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Bovine colostrum antibodies against human viral antigens

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ABSTRACT

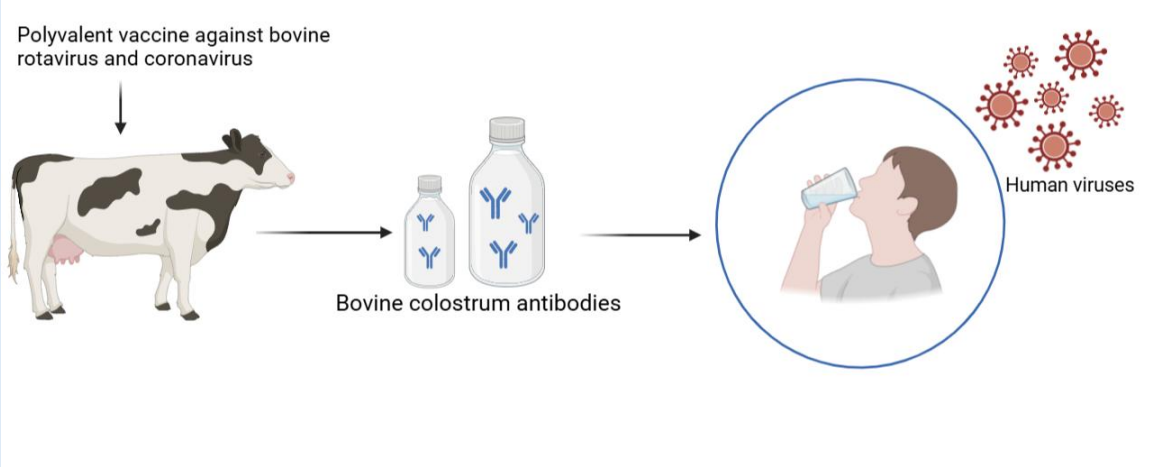
Background: Bovine colostrum (BC) is the first milk produced by a cow postpartum. It is a unique substance rich in antibodies that protects the body against bacterial and viral pathogens. BC significantly contributes to human nutrition and health, as its effectiveness against certain human viruses is well-studied. Locally, cows are vaccinated with a polyvalent inactivated vaccine against viral diarrhea, bovine escherichiosis, bovine rotavirus (BRoV), and bovine coronavirus (BCoV). BC contains high levels of antibodies against these antigens, which provide advanced immune protection to the body.

Methods: The study's goal was to determine whether BC fractions from cows contain antibodies with cross-reactivity against recombinant human proteins represented by receptor-binding domain (RBD) and nucleocapsid (NP) proteins of various strains of human SARS-CoV-2 coronavirus, including the Wuhan, Lambda, Mu, and Omicron variants. This study was conducted using the ELISA method.

Results: Specific antibodies against BRoV and BCoV were detected in significant amounts. The study found that antibodies against all the variants of human coronavirus SARS-CoV-2 studied were present in the BC evaluated. Furthermore, antibodies produced from the Rotateq vaccine, containing human and bovine hybrid rotavirus strains, were present.

Conclusions: Detection of antibodies in the early milk of cows indicates a potential of BC as a food supplement for some human viral diseases due to the presence of conserved epitopes shared by human and bovine rotaviruses and coronaviruses.

Key words: bovine colostrum, antibodies, heterologous immune response, antigens, functional food, antigen



Graphical Abstract: Bovine colostrum antibodies against human viral antigens

INTRODUCTION

Bovine colostrum is produced in the first hours postpartum. BC contains nutrients, regulatory molecules, vitamins, and antioxidants essential for calf nutrition. As this product contains a significant amount of antibodies (up to 50 mg/mL), BC is a heterologous vehicle for passive immunity across species. BC provides the calf with vital nutrients, antioxidants, and vitamins [1-2]. The major immunoglobulins found in colostrum include IgG, IgA, IgM, and IgD isotypes and their subclasses of IgG1-4 and IgA1-2. However, IgG is the predominant type of immunoglobulin in bovine colostrum [3]. Colostrum, milk from humans, and milk from vaccinated animals exhibit similar immunological properties.

