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Short communication

Antibodies against spike protein of SARS-CoV-2 variants in bovine whey IgG enriched fraction

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ABSTRACT

Bovine whey IgG enriched fraction contains IgG antibodies against bacterial and viral pathogens, including antibodies against the spike protein [amino acids (aa) 1–1274] of SARS-CoV-2 Wuhan strain (2019-nCoV WHU01). To date, 13 SARS-CoV-2 variants have been identified, including gamma, delta, kappa, and omicron, which contain 10, eight, seven, and over 30 mutations in the spike protein, respectively. We investigated whether bovine whey IgG enriched fraction contains antibodies against spike proteins of these variants, specifically recombinant partial length spike proteins (aa 177–512, aa 509–685, aa 177–324, aa 250–410 and aa 387–516) of these variants. Direct enzyme-linked immunosorbent assays revealed bovine whey IgG enriched fraction contained antibodies against all recombinant spike proteins of these variants with highest reactivity against aa 177–512 region of omicron spike protein. These results indicate bovine whey IgG enriched fraction contains antibodies against spike proteins of several SARS-CoV-2 variants, including omicron.

1. Introduction

Bovine whey IgG enriched fraction of contains antibodies against several bacterial pathogens, as well as rotaviruses, which cause gastrointestinal infections (Ulfman, Leusen, Savelkoul, Warner, & van Neerven, 2018). This fraction also contains antibodies that bind to influenza and human respiratory syncytial viruses (Hartog et al., 2014). Bovine colostrum obtained from cows immunised with antigens of gastrointestinal pathogens has been called "hyperimmunised milk", with high antibody activities against these antigens (Golay, Ferrara, Felber, & Schneider, 1990). Immune cow colostrum was found to shorten the duration of gastrointestinal infections (Ulfman et al., 2018), and milk products containing colostrum derived from healthy non-immunised pasture fed cows provided immunity against *Salmonella* infection in calves (Griffiths, 1969; Royal, Robinson, & Duganzich, 1968). Immunoglobulin preparations from non-immunised cows contained high levels of antibodies and neutralising activity against verotoxin of *Escherichia coli* O157:H7 (Lissner, Schmidit, & Karch, 1996). In addition, bovine whey IgG enriched fraction was found to protect mice against food-borne infections with enterohaemorrhagic *E. coli* O157:H7 and against *Salmonella* associated enteritidis (Funatogawa, Tada, Kuwahara-arai, Kirikae, & Takahashi, 2019).

Bovine colostrum provides a medium for the heterologous transfer of passive immunity and may offer disease protection in a range of species (Hurley & Theil, 2011). There is a potential role of bovine in providing humans with specific antibodies against human viruses (Saied & Metwally, 2019). In some human viruses bovine has contributed to the acquisition of new knowledge to improve human health against viral infections (Saied, Metwally, Mohamed, & Haridy, 2021). Although ingesting bovine colostrum is beneficial to immunity of the human respiratory system, the mechanisms involved remain unknown and further studies are needed (Batista da Silva Galdino et al., 2021). Bovine rotavirus vaccine is sufficient to enhance the anti-human rotavirus protective efficacy of bovine colostrum (Civra et al., 2019). Potential benefits of using bovine-derived antibodies in countering SARS-CoV-2 and its emerging variants and mutants have been reviewed (Saied et al., 2022).

The novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is responsible for the coronavirus

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disease-2019 (COVID-19) pandemic (WHO, 2020). To date, 13 SARS-CoV-2 variants have been identified, including the gamma (P.1), delta (B.1.617.2), kappa (B.1.617.1) and omicron (B.1.1.529) variants. Compared with the original SARS-CoV-2 Wuhan strain, these variants had acquired several amino acid substitutions, deletions and/or insertions in their spike proteins. The spike protein is composed of S1 and S2 subdomains. The receptor binding domain (RBD) of S1 interacts with angiotensin-converting enzyme 2 (ACE2) to enter cells (Walls et al., 2020). Compared with the original Wuhan strain, the gamma, delta, and kappa variants had ten, eight, and seven mutations, respectively, in the S1 subdomain, whereas the omicron variant had more than 30 mutations in the S1 subdomain (Fig. 1) (WHO, 2022). Bovine whey enriched fraction was recently reported to contain antibodies against the spike protein [amino acids (aa) 1–1274] of the original SARS-CoV-2 Wuhan strain (Oshiro et al., 2021). The present study assessed whether these antibodies recognised spike proteins of the SARS-CoV-2 variants, including the gamma, delta, kappa and omicron variants.

