

Immunogenic activity of viral antigens in the production of environmentally safe serums for the prevention of diseases in young animals

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ABSTRACT

We present the results of optimizing a process for producing polyvalent viral antigens in a passaged MDBK cell culture for the hyperimmunization of production animals. The study aims to improve the production of specific biological preparations for the prevention and treatment of viral infections in young farm animals (including viral diarrhea, rotavirus and coronavirus infections, infectious rhinotracheitis, and bovine parainfluenza-3). The authors determined the optimal parameters for virus propagation, selected a balanced composition for the polyvalent antigen using a 6% aluminum oxide hydrate gel, and established an effective immunizing dose (0.75 mL). The stability of the experimental antigen samples was demonstrated over a 24-month storage period at temperatures ranging from 2 to 8 °C. In experiments on producer animals (producing bulls), the high immunogenicity of the developed preparation was confirmed, resulting in a multiple-fold increase in virus-neutralizing antibody titers. Clinical trials of the resulting hyperimmune serum in neonatal calves demonstrated its high preventive efficacy, which allows us to recommend the developed technology for widespread implementation in veterinary practice to reduce morbidity and mortality in young stock.

Keywords: Immunogenic activity, Viral antigens, Humoral immune response, Antibody titer, Immunization of producers.

Article type: Research Article.

INTRODUCTION

Viral infections in young livestock, characterized by injury to the digestive and respiratory systems, are associated with pathological effects on livestock health and future productivity (Amer *et al.* 2013; Khussainov *et al.* 2024; Hemmatzadeh *et al.* 2025; Sansyzybay *et al.* 2025). Neonatal mortality in calves and lambs is one of the most significant threats to the industry, as it is a multifactorial problem, but neonatal diarrhea is the most common cause (Franzoni *et al.* 2025, Zhu *et al.* 2026). Infectious diarrhea caused by bacterial and viral infections occurs during the first three weeks of life and accounts for 25% of cases, making it the most common form (Gascoigne *et al.* 2017). Many of the infections, such as infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea (BVD), are widespread throughout the world, including the Republic of Kazakhstan. According to serological studies conducted between 2021 and 2022 in four regions (Akmola, Kostanay, Pavlodar, and North Kazakhstan), the total proportion of IBR-seropositive animals exceeded 45% (Omarova *et al.* 2024, Bulatov *et al.* 2025). In South Korea, a study of calves with diarrhea using high-throughput sequencing identified 10 different viruses (BAstV, BEV,