Consumption of bovine colostrum from cows immunized against bovine rotavirus has been shown to reduce the severity of intestinal infections caused by human rotavirus [43-54]. Both colostrum and colostrum-derived products can be used as complementary food sources to meet additional metabolic needs that support health. These properties also contribute to controlling viral infections as BV contains many specific antibodies [6-9]. Controlling the pathogenesis of viral infections that present challenges to the health care system will require new approaches [10]. Therefore, using functional foods that provide health benefits may also alleviate symptoms of viral diseases. Functional foods are natural or processed foods containing biologically active compounds that, at certain effective levels, could provide a health benefit beyond basic nutrition. This could be a promising tool for treating human viral infections [11-14].

In Russia, calves are vaccinated with a polyvalent inactivated vaccine against viral diarrhea, bovine escherichiosis, bovine rotavirus, and bovine coronavirus (Combovak-K vaccine). This vaccine contains inactivated coronavirus virions (KV-90 strain), according to Order of the Ministry of Agriculture of the Russian Federation of October 21, 2020, N 622 "On Approval of Veterinary Rules for keeping cattle for reproduction, breeding and sale". Milk from animals immunized with this vaccine must contain high antibodies against the antigens in the Combovak-K vaccine. This study focused on specific antibodies capable of cross-reacting with human viral proteins. Six recombinant proteins of human coronavirus SARS-CoV-2 and the American Rotateq vaccine containing reassorting human and bovine rotavirus strains were selected as potential antigens to investigate this hypothesis.

Since rotavirus has a fragmented genome consisting of eleven segments of double-stranded RNA, hybrids containing proteins from both parental strains are easily formed. This property allows for the use of reassortants as the primary research model. There are five live reassortants in the Rotateq vaccine, all hybrids containing surface proteins from human and bovine rotaviruses. One of the outer capsid proteins, VP7 (serotypes G1, G2, G3, or G4) of the human parental rotavirus, and the VP4 protein (serotype P7[5]) of the bovine parental rotavirus are expressed on the surface of four vaccine reassortants. The VP4 protein is responsible for attachment of the rotavirus to the cell surface. Furthermore, the VP4 protein (serotype P1A[8]) of the human parental rotavirus and the outer capsid protein VP7 (serotype G6) of the bovine parental rotavirus are expressed on the surface of the fifth virus reassortant. According to the manufacturer, the vaccine induces an immune response, which promotes the production of serum neutralizing antibodies against the five capsid proteins of human rotaviruses contained in the vaccine reassortants (G1, G 2, G,3, G 4 and P1A [8]).

MATERIALS AND METHODS

Colostrum samples: The «Pobeda-1» company provided samples 2–6, which were composed of pooled colostrum from various farms. Sample 1 is the lyophilized arrangement of sample 2. To obtain the colostrum fraction, cows were milked six hours postpartum. As birth occurs at different times in different animals, colostrum samples were frozen at -70°C. When pooled together, samples were thawed at +4°C for 12 hours, aliquoted, and then re-frozen for further analysis using the ELISA method.

Sample 1 was diluted with PBS buffer (pH=7.4) to obtain the protein concentration of 50 mg/mL before involvement in the study. A commercial pasteurized milk sample was used as a negative control.

IgG fraction isolation from a pooled bovine colostrum sample: The second milk sample was centrifuged at 4000 g, +4°C, for 30 minutes to remove the fat fraction. Dry ammonium sulfate (Himmed, Moscow, Russia) was added to the final product at 55% saturation, incubated for 18 hours, then 12,000 grams was centrifuged at +4°C for 40 minutes. The sediment produced was redissolved in a phosphate buffer (pH=7.4), diluted 8-fold, and then processed using an ultrasound. The immunoglobulin G fraction was isolated through affinity chromatography. The sample was applied to a protein A Sepharose column (cat. no S-E Paa, Imtek, Moscow, Russia) and pre-equilibrated with phosphate buffer (pH=7.4). The protein

concentration was determined using a NanoPhotometer NP80 spectrophotometer (Implen) at 280 nm against the buffer where the sample was dissolved. The average molar extinction coefficient for IgG was considered in this process. The IgG fraction was stored at -20 °C in 50% glycerol solution (G7893, Sigma-Aldrich).

Vaccines: The polyvalent vaccine Combovac K (Vetbiochem, Russia) and the human rotavirus vaccine "Rotateq" (MERCK SHARP & DOHME, LLC, USA) were used in the study. The Rotateq vaccine contained human and bovine rotavirus reassortants G1, G2, G3, G4, P1[A8].