2. Materials and methods

2.1. Construction and purification of recombinant SARS-CoV-2 spike proteins

Genes encoding the spike proteins of the SARS-CoV-2 Wuhan (accession no. MN988668) and omicron B.1.1.529 (accession no. EPI_ISL_6640917) variants were synthesised based on their published sequences. Genes encoding the spike proteins of the SARS-CoV-2 gamma (accession no. OK189450.1), delta (accession no. MZ377108.1), and kappa (accession no. MZ571142.1) variants were constructed from the spike protein gene of the SARS-CoV-2 Wuhan strain using a Quickchange site-directed mutagenesis kit

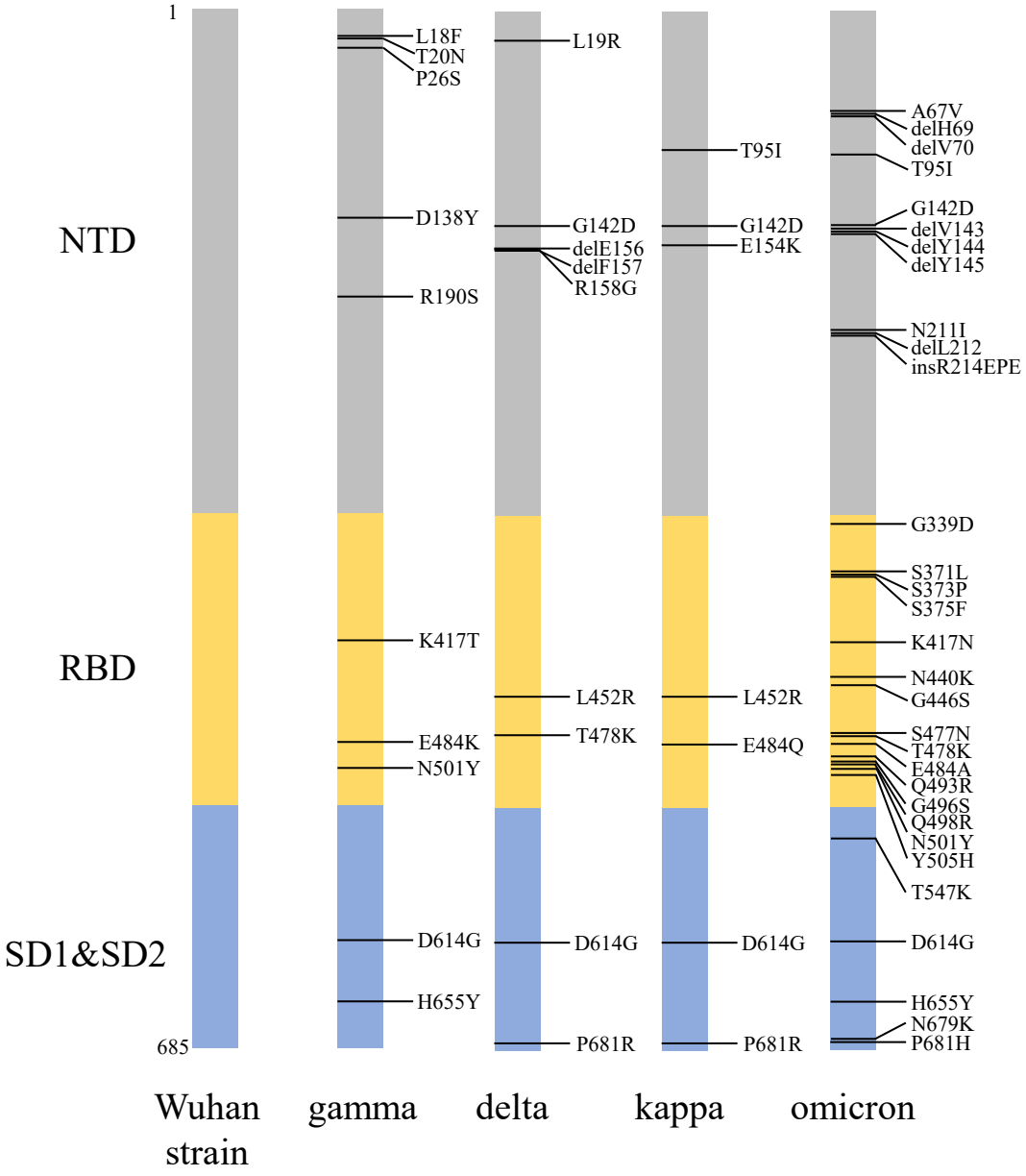

Fig. 1. Topology of the SARS-CoV-2 spike proteins of the original SARS-CoV-2 Wuhan strain and the gamma, delta, kappa and omicron variants and distributions of the amino acid mutations in the spike proteins of the four variants: NTD, N-terminal domain; RBD, receptor binding domain; SD1, subdomain 1; SD2, subdomain 2.