BCoV, BNeV, BNoV, BooV, BParV, BToV, CSpV1, and hunniavirus; Chae *et al.* 2025). Bovine enterovirus (genus *Enterovirus*, family Picornaviridae) has been reported to be associated with diarrhea (Li *et al.* 2012). Bovine enterovirus (BEV) causes both diarrhea and respiratory symptoms in cattle (Blas-Machado *et al.* 2011). The causative agent of bovine viral diarrhea (BVDV) is a virus that is spread worldwide. BVDV is one of the few viruses capable of crossing the placenta and infecting the fetus during pregnancy. BVDV can cause a wide range of clinical symptoms, from mild fever and diarrhea to severe respiratory disease, hemorrhagic lesions, reproductive disorders, and fatal mucosal disease (MD) in persistently infected animals (Lanyon *et al.* 2014). To counteract the significant impact of BVDV, several countries have implemented control programs, which are structured, long-term efforts aimed to eradicate the virus in cattle populations (Moennig *et al.* 2018). Rotavirus A (RVA) and bovine coronavirus (BCoV) are two well-known and common viral causes of diarrhea in newborn calves. RVA is a group of segmented double-stranded RNA viruses that can infect a wide range of hosts, including humans. In calves, they are considered a major intestinal pathogen and often cause diarrhea in calves under two weeks of life (Cho *et al.* 2014). BCoV is a single-stranded RNA virus associated with both gastrointestinal and respiratory diseases in cattle (Panciera *et al.* 2010; Desselberger 2014; Amer 2018; Vlasova *et al.* 2021). One of the most serious diseases of cattle worldwide is bovine respiratory disease complex (BRDC), whose pathogenic complexity and economic threat have been the focus of veterinary epidemiological research. The most commonly detected viruses in BRDC are bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus type 3 (BPIV3), bovine alpha herpesvirus 1 (BoAHV-1), and bovine viral diarrhea virus (BVDV; Hay *et al.* 2016). One of the most effective methods for the emergency prevention and treatment of viral infections in young animals is the use of specific sera and immunoglobulins (Tutkyshbay *et al.* 2025; Khussainov *et al.* 2025). In this regard, the production of high-quality viral antigens is a key step in the technology for manufacturing hyperimmune biological preparations. The development of specific prophylactic measures for infectious diseases in young livestock is impossible without the use of effective methods for the production of viral biomass. The effectiveness of the technology for its production is determined by the cell model used, the virus strain, the method of cell and virus cultivation, the conditions of their growth, as well as a number of other factors that are particularly important in the production of inactivated antigens (Krykbayev *et al.* 2024; Uzar *et al.* 2025). Despite significant progress in the field of cell biotechnology, obtaining viral raw materials from passaged cell cultures for the production of antiviral drugs has so far been fraught with a number of difficulties. Only a few animal cell lines (BHK-21/13, PK-15, MDBK, etc.) are adapted to highly productive suspension culture, and their sensitivity spectrum is highly specific, which does not always allow existing needs to be fully met (Alfano *et al.* 2020; Mukhammadiev *et al.* 2025). Currently, cell-based virus cultivation is considered the most effective method for producing virus-containing biomass with a low ballast protein content suitable for the production of biopharmaceuticals. However, one of the significant problems with this technique is the contamination of cell cultures with bacteria, certain fungi, including yeast and mold, which cause culture medium turbidity without causing cytopathic effects. In addition, stable cell lines undergoing long-term passaging under standard conditions are often contaminated with mycoplasma (Urban-Chmiel *et al.* 2014). One of the key factors in obtaining viral biomass for the production of inactivated antigens is the selection of virus strains with high immunogenicity. For example, the supercapsid shell of virions largely determines the virulence and immunogenic properties of herpesviruses. The presence of at least 10% virions with a supercapsid shell in the initial suspension used to produce an immunogenic preparation of inactivated cattle epizootic virus is sufficient to induce the synthesis of virus-neutralizing antibodies in animals (Abd El Fadeel *et al.* 2022). When developing a technology for producing viral biomass, it is necessary to consider a set of technological parameters related to the selection of optimal virus cultivation parameters. This requires examination of the effect of cell culture age, inoculation concentration and cell infection conditions, medium composition and its use in the cultivation process, as well as the role of serum, various stimulants and antibiotics, pH level, cultivation temperature and other factors on virus accumulation (Hasoksuz *et al.* 2007). Despite a significant number of studies devoted to individual cattle viruses, the development of a comprehensive technology for producing polyvalent viral antigens intended for the hyperimmunization of producer animals in the manufacture of hyperimmune sera remains insufficiently studied. Of particular interest is the optimization of culture conditions for multiple viruses in a unified cell system, followed by the selection of an optimal composition for a polyvalent antigen that exhibits high immunogenicity and storage stability. The aim of this study was to optimize the technology for producing polyvalent viral antigens in MDBK cell cultures for the hyperimmunization of producer animals in the production of serum intended for the prevention of viral diseases in young cattle.

To achieve this objective, the following tasks were addressed:

1. To study the patterns of viral accumulation as a function of cell seeding concentration, infectious dose, and duration of cultivation
2. To investigate the effect of physicochemical parameters (temperature, pH, and nutrient medium composition) on the reproductive activity of viruses in MDBK cell culture
3. To optimize the parameters for obtaining polyvalent viral antigens with high immunogenic properties for use in the production of hyperimmune sera.

MATERIALS AND METHODS

Study site

The study was conducted in 2026 at the “Biogenic Preparations” laboratory of the “Zhalyn” Scientific and Technical Research Center LLP (Almaty, Republic of Kazakhstan). All work with infectious materials was performed in a Class II biological safety cabinet in accordance with BSL-2 biosafety requirements, using personal protective equipment.