ELISA for antibody level determination: The inactivated antigen of bovine rotavirus in the production strain No. 101 of the All-Russian Research Institute of Animal Health had an infectious activity (before inactivation on cell culture) of 8 lgTCD50/cm3. A concentration of 8 lgTCD50/cm3 of inactivated bovine coronavirus antigen from the production strain developed by FGBI "ARRIAH" (Russia) was utilized. Before activation, the infectious activity of the virus within the cell culture must be not less than 7 lgTCD50/cm3 (FGBI "ARRIAH", Russia). The recombinant nucleoprotein (NP) of the SARS-CoV-2 strains, Omicron, and the receptor-binding domain (RBD) of SARS-CoV-2 strains Lambda, Mu, and Omicron (cat. no YVV00802, EVV00317, EVV00316, EVV00319, AntibodySystem, Schiltigheim, France) were used in this study. Recombinant RBD and NP proteins of the SARS-CoV-2 strain, Wuhan, were used (cat. no. 8COV1, 8COV3 Hytest; Moscow, Russia).

The BRoV and BCRoV (1:200) antigens, Combovac K vaccine (1:200), "Rotateq" vaccine (1:200), RBDs, and NPs (1 µg/mL, in PBS) were incubated in microplate wells at 4 °C overnight. The wells were washed 4 times to remove unbound molecules, using PBS containing 0.05% v/v Triton X-100 (PBST). Then, colostrum samples were diluted with PBST (1:200–1:2,000,000), and an IgG sample was diluted with PBST (10-0,0001 µg/mL). These samples were then placed into wells, and the microplates were incubated for 1 hour at 37 °C. Following this step, the microplates were washed, and rabbit antibody against bovine immunoglobulins conjugated with horseradish peroxidase (dilution of 1:5000 in PBST, cat. no P-RAB Iss, Imtek; Moscow, Russia) was added to each well. The microplates were incubated for 1 hour at 37°C. After washing the microplates, the enzyme activity of the bound peroxidase was evaluated. A substrate mixture containing 0.4 mM TMB and 3 mM H2O2 in a 40 mM sodium citrate buffer (pH 4.0) was added to evaluate the enzyme activity. Following incubation at room temperature for 15 minutes, the reaction was terminated by adding 1 M H_2SO_4 (Himmed; Moscow, Russia) to the substrate, and the mixture was diluted to a 1:2 (v/v) ratio. Finally, the absorption at A450 nm was measured using an Omega Star microplate photometer (BMG Labtech; Germany). All solutions were prepared using Milli-Q water (Millipore; Burlington, MA, USA).

The results were obtained in three independent assays and expressed as the mean ± SD.

RESULTS

Detection of antibodies in bovine colostrum samples against Combovac K vaccine, bovine rotavirus, and bovine coronavirus: The presence of antibodies was determined by the ELISA method. The results are presented in Figs. 1 and 2 (2a-2b).

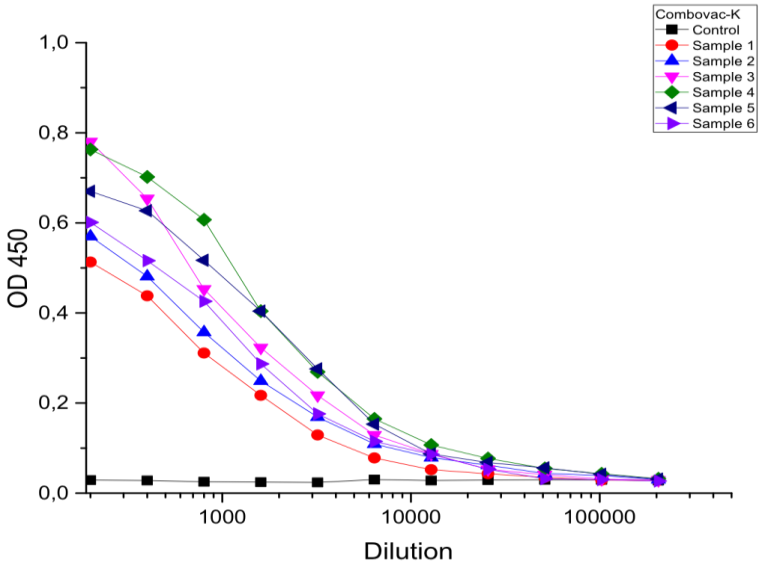


Fig.1 Analysis of pooled colostrum samples from different farms (1:200–1:2,000,000) by the ELISA method. Colostrum samples were titrated with the Combovac K vaccine as the antigen. Data are represented as the mean ±SD (n=3). OD, optical density.

