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Optimization of viral antigen purification for the production of immunological agents used for treatment and prevention of young livestock infections

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ABSTRACT

Gastrointestinal diseases in neonatal calves cause significant economic losses to the agricultural sector worldwide. Commonly identified causative agents include viral pathogens such as bovine viral diarrhea virus (BVDV), bovine rotavirus group A (BRVA), bovine coronavirus (BCoV), bovine alpha-herpesvirus type 1 (BoAHV-1), and bovine parainfluenza virus type 3 (BPI3V). Clinical manifestations of the infections include diarrhea, dehydration, weight loss, reduced milk production, and, in severe cases, fatal outcomes, primarily among calves under one month of life, with mortality rates reaching 50%. The production of effective hyperimmune sera and immunological drugs requires high-quality viral antigens with enhanced immunogenic properties, which determines the need to develop optimized technologies for virus purification and concentration. This study was conducted in 2025 at the "Biogenic Preparations" laboratory of the "Zhalyn" Scientific-Production Technological Center in Almaty, Kazakhstan, to evaluate and optimize various methods for purifying viral antigens used in the production of immunological preparations. Five major viral pathogens of cattle were cultivated in MDBK cell cultures and treated with four different concentration methods: precipitation with polyethylene glycol (PEG) of various molecular weights, ammonium sulfate precipitation, ultrafiltration, and high-speed ultracentrifugation.

The experimental results demonstrated all tested methods increased the concentration of viral particles and enhanced the infectious activity of the suspension, in varying degrees. Precipitation with PEG molecular weight 6,000 at a concentration of 7.5% yielded optimal results, providing a significant increase in infectious activity of 2 log units. Precipitation with ammonium sulfate led to a moderate increase in concentration with an enhancement of viral activity by approximately 1 log unit. Ultrafiltration demonstrated exceptional efficacy, achieving concentration factors ranging from 10 to 50 times with maximum activity values comparable to PEG purification, while providing the advantages of purification through the removal of minor impurities. Ultracentrifugation, while demonstrating a less significant increase in titer (by 0.5–0.7 log units), provided superior purification quality, which is critical for high-quality therapeutic products. Statistical analysis using ANOVA and Tukey's multiple comparison tests confirmed the significance of the differences observed ($p < 0.05$). These findings demonstrate that ultrafiltration and PEG precipitation represent the most effective methods for increasing viral titer with minimal loss of biological activity, making them ideal for large-scale production of diagnostic and therapeutic products. Ultracentrifugation, despite lower concentration rates, provides levels of purity essential for research and specialized therapeutic applications. The study demonstrates that an integrated strategy combining multiple concentration methods optimizes both the quality and activity of viral preparations. The study's findings present

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opportunities for optimizing biotechnology production and veterinary pharmaceuticals by standardizing protocols for the concentration of viral antigens used to prevent and treat gastrointestinal diseases in cattle.

Key words: Viral antigen purification, PEG percipitation, Ammonium sulphate percipitation, Ultracentrifugation, Ultrafiltration, MDBK, BVDV.
Article type: Research Article.