Viral strains and cell cultures

The study utilized reference strains of viruses that cause the major viral diseases of cattle:

- Bovine viral diarrhea virus (BVDV): strains NADL (cytopathogenic, genotype 1), Oregon C24V, and Singer;
- bovine rotavirus (BRVA): strain TM-91 (serotype G6P1), as well as reference serotypes G6 and G10;
- bovine coronavirus (BCoV): strain C-197 and the Hansen isolate;
- bovine alpha-herpesvirus type 1 (BoAHV-1/IRT virus): strains LA (Los Angeles), Clorado, and PI;
- bovine parainfluenza-3 virus (BPI3V): SF-4 (Reisinger).

For virus propagation, we used a subcultured MDBK (Madin-Darby Bovine Kidney) cell line of 75–80 passages, obtained from the Republican Collection of Microorganisms (Astana, Republic of Kazakhstan).

Determination of cell concentration and viability

Cell concentration and viability were determined by staining with a 0.5% trypan blue solution followed by cell counting in a Goryaev chamber. The cell suspension was mixed with the dye in a 1:1 ratio and incubated for 1–2 minutes at room temperature. Unstained (viable) cells were used to standardize the density prior to viral infection.

Cell and virus culture conditions

MDBK cells were cultured in Eagle’s Minimum Essential Medium (MEM) with L-glutamine at a pH of 7.5–7.6, a temperature of 37 °C, and 5% CO₂ in the atmosphere. For passaging, the following were used: Medium 199, balanced Hank’s solution, a 0.5% lactalbumin hydrolysate solution, trypsin solutions (0.2% and 0.3%), Versen solution (0.02%), and bovine serum.

Virus replication was carried out on an established MDBK monolayer with 80–90% confluence. Cultivation parameters were selected based on the biological characteristics of each virus:

- BoAHV-1: The virus was added to a 24–48-hour-old monolayer at an infection titer of 0.1–1.0 TCID₅₀/cell. After adsorption for 45–60 minutes at 37 °C, the cells were cultured in a maintenance medium containing 1–2% serum. Viral material was harvested when cytopathic effects developed in at least 80% of the cell monolayer.
- BPI3V: Infection was performed simultaneously with cell seeding. Viral replication was monitored by the formation of characteristic multinucleated symplasts.
- BVDV: A 24-hour-old MDBK monolayer was used. The development of cytopathic effects was observed 48–72 hours after infection.
- BRVA: Prior to infection, the viral suspension was activated with trypsin at a concentration of 5–10 µg mL⁻¹ at 37 °C for 30–45 minutes. After activation, the virus was applied to a monolayer of MDBK cells that had been pre-washed to remove serum; further cultivation was carried out in serum-free MEM medium supplemented with trypsin at a concentration of 0.5–1.0 µg mL⁻¹.
- BCoV: proteolytic enzymes were added to the culture medium. The adsorption period lasted 60–90 minutes, after which the cells were incubated until the appearance of characteristic cytopathic effects, which were observed after 3–5 days.

Each virus was cultured separately at 37 °C and a medium pH of 7.2–7.4. Viral material was harvested during the period of maximum infectious titer.

Optimization of virus cultivation conditions

Virus cultivation processes were optimized through a comprehensive assessment of the effects of cell monolayer age, cell seeding density, multiplicity of infection, the composition of growth and maintenance media, medium pH, temperature, cultivation duration, and passage number.

Determination of viral infectious activity

Viral infectious activity was determined by titration in MDBK cell culture, with calculation of the 50% tissue cytopathic dose (TCID₅₀). Serial 10-fold dilutions of viral suspensions (10^{-1} – 10^{-8}) were added to 96-well plates containing a pre-formed monolayer of MDBK cells at a volume of 0.1 mL per well. After incubation at 37 °C in a 5% CO₂ atmosphere for 3–6 days, the development of cytopathic effects was assessed. Virus titers were calculated using the Reed–Munch method and expressed in lg TCID₅₀ mL⁻¹.