A comparison of the antibody profiles of all pooled colostrum samples revealed that they were comparable, except for the lyophilized sample (sample 1). With respect to the Combovac K vaccine, a high antibody titer was observed in sample 1. The antibody titer ranged from 1:50,000 to 1:200,000 for different samples.

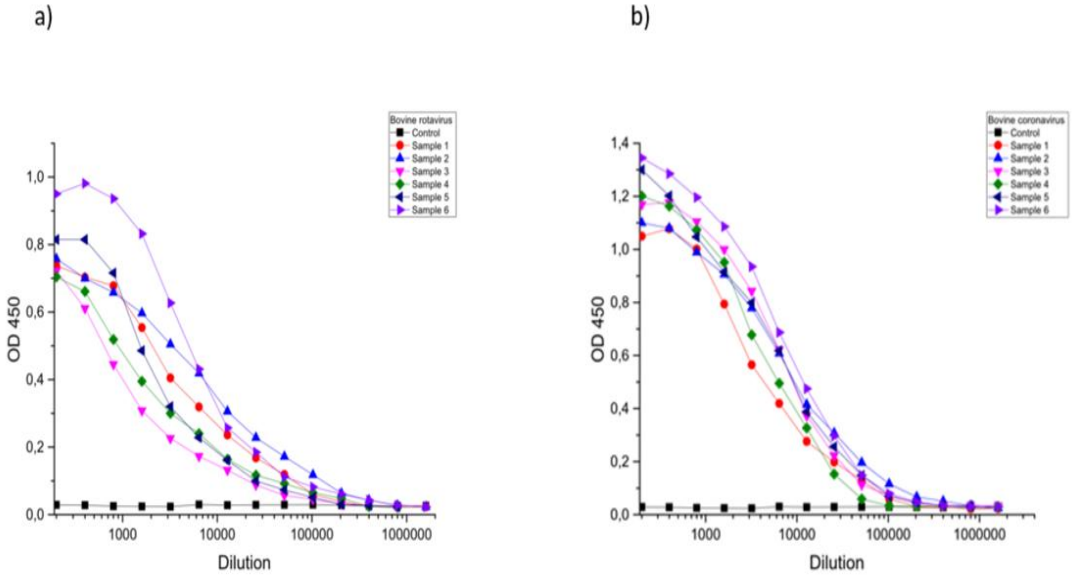


Figure 2. The ELISA method analyzes pooled colostrum samples from different farms (1:200–1:2,000,000). Colostrum samples were titrated on bovine rotavirus (a) and bovine coronavirus antigens (b). Data are represented as the mean ±SD (n=3). OD, optical density. **Figure 2.** All colostrum samples, including lyophilized sample 1, showed antibodies to BRoV and BCoV antigens. The antibody titer ranged from 1:300,000 to 1:1,600,000 for different samples for BRoV and from 1:100,000 to 1:1,600,000 for BCoV.

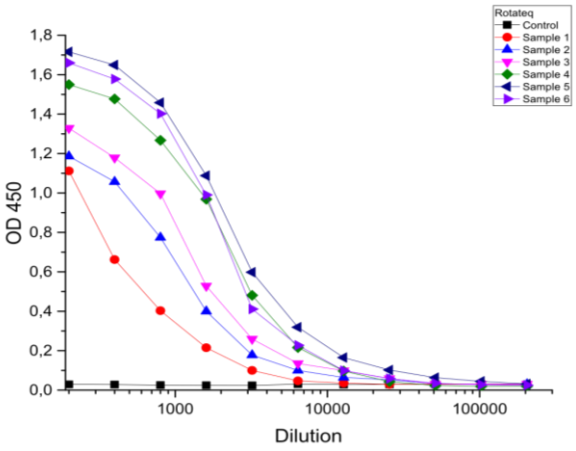


Figure 3. The ELISA method analyzes pooled colostrum samples from different farms (1:200–1:2,000,000). Colostrum samples were titrated on the Rotateq vaccine as the antigen. Data are represented as the mean ±SD (n=3). OD, optical density.

Detection of antibodies in bovine colostrum samples against Rotateq vaccine: All colostrum samples contained antibodies to the hybrid variants of rotavirus in the Rotateq vaccine (Fig. 3).

All the profiles maintained a similar shape. All the samples contained antibodies, whose titers ranged from 1:50,000 to 1:200,000 in different pooled colostrum samples.