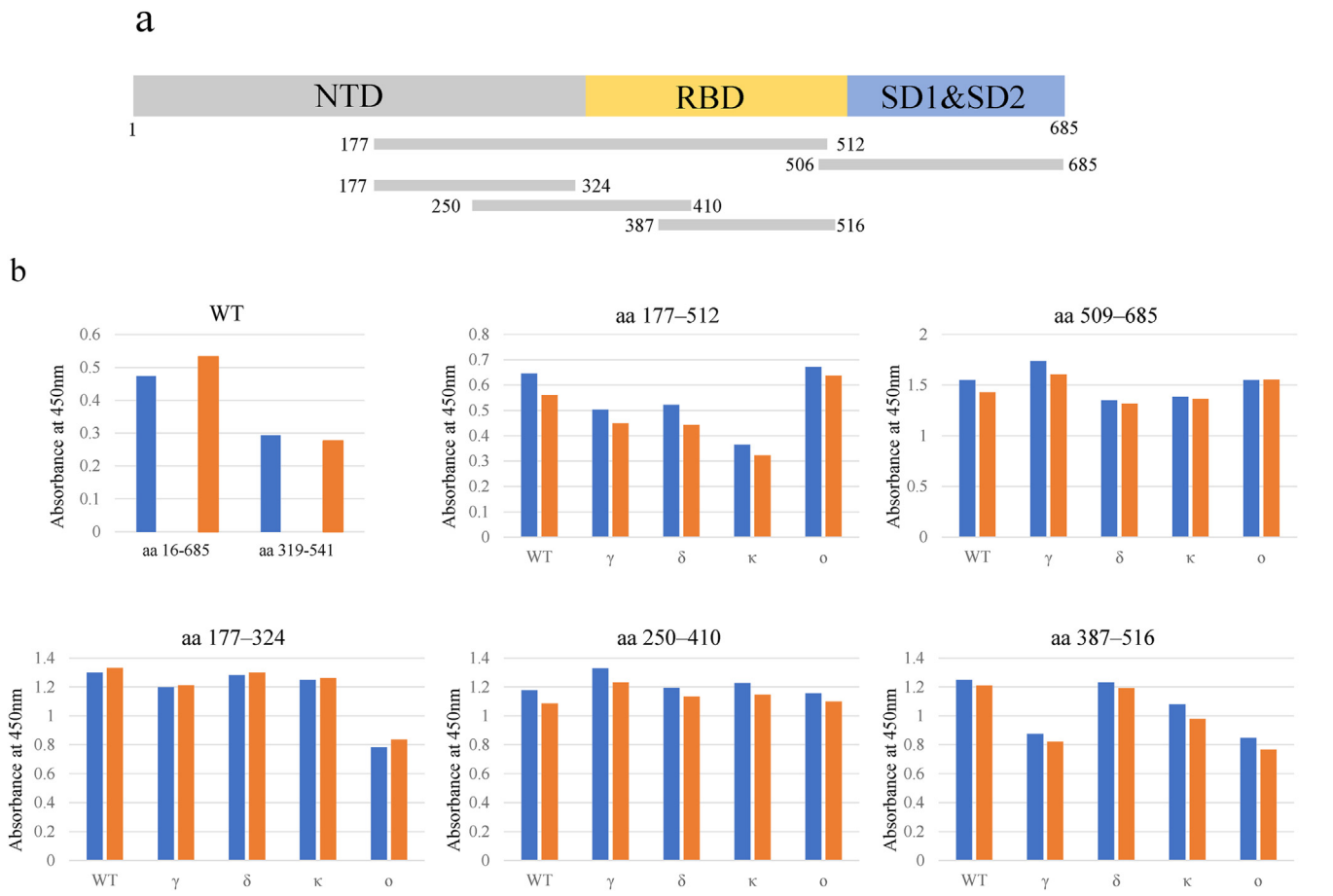

Fig. 2. Bovine whey IgG enriched fraction containing antibodies against the spike proteins of the five SARS-CoV-2 strains: (a) topology of recombinant protein covering S1 subunit (aa 16–685), RBD (aa 319–541) of SARS-CoV-2 Wuhan strain and the five regions of recombinant spike protein (aa 177–512, 509–685, 177–324, 250–410 and 387–516) of the five SARS-CoV-2 strains; (b) two lots (lot A, blue; lot B, orange) of bovine whey IgG enriched fractions that were tested.