Virus inactivation

Virus suspensions were inactivated using a 37% formalin solution. For BoAHV-1, the exposure time was 72 hours at 37 °C; for BVDV, BRVA, BCoV, and BPI3V, it was 24 hours at the same temperature. The completeness of inactivation was verified by four consecutive passages of the treated material through a susceptible MDBK cell culture. Inactivation was considered complete when there were no signs of viral replication or cytopathic effects in any of the passages.

Sterility control

Sterility control of viral materials and experimental antigens was performed at all stages of the study in accordance with the requirements of GOST 28085, “Biological Preparations: Methods for Bacteriological Sterility Control.”

Safety assessment of experimental antigens

The safety of the experimental antigens was studied in laboratory animals (white mice and guinea pigs), as well as in naturally susceptible animals—cattle. The preparation was administered subcutaneously to guinea pigs at a dose of 1.0 mL and intramuscularly to cattle at a dose of 4.0 mL. The clinical condition of the animals was assessed daily for 10 days following administration of the preparation, considering their general condition, body temperature, appetite, local reaction at the injection site, and possible side effects.

Determination of specific activity

The immunogenic activity of the experimental antigens was assessed based on the levels of specific antibodies in the animals' blood serum. For serological monitoring, the neutralization test (NT) and the hemagglutination inhibition test (HI) were used, both performed according to standard procedures.

Statistical analysis

All experiments were performed in at least three independent replicates. Quantitative data are presented as the mean and standard deviation ($M \pm SD$). The normality of the distribution was tested using the Shapiro-Wilk test. To assess between-group differences, one-way analysis of variance (ANOVA) was used, followed by Tukey's post hoc test. When the data were not normally distributed, the nonparametric Mann-Whitney U test was used. Statistical analysis of the results was performed using SPSS Statistics 20/0 (IBM Corp., USA) and GraphPad Prism 6.01 (GraphPad Software Inc., USA). Differences were considered statistically significant at $p < 0.05$.

RESULTS

During the first stage, the technological parameters for the replication of BVDV, BRVA, BCoV, BoAHV-1, and BPI3V viruses in MDBK cell culture were optimized. It was established that optimal conditions for viral biomass accumulation are achieved using a 24- to 48-hour-old cell monolayer, an infectious dose of 0.1 lg TCID₅₀ mL⁻¹, and incubation at 37 °C in a serum- and glutamine-free maintenance medium (pH 7.0–7.2). Virus-containing material was harvested upon the development of pronounced specific cytopathic effects in 75–80% of the cell monolayer. To develop the optimal composition of the polyvalent antigen, five experimental formulations with different ratios of viral components and adjuvant (6% aluminum oxide hydrate [AOH] gel) were prepared and tested. Antigenic activity was assessed based on the level of humoral immune response in laboratory animals (guinea pigs, $n = 10$) 21 days after a single subcutaneous injection of 0.5 mL. The results of the studies are presented in Tables 1 and 2. Therefore, all studied antigen compositions were characterized by high immunogenic activity against the viral components of BVDV, BRVA, BCoV, BoAHV-1, and BPI3V. The immunogenic activity of the first four vaccine variants was practically identical for all components. At the same time, the fifth vaccine

variant showed a decrease in immunogenic activity, which is probably related to an increase in the aluminum oxide hydrate content to 30%, due to its excessive concentration. The dose-dependent immunogenicity of the experimental vaccines was evaluated in guinea pigs. The animals, divided into groups similar in age and weight ($n = 10$ in each group), were immunized with the prepared antigen (composition 3) by a single subcutaneous injection at doses of 0.25, 0.5, and 1.0 mL. Intact animals were used as controls. Blood samples were collected on the 21st day after immunization. The guinea pig blood serum obtained was tested in a neutralization reaction. The results of the studies are presented in Table 3.

Table 1. Composition of polyantigens ($n = 10$, $p < 0.05$).