Detection of antibodies in bovine colostrum samples against recombinant recNP and recRBD proteins of different SARS-CoV-2 variants: The antibody profiles are presented in Fig. 4 and Fig. 5. All six colostrum samples contained antibodies against recNP SARS-CoV-2 (Wuhan and Omicron) (Fig. 4a, 4b). The titer of antibodies to recNP Omicron ranged from 1:100,000 to 1:2,000,000 for liquid samples. The titer of antibodies to recNP Omicron ranged from 1:25,600 within the lyophilized sample. The titer of antibodies to recNP Wuhan ranged from 1:100,000 to 1:1,000,000 for liquid samples. The titer of antibodies to recNP Wuhan ranged from 1:25,600 for the lyophilized sample.

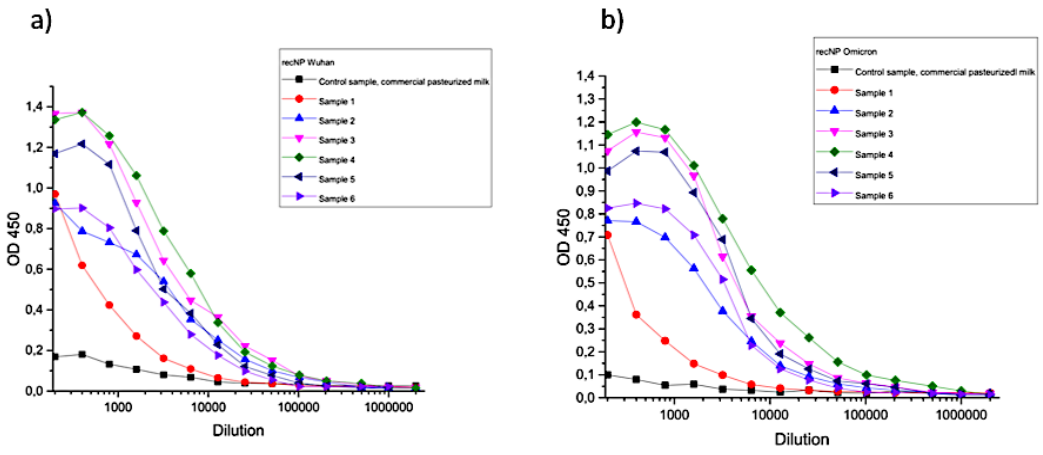


Figure 4. Analysis of pooled colostrum samples from different farms (1:200–1:2,000 000) by the ELISA method. Colostrum samples were titrated on recNP SARS-CoV-2 Wuhan (a) and recNP Omicron (b) antigens. Data are represented as the mean ± SD (n=3). OD, optical density.

Similarly, the presence of antibodies against recombinant RBD proteins of different SARS-CoV-2 strains was tested. It was shown that all colostrum samples contained antibodies against recRBD SARS-CoV-2 (Wuhan, Omicron, Lambda, Mu) (Fig. 5a-5d).