(Stratagene, USA), and the primers listed in Supplementary material Table S1. Five sequences encoding each SARS-CoV-2 spike protein variant, consisting of nucleotides (nt) 529–1536, 1525–2055, 529–972, 748–1230 and 1159–1548 and corresponding to amino acids (aa) 177–512, 509–685, 177–324, 250–410 and 387–516, respectively, were cloned into the pET28a expression vector (Novagen, USA). *E. coli* BL21-CodonPlus (DE3)-RIP (Agilent Technologies, USA) was transformed using the constructed plasmids. These five recombinant SARS-CoV-2 spike proteins contained an adjacent region of RBD or an internal region of RBD (Fig. 2a). Recombinant SARS-CoV-2 spike proteins were purified using TALON Metal Affinity Resin, according to the manufacturer's instructions (Clontech Laboratories, USA), and coated onto direct enzyme-linked immunosorbent assay (ELISA) plates. Thus, 25 recombinant spike proteins were tested, consisting of five regions of each of the five variants of SARS-CoV-2 (Fig. 2a). Recombinant protein covering S1 subunit (aa 16–685) and RBD (aa 319–541) of SARS-CoV-2 Wuhan strain were purchased from Sino Biological Inc, USA and used for ELISA.

2.2. Bovine whey IgG enriched fraction

Bovine whey IgG enriched fraction (IgG30^{+}; Aotearoa Co., Tokyo) was obtained from milk of pasture fed, non-immunised healthy New Zealand cows by New Zealand Dairy Group in 2018 and 2019 (Oshiro et al., 2021). Two lots of this fraction (lots A and B) were used in the present study.

2.3. Direct enzyme-linked immunosorbent assays

Direct ELISA assays, using partial-length recombinant SARS-CoV-2 spike proteins as coating antigens, were performed as described (Oshiro et al., 2021).

3. Results and discussion

3.1. Detection of antibodies against spike proteins of SARS-CoV-2 variants

Both lots of bovine whey IgG enriched fraction contained antibodies against recombinant S1 subunit of SARS-CoV-2 spike protein (aa 16-685) and RBD of SARS-CoV-2 spike protein (aa 319–541) (Fig. 2b). Both lots of bovine whey IgG enriched fraction contained antibodies against all the recombinant proteins tested, corresponding to aa 177–512, 509–685, 177–324, 250–410 and 387–531 of the SARS-CoV-2 Wuhan strain and the gamma, delta, kappa and omicron variants, with both lots showing similar immunoreactivity profiles against these recombinant proteins (Fig. 2b). The IgG enriched fractions showed the highest reactivity against recombinant aa 177–512 of the omicron variant and the lowest reactivity against the same region of the kappa strain. The IgG enriched fraction showed similar levels of reactivity against aa 509–685 and aa 250–410 of all five strains, lower reactivity against aa 177–324 of omicron than against aa 177–324 of the other four strains, and higher reactivity against aa 387–516 of the Wuhan and

delta strains than against the same region of the gamma, kappa, and omicron strains.

The findings in this study suggest that pasture-fed healthy New Zealand cows are exposed to viruses that cross react with the spike protein of SARS-CoV-2. The cows from which milk had been collected were likely not infected by SARS-CoV-2 because the two lots of bovine IgG enriched fraction were prepared in November 2018 and August 2019, respectively, which predate the emergence of SARS-CoV-2 that was in December 2019. Although the ability of this virus to infect cows has not been determined, antigen against these antibodies in bovine whey enriched IgG fraction might be more similar to spike protein of the omicron variant than that of the SARS-CoV-2 Wuhan strain.

Bovine coronavirus has been shown to cause respiratory and enteric infections in cows (Saied et al., 2021; Saif, 2010), suggesting that these New Zealand cows were likely infected with bovine coronavirus. However, the amino acid sequence of the spike protein of bovine coronavirus (accession no. AAA66399.1) differed from the amino acid sequences of the spike proteins of the Wuhan and omicron strains of SARS-CoV-2, with homologies of only 29.59% and 29.27%, respectively. The cows may have been infected with several viruses, including bovine coronavirus, and acquired various antibodies against epitopes of SARS-CoV-2 spike proteins from several variants.

Author contributions

SO and NM performed experiment and created the research data. SO and TT wrote the first version of the manuscript. All authors read, made significant edits, and approved the final manuscript.