Component	Titer	Proportion (% vol.)				
		lg TCID ₅₀ cm ⁻³	Composition 1	Composition 2	Composition 3	Composition 4
BVDV	7.5-8.0	-	-	20	25	-
	8.0-8.5	-	15	-	-	10
	8.5-9.0	5	-	-	-	-
BRVA	7.5-8.0	-	-	15	-	25
	8.0-8.5	10	-	-	20	-
	8.5-9.0	-	10	-	-	-
BCoV	7.5-8.0	-	-	-	15	20
	8.0-8.5	20	15	-	-	-
	8.5-9.0	-	-	10	-	-
BoAHV-1	7.5-8.0	25	25	-	-	-
	8.0-8.5	-	-	15	-	10
	8.5-9.0	-	-	-	5	-
BPI3V	7.5-8.0	30	20	-	-	-
	8.0-8.5	-	-	20	10	-
	8.5-9.0	-	-	-	-	5
Al(OH) ₃ gel		10	15	20	25	30

Table 2. Antigenic activity of different polyantigenic compositions ($n = 10$, $p < 0.05$).

Composition	Antibody titer (M ± SD)				
	BVDV	BRVA	BCoV	BoAHV-1	BPI3V
1	86.8 ± 6.4	102.5 ± 10.3	93.8 ± 7.8	94.6 ± 7.8	98.6 ± 4.6
2	99.6 ± 8.5	113.2 ± 11.6	102.4 ± 11.2	102.0 ± 8.4	86.2 ± 5.2
3	102.6 ± 9.2	116.8 ± 14.6	117.1 ± 10.6	116.4 ± 9.2	95.4 ± 8.4
4	97.2 ± 7.1	111.3 ± 10.5	98.6 ± 8.4	105.7 ± 11.3	92.0 ± 9.7
5	74.6 ± 5.6	94.6 ± 9.2	78.5 ± 8.2	86.3 ± 7.1	78.6 ± 4.6
Control	-	-	-	-	-

Table 3. Dose dependence in antigenic activity of polyantigenic composition ($n = 10$, $p < 0.05$)

Dose (mL)	Antibody titer (M ± SD)				
	BVDV	BRVA	BCoV	BoAHV-1	BPI3V
0.25	67.6 ± 4.7	74.5 ± 6.7	75.4 ± 6.2	79.4 ± 6.7	69.3 ± 7.5
0.5	89.3 ± 7.1	98.2 ± 6.1	96.5 ± 8.4	94.5 ± 9.1	93.5 ± 6.4
0.75	106.4 ± 8.5	106.8 ± 8.6	101.6 ± 9.5	105.3 ± 4.5	102.5 ± 6.1
1.0	88.6 ± 7.6	83.2 ± 7.8	89.3 ± 7.5	85.1 ± 7.4	87.3 ± 8.2
Control	0	0	0	0	0

Analysis of the data presented in Table 3 shows that the 0.25 mL antigen dose is insufficient for the immune response to BVDV, BRVA, BCoV, BoAHV-1, and BPI3V viruses. When doses of 0.5 and 1.0 mL were administered, antibody formation was observed at comparable levels. However, the use of a 1.0 mL dose is considered unsuitable, as it results in excessive antigenic burden for the animals and overuse of the drug components. The most significant immune response was observed using a 0.75-mL dose. The development and veterinary application of new polyspecific sera, in addition to ensuring the safety of antigens for hyperimmunization, requires the preservation of their antigenic and immunogenic properties during storage, which determines the effectiveness of the immunization of animals. Therefore, a secondary objective of the study was to examine the antigenic and immunogenic activity of the preparations during storage and to determine their shelf life. To solve these problems, experimental samples of antigens, previously tested for basic quality indicators (harmlessness, antigenic and immunogenic activity), were stored for 24 months at a temperature range of 2 °C to 8 °C. During storage, monthly visual examinations of the preparations were carried out to identify possible changes in appearance and the degree of resuspension of the sediment. The antigenic activity of the tested

preparations was assessed immediately after production, as well as dynamically during storage at 6-month intervals, based on the level of antibodies in the blood serum of immunized laboratory animals. Guinea pigs ($n = 5$) were used as a model. Guinea pigs were immunized with experimental antigen samples with different storage periods via a single subcutaneous injection at a dose of 0.5 mL. After 21 days of immunization, blood was collected from the animals, and the serum was tested in a neutralization reaction for the presence of virus-neutralizing antibodies to BVDV, BRVA, BCoV, BoAHV-1, and BPI3V viruses. The results of the studies are presented in Table 4.