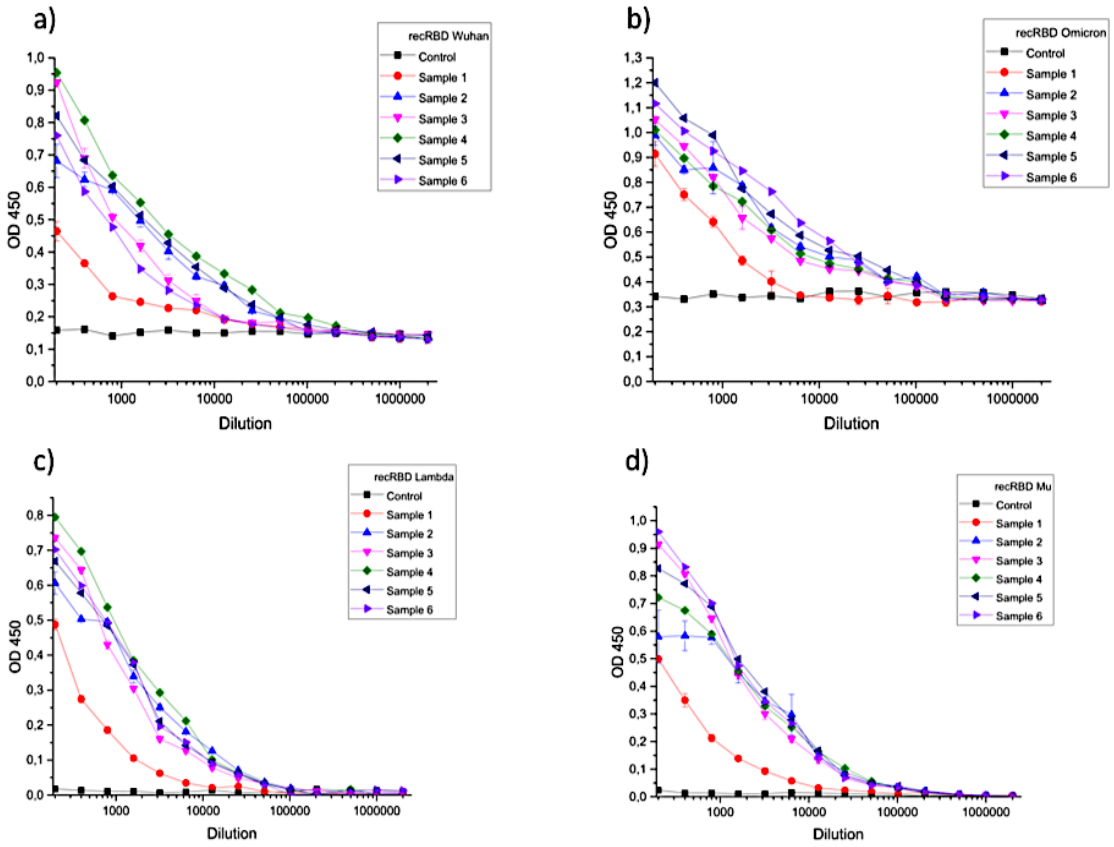


Figure 5. The ELISA method analyzes pooled colostrum samples from different farms (1:200–1:2,000,000). Colostrum samples were titrated on recRBD SARS-CoV-2 Wuhan (a), Omicron (b), Lambda (c), and Mu (d) antigens. Data are represented as the mean ± SD (n=3). OD, optical density.

The titer of antibodies to recRBD Omicron was about 1:150,000 for liquid samples and 1:25,600 for the lyophilized sample. For recRBD Wuhan, titers ranged from 1:200,000 to 1:500,000 and 1:200,000 for the lyophilized sample. For recRBD Lambda, the titer was 50,000 for the lyophilized sample, and 1:100,000-1:150-000 for the liquid sample. For recRBD Mu, the titer was 1:100,000 for the lyophilized sample and 1:500,000 for other samples.

While samples 2-6 did not show a significant difference, they did exhibit similar reactivity profiles against recombinant RBD proteins. Sample 1 of the lyophilized colostrum displayed lower antibodies against recombinant RBD proteins than the liquid samples (Fig. 4). This was also found with NP proteins (Fig. 4).

Isolation of immunoglobulin G fraction from bovine colostrum: The immunoglobulin G (IgG) fraction was isolated from a liquid pooled colostrum sample and tested on recNP and recRBD SARS-CoV-2, Wuhan. The results of the ELISA indicated the presence of IgG antibodies in the bovine IgG fraction isolated from the liquid colostrum sample. This suggests an immune response to SARS-CoV-2 Wuhan, by RBD and NP proteins (Fig. 6).

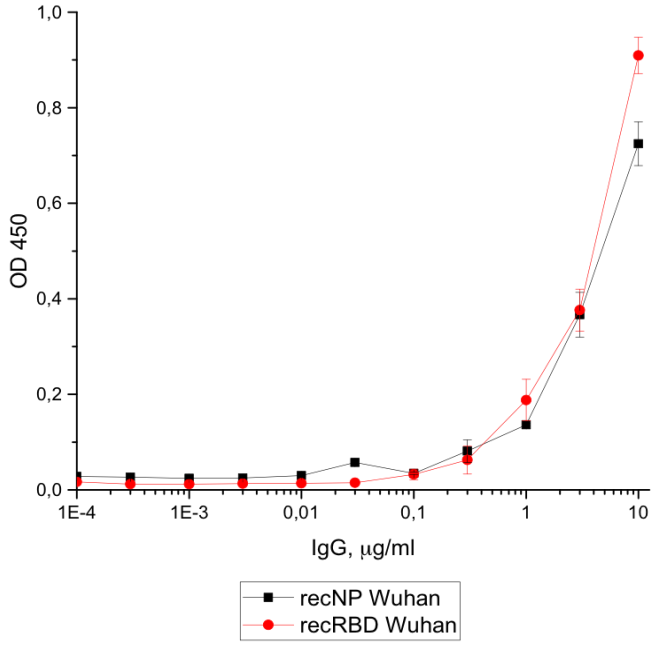


Figure 6. Analysis of IgG sample from the colostrum sample 2, isolated by affinity chromatography (10-0,0001 μg/mL) by the ELISA method. IgG from sample 2 was titrated on recRBD and recNP SARS-CoV-2 (Wuhan) antigens. Data are represented as the mean ± SD (n=3). OD, optical density.