INTRODUCTION

Gastrointestinal diseases in neonatal calves cause significant economic losses to agricultural producers. These diseases are caused by viral and bacterial pathogens, and also by failure to maintain proper environmental conditions in cattle housing, improper feeding, and poor housing conditions for pregnant cows and newborn calves (Khussainov *et al.* 2024; Sedky *et al.* 2025). The bovine diarrhea virus, rotaviruses, coronaviruses, herpesviruses, *Escherichia coli*, and *Salmonella* play a significant role in the etiology of these diseases, and the diseases often have a mixed clinical course (Bulatov *et al.* 2025). Bovine viral diarrhea (BVD) is a widespread endemic disease affecting cattle and other ruminants worldwide. The causative agent of the disease is the bovine viral diarrhea virus (BVDV)—an RNA-containing virus from the Flaviviridae family, genus Pestivirus (Kincade *et al.* 2025). Persistently infected (PI) calves account for approximately 0.5–2% of the total herd, but less than 1% of them survive to adulthood (La Polla *et al.* 2022). Such animals are unable to mount an immune response and shed the virus into the environment throughout their lives via various bodily fluids—mucus, milk, saliva, tears, urine, semen, and follicular fluid (Fray 2000). Bovine rotavirus group A (BRVA) is recognized as one of the most common viral causes of severe gastroenteritis in dairy calves, especially during the preweaning period (Huang *et al.* 2025). This virus, belonging to the genus Rotavirus of the family Reoviridae, has a characteristic wheel-like shape when viewed under an electron microscope. Its genome consists of 11 segments of double-stranded fragmented RNA (Chae *et al.* 2025). Bovine coronavirus (BCoV), which belongs to the beta-coronaviruses, can cause both intestinal and respiratory forms of disease (Furlan *et al.* 2025a). It affects both calves and adult animals, causing acute diarrhea in young stock and winter dysentery in adult herds, leading to significant losses in milk and meat production (Brandão *et al.* 2007; Stipp *et al.* 2009). Bovine alpha-herpesvirus type 1 (BoAHV-1) is one of the key viral pathogens belonging to the genus Varicellavirus, family Herpesviridae, subfamily Alphaherpesvirinae. This virus poses a serious threat to livestock farming, causing significant economic losses on a global scale (Liu *et al.* 2023). Bovine parainfluenza virus type 3 (BPI3V), belonging to the family Paramyxoviridae, subfamily Paramyxovirinae, is a common causative agent of respiratory diseases in cattle. It manifests as rhinitis, fever (acute or subclinical), and profuse nasal discharge (Ostler & Jones 2023). This infection often leads to secondary bacterial infections and can be exacerbated by other viral agents, in some cases resulting in the death of animals. In the production of hyperimmune sera, the most critical step is obtaining an active, highly specific antigen capable of inducing a potent immune response. An integral part of this process is the high-quality purification of the antigen from non-antigenic impurities. To enhance the antigen's immunogenicity and ensure the high efficacy of the finished serum, modern adjuvants and stabilizers are used (Levitz & Golenbock 2012).

Although PEG precipitation, ammonium sulfate precipitation, ultrafiltration, and ultracentrifugation have been widely applied for virus concentration and purification, comparative studies evaluating these methods under identical experimental conditions for multiple bovine viral pathogens remain limited. Furthermore, the production of polyvalent immunological agents requires simultaneous processing of antigenically distinct viruses while maintaining their biological activity and immunogenic properties. Therefore, selecting an optimal concentration strategy is critical for large-scale production of veterinary immunobiological preparations. The aim of this study was to compare PEG precipitation, ammonium sulfate precipitation, ultrafiltration, and ultracentrifugation for the concentration and purification of BVDV, BRVA, BCoV, BoAHV-1, and BPI3V and to identify the most effective approach for the production of polyvalent immunological agents intended for prevention and treatment of enteric and respiratory diseases in calves.

MATERIALS AND METHODS

The studies were conducted at the "Biogenic Preparations" laboratory of the "Zhalyn" Scientific and Production Technology Center LLP, Almaty, Republic of Kazakhstan, in 2025.

Preparation of virus-containing suspensions

Each of the studied viruses—BVDV, BRVA, BCoV, BoAHV-1, and BPI3V—was cultivated in MDBK cell cultures. Viral strains of BVDV, BRVA, BCoV, BoAHV-1, and BPI3V were obtained from the virus collection