Table 4. Polyvalent antigen activity dynamics during storage time ($n = 5$, $p < 0.05$).

Virus type	Antibody titer ($M \pm SD$)				
	Storage time				
	Initial	6 months	12 months	18 months	24 months
BVDV	97.2 $\pm$ 8.6	93.4 $\pm$ 8.4	87.5 $\pm$ 8.6	65.2 $\pm$ 7.1	43.4 $\pm$ 5.2
BRVA	104.3 $\pm$ 9.5	98.7 $\pm$ 9.5	86.3 $\pm$ 7.5	64.8 $\pm$ 6.5	38.7 $\pm$ 2.9
BCoV	114.5 $\pm$ 10.3	106.7 $\pm$ 10.1	93.8 $\pm$ 7.8	75.1 $\pm$ 6.3	47.3 $\pm$ 3.8
BoAHV-1	118.7 $\pm$ 10.1	104.9 $\pm$ 9.7	97.1 $\pm$ 8.7	82.9 $\pm$ 6.7	51.4 $\pm$ 5.7
BPI3V	106.3 $\pm$ 9.7	97.8 $\pm$ 8.2	88.6 $\pm$ 7.4	73.4 $\pm$ 6.1	43.1 $\pm$ 5.5

Based on the data in Table 4, immunizing guinea pigs with the polyvalent antigen throughout the storage period at temperatures range from 2 °C to 8 °C led to the formation of antiviral antibodies against the experimental antigens. The highest antibody levels were observed immediately after the antigen was produced and during the first 12 months of storage. By the 18th month, antibody titers had decreased, but remained sufficiently high in all animals in the experimental group. By the 24th month of storage (observation period), the decrease in antibody titers was significant and for individual antigenic components was 2–3 times. An important indicator of antigen efficacy is the ability to induce high levels of antibodies to all components of the antigen. Animal studies allow the determination of optimal doses and immunization schedules. The primary objectives of the research were to evaluate the humoral immune response and determine the immunizing doses of antigens. Experimental samples of antigens were produced in accordance with the proposed technology and tested for all quality indicators, including sterility, complete virus inactivation, safety, and antigenic activity in lab animals. To assess safety, the test samples were administered once subcutaneously to 2–3-year-old male calves at a dose of 4.0 mL. The experiment used 10 animals. The calves were observed for 10 days, during which time all animals remained clinically healthy throughout the observation period. Antigen was administered subcutaneously ten times with an interval of 21 days between the first and second injections, and then with an interval of 7 days, in increasing doses from 5.0 to 20.0 mL (5–6–7–8–10–12–14–16–18–20 mL). After the tenth administration, blood was collected and serum was tested for antibodies to BVDV, BRVA, Bo, BoAHV-1, and BPI3V using a neutralization test (Table 5).

Table 5. Antigen activity in producing animals ($n = 10$, $p < 0.05$).

Virus type	Antibody titer ($M \pm SD$)				
	Days of immunization				
	1	21	35	45	60
BVDV	044.7 $\pm$ 2.5	152.8 $\pm$ 10.6	483.4 $\pm$ 24.6	827.9 $\pm$ 53.6	
BRVA	038.5 $\pm$ 2.4	164.9 $\pm$ 10.2	457.6 $\pm$ 21.9	947.6 $\pm$ 57.9	
BCoV	043.6 $\pm$ 3.7	148.4 $\pm$ 9.3	487.6 $\pm$ 27.4	886.1 $\pm$ 59.2	
BoAHV-1	041.7 $\pm$ 3.6	146.8 $\pm$ 8.9	507.4 $\pm$ 28.3	1004.7 $\pm$ 64.9	
BPI3V	044.9 $\pm$ 4.1	158.5 $\pm$ 10.6	501.9 $\pm$ 29.2	1007.2 $\pm$ 64.3	