The recNP Wuhan and recNP Omicron samples' antibody curves exhibited similar profiles, suggesting comparable immune potential against both proteins.

DISCUSSION

Steer cows in Russia are routinely vaccinated with the Combovac K vaccine to control veterinary infections. Consequently, bovine colostrum contains significant amounts of antibodies to the bovine rotavirus and bovine coronavirus, which are present in the vaccine. The maximum antibody titer for the bovine vaccine Combovac K was 1:200,000. The maximum titer for individual antigens of bovine rotavirus and coronavirus was 1:1,600,000. This discrepancy can be explained by the low content of individual viral antigens (up to 20%) in the combined bovine vaccine, compared to pure inactivated bovine viruses as antigens. This study confirmed that bovine colostrum contained high antibodies against bovine antigens. It was observed that samples 1,3,4, and 5, as well as samples 2 and 6 of the pooled colostrum, showed similar curve shapes for both recNP Wuhan and recNP Omicron. Samples 3 and 4 displayed the highest optical density, possibly due to the individual immunological characteristics of the animals from farms 3 and 4. Despite the initial optical density values of samples 1, 2, and 6 being similar, the profile of the lyophilized colostrum sample differed significantly from the liquid colostrum samples. The lyophilized colostrum contained a notably lower amount of antibodies. However, this study found that the BC evaluated contained antibodies capable of reacting against human SARS-CoV-2 proteins. These antibodies have been shown to interact with conserved epitopes common to both bovine and human viruses. The epitope sequence alignment of the SARS-CoV-2 spike proteins had been analyzed, and a high degree of similarity (57 to 83%) with BCoV was found [15]. It is plausible that two unrelated viruses may contain the same antigenic peptide, even though the peptide can be found in

unrelated proteins. However, T cells are characterized by recognizing peptides rather than the protein itself. This concurrence may activate memory T cells formed by the virus, not by a protein, but by a peptide. This synergistic effect may also activate memory T cells that form peptides. *In vivo*, this could activate memory T cells formed for the first pathogen when the second pathogen enters the body.

The antibody curves of the recNP Wuhan and recNP Omicron samples exhibited similar profiles, suggesting comparable immune potential against both proteins. These data can conclude that IgG antibodies form the foundation of the antiviral potential of colostrum samples, offering a potential therapeutic effect against human viruses.

The observed cross-reactivity of bovine colostrum antibodies against SARS-CoV-2 is consistent with the findings of previous studies [16-18]. The results of direct enzyme-linked immunosorbent assays (ELISA) demonstrated that the bovine whey IgG-enriched fraction contained antibodies against all recombinant spike proteins and nucleoproteins of the Wuhan, Gamma, Delta, Kappa, and Omicron variants [19-20]. Another study demonstrated that the bovine coronavirus spike protein may provide protective immunity against SARS-CoV-2. In a related study, BALB/c mice vaccinated with bovine coronavirus spike protein exhibited induced cell-mediated and humoral immune responses that were found to cross-react with the SARS-CoV-2 spike protein [21].

Following the entry into the body, antibodies may bind specifically to shared antigenic structures of SARS-CoV-2. This interaction may lead to complete or partial inactivation of the virus. This facilitates the processing of SARS-CoV-2 antigens by the gut-associated lymphoid tissue (GALT). Furthermore, this process promotes the formation of immune complexes that could activate the antiviral signaling cascade, complement fixation, and binding to Fc receptors (FcRs) [22]. These FcRs are crucial for humoral immunity and are essential for an appropriate response to infections. Since coronaviruses are predominantly pneumoenteric viruses, GALT and other mucosa-associated lymphoid tissues are vital in infection control. Reducing active viral particles in the intestinal mucosa and activating other immune mechanisms in the GALT could enhance the specific immune response against SARS-CoV-2 infection [23]. This could improve immune system regulation [23].