maintained at the Biogenic Preparations Laboratory of the Zhalyn Scientific and Production Technology Center. Prior to use, the viruses were propagated in MDBK cells and stored at −80 °C. A roller system with bottles 130 ± 2 mm in diameter and 375 ± 3 mm in height was used for cell and virus cultivation. Cells growth was performed at 37 °C, and 5% CO_2. MDBK cells were cultivated following established procedures with Eagle's minimal essential medium (MEM) (Capricorn scientific) supplemented with 10% fetal bovine serum (FBS; Capricorn Scientific), 1.5 g L^{-1} of $NaHCO_3$, 0.11 g L^{-1} of sodium pyruvate and 1g L^{-1} *Mycoplasma* Prevention Reagent (Abbexa). MDBK cells routinely tested and confirmed to be free of mycoplasma contamination. Cell viability before infection exceeded 95%. A virus stock in MEM + 10% FBS constituted at 4th passage with a titer of 8.16 log10 $TCID_{50}$ mL^{-1}, frozen at −80 °C, was used in all the experiments. Viable cell concentration was determined by cell counting in a hemocytometer using trypan blue exclusion. Viral infectivity was determined by endpoint titration in MDBK cell cultures using ten-fold serial dilutions. Each dilution was tested in quadruplicate wells. The 50% tissue culture infectious dose ($TCID_{50}$ mL^{-1}) was calculated according to the Reed–Muench method.
After the cytopathic effect (CPE) appeared, the culture supernatant was collected and centrifuged using a low-speed centrifuge (Drawell, PRC) at 3,000 rpm for 15 minutes to remove cell debris. The supernatant (virus-containing suspension) was used for further processing.

Concentration of viruses using polyethylene glycol (PEG)

Various molecular weights of PEG—3,000, 6,000, and 20,000 daltons—were used for virus precipitation at concentrations of 5.0%, 7.5%, and 10.0%. The virus-containing suspension was mixed with the required amount of PEG. The mixture was incubated at 4 °C for 12–16 hours, stirring constantly at a slow rate (300 rpm). After incubation, centrifugation was performed at 10,000 rpm (or 10,000 g) for 30 minutes at 4 °C. The precipitate was resuspended in phosphate-buffered saline (PBS) or in a minimal volume of culture medium (pH 7.1). The final volume of the concentrate was 0.66 ± 0.03 mL. The resulting concentrate was used to assess the degree of precipitation. For quantitative assessment, the infectious dose ($TCID_{50}$ mL^{-1}) was calculated before and after concentration.

Virus precipitation with ammonium sulfate

Ammonium sulfate [$(NH_4)_2SO_4$] was slowly added to the purified viral suspension to a final concentration of 40% (w/v) while stirring continuously for homogeneous distribution of the reagent. The mixture (pH 7.5) was incubated at 4 °C for 12–16 hours to achieve maximum precipitation of viral particles. After incubation, the suspension was centrifuged at 10,000 rpm (approximately 10,000 g) for 30 minutes at 4 °C. The resulting precipitate was resuspended in a minimal volume of phosphate-buffered saline (PBS). Quantitative determination of viral activity was performed by virus titration (e.g., determination of $TCID_{50}$) on susceptible cell cultures. The preparations were stored at −70 °C until further use.

Ultrafiltration Process

Virus concentration was performed using an UPM 10 ultrafiltration polyamide membrane with a molecular cut-off of 10 kDa. The virus-containing suspension was passed through the membrane at an average flow rate of 1.8 mL min^{-1}. Filtration continued until the required concentration factor was reached, as monitored by the volume of accumulated filtrate collected in a measuring vessel at the outflow of the ultrafiltration cell. The concentrated viral preparation was collected from the membrane and resuspended in a minimal volume of a suitable buffer (e.g., PBS) for further analysis. To determine the concentration factor, the volumes of the initial suspension and the final concentrate were measured. The infectious activity of the viruses was assessed by titration (e.g., $TCID_{50}$) before and after ultrafiltration.