Conflict of interest

S. J. works for Kohjin Bio Co., Ltd.
M.T. works for Aotearoa Co., Ltd.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2022.105436.

References

Batista da Silva Galdino, A., do Nascimento Rangel, A. H., Buttar, H. S., Lima Nascimento, M. S., Cristina Gavioli, E., de Paula Oliveira, R., et al. (2021). Bovine colostrum: Benefits for the human respiratory system and potential contributions for clinical management of COVID-19. *Food and Agricultural Immunology, 32*, 143–162.

Civra, A., Altomare, A., Francese, R., Donalisio, M., Aldini, G., & Lembo, D. (2019). Colostrum from cows immunized with a veterinary vaccine against bovine rotavirus displays enhanced in vitro anti-human rotavirus activity. *Journal of Dairy Science, 102*, 4857–4869.

Funatogawa, K., Tada, T., Kuwahara-arai, K., Kirikae, T., & Takahashi, M. (2019). Enriched bovine IgG fraction prevents infections with enterohaemorrhagic *Escherichia coli* O157:H7, *Salmonella enterica* serovar Enteritidis, and *Mycobacterium avium*. *Food Sciences and Nutrition, 7*, 2726–2730.

Golay, A., Ferrara, J. M., Felber, J. P., & Schneider, H. (1990). Cholesterol-lowering effect of skim milk from immunized cows in hypercholesterolemic patients. *American Journal of Clinical Nutrition, 52*, 1014–1019.

Griffiths, O. V. (1969). Colostral immunity against salmonella infection in calves. *New Zealand Veterinary Journal, 17*, 50.

Hartog, G. D., Jacobino, S., Bont, L., Cox, L., Ulfman, L. H., Leusen, J. H. W., et al. (2014). Specificity and effector functions of human RSV-specific IgG from bovine milk. *PLoS One, 9*, Article e112047.

Hurley, W. L., & Theil, P. K. (2011). Perspectives on immunoglobulins in colostrum and milk. *Nutrients, 3*, 442–474.

Lissner, R., Schmidit, H., & Karch, H. (1996). A standard immunoglobulin preparation produced from bovine colostra shows antibody reactivity and neutralization activity against Shiga-like toxins and EHEC-hemolysin of *Escherichia coli* O157: H7. *Infection, 24*, 378–383.

Oshiro, S., Tada, T., Mizutani, N., Funatogawa, K., Sekiguchi, J. I., Takahashi, M., et al. (2021). Presence of antibodies against SARS-CoV-2 spike protein in bovine whey IgG enriched fraction. *International Dairy Journal, 117*, 105002.

Royal, W. A., Robinson, R. A., & Duganzich, D. M. (1986). Colostral immunity against salmonella infection in calves. *New Zealand Veterinary Journal, 16*, 141–145.

Saied, A. A., & Metwally, A. A. (2019). Bovine-origin human therapy; need more attention. *International Journal of Current Microbiology and Applied Sciences, 8*, 2766–2770.

Saied, A. A., Metwally, A. A., Alobo, M., Shah, J., Sharun, K., & Dhama, K. (2022). Bovine-derived antibodies and camelid-derived nanobodies as biotherapeutic weapons against SARS-CoV-2 and its variants: A review article. *International Journal of Surgery, 98*, 106233.

Saied, A. A., Metwally, A. A., Mohamed, H. M. A., & Haridy, M. A. M. (2021). The contribution of bovines to human health against viral infections. *Environmental Science and Pollution Research International, 28*, 46999–47023.

Saif, L. J. (2010). Bovine respiratory coronavirus. *Veterinary Clinics: Food Animal Practice, 26*, 349–364.

Ulfman, L. H., Leusen, J. H. W., Savelkoul, H. F. J., Warner, J. O., & van Neerven, R. J. J. (2018). Effects of bovine immunoglobulins on immune function, allergy, and infection. *Frontiers in Nutrition, 5*. Article 52.

Walls, A. C., Xiong, X., Park, Y. J., Tortorici, M. A., Snijder, J., Quispe, J., et al. (2020). Unexpected receptor functional mimicry elucidates activation of coronavirus fusion. *Cell, 176*, 1026–1039.

WHO. (2019). *Coronavirus disease 2019 (COVID-19) situation report*. Geneva, Switzerland: World Health Organisation. https://www.who.int/docs/default-source/coronaviruse/situation-reports.

WHO. (2022). *Tracking SARS-CoV-2 variants*. Geneva, Switzerland: World Health Organisation. https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/.