass-like (B-48) and 793/B-like (1/96) vaccine kinetics after combined administration at 1 day of age. *Poult Sci*. 2018;97(10):3501-3509.
9. Rohaim MA, El Naggar RF, Abdelsabour MA, Mohamed MH, El-Sabagh IM, Munir M. Evolutionary analysis of infectious bronchitis virus reveals marked genetic diversity and recombination events. *Genes*. 2020;11(6):605.
10. Valastro V, Holmes EC, Britton P, et al. S1 gene-based phylogeny of infectious bronchitis virus: an attempt to harmonize virus classification. *Infect Genet Evol*. 2016;39:349-364.
11. Korath AD, Janda J, Untersmayr E, et al. One health: EAACI position paper on coronaviruses at the human-animal interface, with a specific focus on comparative and zoonotic aspects of SARS-Cov-2. *Allergy*. 2022;77(1):55-71.
12. Tizard IR. Vaccination against coronaviruses in domestic animals. *Vaccine*. 2020;38(33):5123-5130.
13. Enjuanes L, Almazán F, Sola I, Zuñiga S. Biochemical aspects of coronavirus replication and virus-host interaction. *Annu Rev Microbiol*. 2006;60:211-230.
14. Shang J, Wan Y, Luo C, et al. Cell entry mechanisms of SARS-CoV-2. *Proc Natl Acad Sci*. 2020;117(21):11727-11734.
15. Perlman S, Netland J. Coronaviruses post-SARS: update on replication and pathogenesis. *Nat Rev Microbiol*. 2009;7(6):439-450.
16. Ge J, Zhang S, Zhang L, Wang X. Structural basis of severe acute respiratory syndrome coronavirus 2 infection. *Curr Opin HIV AIDS*. 2021;16(1):74-81.
17. Spiga O, Bernini A, Ciutti A, et al. Molecular modelling of S1 and S2 subunits of SARS coronavirus spike glycoprotein. *Biochem Biophys Res Commun*. 2003;310(1):78-83.
18. Shang J, Zheng Y, Yang Y, et al. Cryo-EM structure of infectious bronchitis coronavirus spike protein reveals structural and functional evolution of coronavirus spike proteins. *PLoS Pathog*. 2018;14(4):e1007009.
19. Cervia C, Nilsson J, Zurbuchen Y, Valaperti A, Schreiner J, Wolfensberger A, Raeber ME, Adamo S, Weigang S, Emmenegger M, Hasler S, Bosshard PP, de Cecco E, Bächli E, Rudiger A, Stüssi-Helbling M, Huber LC, Zinkernagel AS, Schaer DJ, Aguzzi A, Kochs G, Held U, Probst-Müller E, Rampini SK, Boyman O Systemic and mucosal antibody responses specific to SARS-CoV-2 during mild versus severe COVID-19. *J Allergy Clin Immunol* 2021;147(2):545–557. e9, 557.e9.
20. Mao T, Israelow B, Suberi A, et al. Unadjuvanted intranasal spike vaccine booster elicits robust protective mucosal immunity against sarbecoviruses. *bioRxiv*. 2022. doi:10.1101/2022.01.24.477597
21. Nguyen-Contant P, Embong AK, Kanagaiah P, et al. S protein-reactive IgG and memory B cell production after human SARS-CoV-2 infection includes broad reactivity to the S2 subunit. *MBio*. 2020;11(5):e01991-e01920.
22. Yu D, Han Z, Xu J, et al. A novel B-cell epitope of avian infectious bronchitis virus N protein. *Viral Immunol*. 2010;23(2):189-199.
23. Yu Y, Wang M, Zhang X, et al. Antibody-dependent cellular cytotoxicity response to SARS-CoV-2 in COVID-19 patients. *Signal Transduct Target Ther*. 2021;6(1):1-10.

24. Yamin R, Jones AT, Hoffmann H-H, et al. Fc-engineered antibody therapeutics with improved anti-SARS-CoV-2 efficacy. *Nature*. 2021;599(7885):465-470.
25. Lee WS, Wheatley AK, Kent SJ, DeKosky BJ. Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies. *Nat Microbiol*. 2020;5(10):1185-1191.
26. Shrock E, Fujimura E, Kula T, et al. Viral epitope profiling of COVID-19 patients reveals cross-reactivity and correlates of severity. *Science*. 2020;370(6520):eabd4250.
27. Brigger D, Horn MP, Pennington LF, et al. Accuracy of serological testing for SARS-CoV-2 antibodies: first results of a large mixed-method evaluation study. *Allergy*. 2021;76(3):853-865.