Based on the data in Table 5, it's clear that the animals had a strong immune response to the antigen. The analysis showed significant increase in antibody levels, which confirmed the optimal doses of antigen selected for its viral components. Newborn calves from unvaccinated cows remain unprotected against a number of viral and bacterial pathogens. In this regard, the administration of serum containing ready-made antiviral antibodies to calves is practically important for the development of passive immunity in young animals. To evaluate the level of passive immunity, calves in two groups ($n = 10$) were administered serum intramuscularly twice: initially on the second day after birth, and then 2–3 days later at a dose of 10 mL. Blood samples were taken before the administration of serum, as well as on the 7th, 14th, and 21st days of life. Calves of similar age not administered with serum were used as controls. The blood samples were tested for antibodies to BVDV, BRVA, BCoV, BoAHV-1, and BPI3V using neutralization assays (Table 6). The data obtained support the recommendation of double prophylactic intramuscular administration of serum in the first 2–3 days of life to prevent viral diseases in young cattle.

Table 6. Preventive serum activity in calves (n = 10, *p* <0.05).

Calves' age (days)	Antibody titer (M ± SD)				
	BVDV	BRVA	BCoV	BoAHV-1	BPI3V
Experimental					
1	12.3 ± 1.18	16.8 ± 1.45	13.5 ± 1.43	12.7 ± 7.6	8.6 ± 0.32
7	87.4 ± 8.5	92.3 ± 9.4	102.4 ± 10.5	108.1 ± 10.4	83.7 ± 7.2
14	63.4 ± 5.1	67.5 ± 4.7	76.4 ± 6.5	87.3 ± 6.5	64.7 ± 3.9
21	43.6 ± 2.7	42.9 ± 3.8	53.8 ± 4.9	62.1 ± 5.5	43.9 ± 4.7
Control					
1	11.4 ± 2.5	28.6 ± 2.7	12.8 ± 1.4	14.7 ± 0.7	19.3 ± 0.8
7	5.1 ± 0.3	12.1 ± 1.4	6.3 ± 0.4	5.9 ± 0.3	4.3 ± 0.2
14	0	0	0	0	0
21	0	0	0	0	0

DISCUSSION

Infection caused by BVDV can be acute or chronic, with animals with persistent infection (PI) serving as the primary reservoir that maintains the virus's presence in the cattle population. Calves and young cattle are more susceptible to severe forms of the infection, including severe diarrhea, dehydration, thrombocytopenia, and high mortality (Agustono *et al.* 2026). Bovine Respiratory Disease Complex (BRDC) and neonatal calf diarrhea (NCD) are the two most important syndromes responsible for the majority of calf mortality. They have a complex etiology involving several viral agents, including bovine coronavirus (BCoV; Socha *et al.* 2026). In nine provinces of the Eastern Anatolia region, where cattle breeding is most intensive in Turkey, PCR analysis of samples collected from 500 calves with neonatal diarrhea revealed viral agents in 44% of cases, bacterial agents in 28%, and protozoa in 28%. The studies identified the following pathogens: 10.8% bovine coronavirus, 11.6% bovine rotavirus, 6.6% *Eimeria zuernii*, 14% *Cryptosporidium*, 1.4% bovine torovirus, 5.8% bovine norovirus, 3.2% BVDV, 2.4% *Salmonella* spp., 17% *E. coli* K99, and 1.4% *C. perfringens* (Erdoğan *et al.* 2025). Studies conducted in various countries around the world to investigate the role of viral associations in the pathology of newborn calves indicate their widespread prevalence, including in Kazakhstan (Bashenova *et al.* 2025, Karabassova *et al.* 2026). To immunize production animals for the production of hyperimmune sera, we developed a polyvalent antigen that was tested for sterility, complete inactivation of viruses, safety, and antigenic activity in laboratory animals. Aluminum hydroxide was used as an adjuvant. The results of the tests showed that the experimental samples of the polyvalent antigen are sterile, safe, and possess pronounced antigenic activity against the viral components of BVDV, BRVA, BCoV, BoAHV-1, and BPI3V. At the same time, it was found that in antigen variants containing 30% aluminum hydroxide, antigenic activity was lower compared to samples containing 20% aluminum hydroxide; furthermore, the increased concentration of aluminum hydroxide led to its inefficient use. When developing and introducing an immunizing antigen into clinical practice, one of the key requirements is the stability of its physicochemical parameters, as well as its antigenic and immunogenic properties, throughout its entire shelf life, which determines the effectiveness of its use. During experiments on laboratory animals, the optimal storage period for the antigen was established as 18 months at a temperature between 2 °C and 8 °C. The next stage of the research involved testing an experimental batch of the inactivated polyvalent antigen on livestock. The antigenic activity of the viral components, the optimal immunization intervals, and the immunizing dose of the experimental antigen were determined in experiments on young bulls. The results of the studies showed that the highest titers of virus-neutralizing antibodies against BVDV, BRVA, BCoV, BoAHV-1, and BPI3V were observed in producer animals following a 10-dose subcutaneous immunization regimen with a 21-day interval between the first and second doses and 7-day intervals for subsequent doses, administered in doses ranging from 5.0 to 20.0 mL according to an ascending schedule (5–6–7–8–10–12–14–16–18–20 mL). Blood sera obtained from the producer animals were tested for their ability to confer passive immunity to newborn calves. The results of the experiments support the recommendation that, for the prevention of viral diarrhea, newborn calves receive two intramuscular injections of polyvalent serum: the first on the second day after birth, and the second 2–3 days later at a dose of 10 mL. Data obtained from experiments on laboratory and naturally susceptible animals indicate the safety and high preventive efficacy of the polyvalent serum against the BVDV, BRVA, BCoV, BoAHV-1, and BPI3V viruses.