This study also revealed the presence of antibodies in the Rotateq vaccine, as all the samples contained antibodies. The lowest antibody titer obtained on the Rotateq vaccine was observed for the lyophilized sample, which was 1:6000. This result was expected due to the technological characteristics of the lyophilization procedure. This procedure is associated with significant destruction and loss of antibodies. The development of technologies in milk processing, using various filtration systems and eliminating high-temperature stages, can potentially prevent substantial losses of proteins and antibodies. This could enable the creation of functional foods with preserved antibody profiles. Since this finding is consistent with the results of other studies, antibodies derived from cows immunized with a veterinary bovine rotavirus vaccine are concluded to show a cross-reactivity to the human rotavirus [24-25]. Bovine and human rotaviruses are highly homologous. The VP7 and VP4 genes of bovine and human rotaviruses were amplified as confirmed by the expected PCR products of 1062bp for the VP7 gene, 856bp for the bovine VP4 gene, and 876bp for the human VP4. The nucleotide sequences obtained following the sequencing of PCR products were subsequently subjected to a sequence analysis, focusing on the VP7 and VP4 genes. This analysis yielded results demonstrating a range of 85% to 99% sequence similarity [26-27]. It was established that the BRoV proteins in the

Rotateq vaccine showed characteristics similar to 94% of human rotavirus strain proteins [28].

A substantial number of studies have demonstrated the efficiency of bovine colostrum in preventing rotavirus infection. In a double-blind, placebo-controlled study, 80 children with rotavirus diarrhea were divided into groups to orally receive 10 grams of immunized bovine colostrum (containing 3.6 grams of antibody) or the same amount of a placebo, for a period of four days. The stool volume, oral rehydration solution consumption, stool frequency, and the presence of rotavirus in the stool for the 4 days during treatment were monitored. The study revealed a substantial enhancement in all parameters [29-30]. However, not all the clinical trials demonstrated unequivocal efficacy of orally administered bovine IgG against human rotavirus infection. Therefore, the level of immune protection against infection does not always correlate with the antibody titer in the pathogen [31].

Although heterologous immune responses may be less effective than specific immune responses against homologous antigens, they can significantly influence the course of infection by enhancing the immune response. In this context, bovine colostrum-based products can be used as an additional source of antibodies. While the presence of antibodies alone may not indicate protection against a pathogen, the results demonstrate that future studies must prioritize the therapeutic potential of bovine colostrum. There is a strong possibility that BC could be used as an additional food supplement to treat some human viral infections.

In this study, several pooled samples from diverse agricultural farms were tested. Furthermore, the lyophilized sample was analyzed against both the same sample in liquid form and other liquid samples. The analysis revealed the presence of substantial amounts of cross-reactive antibodies within all samples. Future research should focus on developing complex preparations containing bovine colostrum antibodies and other antibacterial and antiviral proteins of natural origin. These could include lysozyme or lactoperoxidase. Technological schemes for colostrum processing should also be developed to preserve all its functional properties.

Despite the recent decline in prevalence of severe acute respiratory syndrome from the coronavirus 2 (SARS-CoV-2) infection, various human viruses persist within the human population. New outbreaks are inevitable. Therefore, there is a growing need to find additional means to control human coronavirus infections. The impact of nutrition quality on the severity of the SARS-CoV-2 infection is gaining increasing attention from the medical community. The immune system is under additional stress when the body encounters infectious agents. Consequently, the assumption that a balanced intake of nutrients may facilitate the production of an adequate immune response by the infected patient could lead to an enhanced ability to heal from infection.

CONCLUSIONS

Colostrum and colostrum-derived products and compounds can be used as supplementary food sources to meet additional metabolic needs. These products are enriched with specific antibodies that contribute to the control of some viral infections. Bovine colostrum-based functional foods have proven economical and efficient compared to other treatment methods for various human viral infections. This application is particularly relevant for certain populations who cannot be immunized due to age or existing medical conditions. These populations include premature infants and newborns under six months of age who cannot receive the coronavirus vaccine and rely on breast milk and infant formula for nutrients and immune components that combat infection. The study's results emphasize the necessity of prioritizing the exploration of novel, cost-

effective, and safe therapeutic strategies for treating human viral infections.

Abbreviations: BC: bovine colostrum, BRoV: bovine rotavirus, BCoV: bovine coronavirus, RBD: receptor-binding domain, NP: nucleocapsid, SD: standard deviation, OD: optical density, GALT: Gut-associated lymphoid tissue.

Competing interests: The Authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions: D.S. and M.Y. experimented with and created the research data. N.V., S.M., and A.F. wrote the first version of the manuscript. All authors read, made significant edits, and approved the final manuscript.

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