Ultracentrifugation

The clarified culture supernatant was placed in special centrifuge PPCO tubes (Zongyi-Biology PRC) suitable for high-speed operation. The viruses were centrifuged at 90,000 RPM for 1 hour at 4 °C using an fixed angle rotor ultracentrifuge (Drawell, PRC). After centrifugation, the supernatant was carefully removed, leaving the viral precipitate. The viral precipitate was resuspended in a minimal volume of phosphate-buffered saline (PBS) or another suitable medium for further analysis and storage. To ensure complete dissolution of the precipitate, gentle mixing or incubation on ice was used. The infectivity of the viruses was assessed by titration (e.g., $TCID_{50}$) before and after ultracentrifugation. The degree of concentration, purity, and integrity of the viral particles were evaluated.

Statistical analysis

All experiments were performed in five independent replicates. Data are presented as mean ± standard deviation (SD). Normality of distribution was assessed using the Kolmogorov–Smirnov test. Differences among concentration methods were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Statistical significance was accepted at $p < 0.05$. All statistical analyses were performed using SPSS version 20.0, GraphPad Prism version 6.01, and Microsoft Excel.

RESULTS

To produce purified and concentrated viral preparations—of bovine viral diarrhea virus (BVDV), bovine rotavirus (BRVA), bovine coronavirus (BCoV), herpesvirus type 1 (BoAHV-1), and bovine parainfluenza-3 virus (BPI3V), a variety of methods were used to isolate the viruses from suspension cultures. During the concentration of viral particles, precipitation conditions using polyethylene glycol (PEG) were studied. Different molecular weights of PEG were tested in the experiments: 3,000, 6,000, and 20,000 daltons at concentrations of 5.0%, 7.5%, and 10.0%. The results are presented in Table 1. Analysis of the experimental data presented in Table 1 showed that the best results for the precipitation of BVDV, BRVA, BCoV, BoAHV-1, and BPI3V viruses were achieved using polyethylene glycol with a molecular weight of 6,000 daltons at a concentration of 7.5%. Under these conditions, a significant increase in infectious activity was observed: for BVDV—from 6.9 ± 0.52 to 8.8 ± 0.65; for BRVA—from 6.8 ± 0.58 to 8.7 ± 0.64; BCoV—from 6.6 ± 0.58 to 8.7 ± 0.72; BoAHV-1—from 6.5 ± 0.53 to 8.6 ± 0.75; and BPI3V—from 6.7 ± 0.57 to 8.7 ± 0.77. To concentrate bovine viral diarrhea virus (BVDV), bovine rotavirus (BRVA), bovine coronavirus (BCoV), bovine herpesvirus type 1 (BoAHV-1), and bovine parainfluenza virus 3 (BPI3V), the precipitation method using salt precipitation was also applied. Ammonium sulfate [$(NH_4)_2SO_4$] at a concentration of 40% was used as the reagent. The experimental results are presented in Table 2. The results presented in Table 2 demonstrate that precipitation of BVDV, BRVA, BCoV, BoAHV-1, and BPI3V viruses using 40% ammonium sulfate and a tenfold concentration leads to a significant increase in infectious activity. Thus, the activity of BVDV increased from 6.9 ± 0.54 to 7.9 ± 0.63, BRVA—from 6.8 ± 0.54 to 7.7 ± 0.69, BCoV—from 6.6 ± 0.89 to 7.9 ± 0.63, BoAHV-1 — from 6.5 ± 0.57 to 7.7 ± 0.64, and BPI3V — from 6.7 ± 0.61 to 7.6 ± 0.62. Ultrafiltration is widely used for virus concentration; this method is based on the separation of viral particles from culture medium using semipermeable membranes with a specified pore size. In this study, the concentration of virus-containing suspensions was performed using UPM 10 ultrafiltration membranes with a molecular weight cut-off of 10 kDa. The average flow rate during the concentration process was 1.8 mL min^{-1}. The dilution factor was determined by the volume of accumulated filtrate in the measuring vessel at the outflow of the ultrafiltration cell. The results are presented in Table 3.