CONCLUSION

The aim of this study was to optimize the technology for producing polyvalent viral antigens in MDBK cell culture for the hyperimmunization of producer animals in the production of serum intended for the prevention and

treatment of viral diseases in young cattle (BVDV, BRVA, BCoV, BoAHV-1, BPI3V). The study was conducted in 2026 under laboratory conditions in compliance with biosafety requirements. Optimization of virus replication. It was established that effective accumulation of viral biomass is achieved using a 24–48-hour-old MDBK cell monolayer, an inoculum dose of 0.1 lg TCID₅₀/mL, a temperature of 37 °C, and a pH of 7.0–7.2 in a serum-free medium without glutamine.

Selection of the polyvalent antigen composition: Of the five experimental variants tested, variant No. 3 was deemed optimal, providing a balanced composition of viral components and an optimal concentration of the adjuvant (6% aluminum oxide hydrate gel). The variant with a 30% AO content (variant No. 5) showed reduced antigenic activity due to an excessive load.

Determination of the immunizing dose: Studies in laboratory animals (guinea pigs) showed that the optimal dose for stimulating a humoral response is 0.75 mL. A dose of 0.26 mL proved insufficient, while 1.0 mL was excessive.

Storage stability: Tests of experimental samples over a 24-month period (at temperatures ranging from 2 °C to 8 °C) confirmed their stability. High antibody titers were maintained for 12 months, and by the 24th month, a natural 2- to 3-fold decrease in titers was observed.

Immunization of breeding animals: Tenfold escalating administration of the polyvalent antigen to breeding bulls (in doses ranging from 5.0 to 20.0 mL) resulted in a robust increase in specific virus-neutralizing antibodies (up to 827–1,007 by day 60), confirming the high immunogenicity of the preparation.

Preventive efficacy of the serum: Administration of the resulting hyperimmune serum to newborn calves (two doses of 10 mL each, administered 2–3 days apart) resulted in the establishment of pronounced passive immunity, which remained at a protective level throughout the first three weeks of life (in contrast to the control group, where antibody titers dropped to zero by day 14).

The developed comprehensive technology for producing a polyvalent viral antigen and hyperimmune serum demonstrated high specific activity, safety, and stability. The resulting preparations effectively induce strong immunity in producer animals and enable the successful establishment of passive protection in newborn calves against a complex of the most dangerous viral and diarrheal infections.

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