28. Tso FY, Lidenge SJ, Pena PB, et al. High prevalence of pre-existing serological cross-reactivity against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in sub-Saharan Africa. *Int J Infect Dis*. 2021;102:577-583.
29. Clustal Omega Multiple Sequence Alignment. https://www.ebi.ac.uk/Tools/msa/clustalo/. Accessed 21/01/2021.
30. Okba NM, Müller MA, Li W, et al. Severe acute respiratory syndrome coronavirus 2– specific antibody responses in coronavirus disease patients. *Emerg Infect Dis*. 2020;26(7):1478-1488.
31. Bates TA, Weinstein JB, Leier HC, Messer WB, Tafesse FG. Cross-reactivity of SARS-CoV structural protein antibodies against SARS-CoV-2. *Cell Rep*. 2021;34(7):108737.
32. Tamminen K, Salminen M, Blazevic V. Seroprevalence and SARS-CoV-2 cross-reactivity of endemic coronavirus OC43 and 229E antibodies in Finnish children and adults. *Clin Immunol*. 2021;229:108782.
33. Mina MJ, Metcalf CJE, De Swart RL, Osterhaus A, Grenfell BT. Long-term measles-induced immunomodulation increases overall childhood infectious disease mortality. *Science*. 2015;348(6235):694-699.
34. Laksono BM, de Vries RD, Verburgh RJ, et al. Studies into the mechanism of measles-associated immune suppression during a measles outbreak in The Netherlands. *Nat Comm*. 2018;9(1):1-10.
35. Mina MJ, Kula T, Leng Y, et al. Measles virus infection diminishes preexisting antibodies that offer protection from other pathogens. *Science*. 2019;366(6465):599-606.
36. Shen X-R, Geng R, Li Q, et al. ACE2-independent infection of T lymphocytes by SARS-CoV-2. *Signal Transduct Target Ther*. 2022;7(1):1-11.
37. Rees-Spear C, McCoy LE. Vaccine responses in ageing and chronic viral infection. *Oxf Open Immunol*. 2021;2(1):iqab007.
38. Bruni M, Cecatiello V, Diaz-Basabe A, et al. Persistence of anti-SARS-CoV-2 antibodies in non-hospitalized COVID-19 convalescent health care workers. *J Clin Med*. 2020;9(10):3188.
39. Chen X, Pan Z, Yue S, et al. Disease severity dictates SARS-CoV-2-specific neutralizing antibody responses in COVID-19. *Signal Transduct Target Ther*. 2020;5(1):1-6.
40. Kowitdamrong E, Puthanakit T, Jantarabenjakul W, et al. Antibody responses to SARS-CoV-2 in patients with differing severities of coronavirus disease 2019. *PLoS One*. 2020;15(10):e0240502.
41. Dugas M, Grote-Westrick T, Vollenberg R, et al. Less severe course of COVID-19 is associated with elevated levels of antibodies against seasonal human coronaviruses OC43 and HKU1 (HCoV OC43, HCoV HKU1). *Int J Infect Dis*. 2021;105:304-306.
42. Yongchen Z, Shen H, Wang X, et al. Different longitudinal patterns of nucleic acid and serology testing results based on disease severity of COVID-19 patients. *Emerg Microbes & Infect*. 2020;9(1):833-836.
43. Yates JL, Ehrbar DJ, Hunt DT, et al. Serological analysis reveals an imbalanced IgG subclass composition associated with COVID-19 disease severity. *Cell Rep Med*. 2021;100329:100329.
44. Loyal L, Braun J, Henze L, et al. Cross-reactive CD4+ T cells enhance SARS-CoV-2 immune responses upon infection and vaccination. *Science*. 2021;374(6564):eabh1823.
45. Kosikova M, Li L, Radvak P, Ye Z, Wan X-F, Xie H. Imprinting of repeated influenza a/H3 exposures on antibody quantity and antibody quality: implications for seasonal vaccine strain selection and vaccine performance. *Clin Infect Dis*. 2018;67(10):1523-1532.
46. Zohar T, Alter G. Dissecting antibody-mediated protection against SARS-CoV-2. *Nat Rev Immunol*. 2020;20:392-394.
47. Lin CY, Wolf J, Brice DC, et al. Pre-existing humoral immunity to human common cold coronaviruses negatively impacts the protective SARS-CoV-2 antibody response. *Cell Host Microbe*. 2022;30(1):83-96.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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