Table 1. Concentration of viral particles with PEG percipitation.

PEG type	PEG concentration (%)	Volume	The infectious dose ($TCID_{50}$ mL^{-1})
BVDV			
None	0	10	6.9 ± 0.54
M-3000	5.0	1	7.1 ± 0.64
	7.5	1	7.3 ± 0.58
	10.0	1	7.5 ± 0.43
M-6000	5.0	1	8.1 ± 0.48
	7.5	1	8.8 ± 0.65
	10.0	1	8.4 ± 0.67
M- 20000	5.0	1	8.3 ± 0.75
	7.5	1	8.1 ± 0.76
	10.0	1	7.6 ± 0.64
BRVA			
None	0	10	6.8 ± 0.54
M-3000	5.0	1	7.4 ± 0.64
	7.5	1	7.6 ± 0.68
	10.0	1	7.8 ± 0.58
M-6000	5.0	1	8.4 ± 0.72
	7.5	1	8.7 ± 0.64
	10.0	1	8.6 ± 0.58
M- 20000	5.0	1	7.9 ± 0.72
	7.5	1	7.7 ± 0.71
	10.0	1	7.4 ± 0.69

bCOV			
None	0	10	6.6 ± 0.89
M-3000	5.0	1	6.8 ± 0.62
	7.5	1	7.2 ± 0.72
	10.0	1	7.4± 0.66
M-6000	5.0	1	8.2 ± 0.74
	7.5	1	8.7 ± 0.72
	10.0	1	8.4 ± 0.54
M- 20000	5.0	1	7.6 ± 0.63
	7.5	1	7.8 ± 0.67
	10.0	1	7.6 ± 0.68
BoAHV-1			
None	0	10	6.5 ± 0.57
M-3000	5.0	1	7.2 ± 0.61
	7.5	1	7.4 ± 0.67
	10.0	1	7.8± 0.69
M-6000	5.0	1	8.1 ± 0.73
	7.5	1	8.6 ± 0.75
	10.0	1	8.5 ± 0.69
M- 20000	5.0	1	7.5 ± 0.67
	7.5	1	7.1 ± 0.61
	10.0	1	6.9 ± 0.56
BPI3V			
None	0	10	6.7 ± 0.61
M-3000	5.0	1	7.1 ± 0.43
	7.5	1	7.5 ± 0.54
	10.0	1	7.8 ± 0.63
M-6000	5.0	1	8.3 ± 0.58
	7.5	1	8.7 ± 0.77
	10.0	1	8.8 ± 0.72
M- 20000	5.0	1	8.3 ± 0.68
	7.5	1	7.9 ± 0.67
	10.0	1	7.3 ± 0.63

Table 2. Concentration of viral particles with $(NH_4)_2SO_4$ solution.

Virus	Preparation	Volume (mL)	The infectious dose ($TCID_{50}$ mL^{-1})
BVDV	Supernatant	10	6.9 ± 0.54
	Precipitant	1	7.9 ± 0.63
BRVA	Supernatant	10	6.8 ± 0.54
	Precipitant	1	7.7 ± 0.69
bCOV	Supernatant	10	6.6 ± 0.89
	Precipitant	1	7.9 ± 0.63
BoAHV-1	Supernatant	10	6.5 ± 0.57
	Precipitant	1	7.7 ± 0.64
BPI3V	Supernatant	10	6.7 ± 0.61
	Precipitant	1	7.6 ± 0.62

The results presented in Table 3 demonstrate that the ultrafiltration method provides a concentration increase of 10 to 50-fold. A tenfold concentration of the viral suspension resulted in an increase in infectious activity: for BVDV, from 6.9 ± 0.56 to 7.4 ± 0.62; BRVA—from 6.8 ± 0.52 to 7.5 ± 0.61; BCoV—from 6.6 ± 0.61 to 7.7 ± 0.71; BoAHV-1 — from 6.5 ± 0.54 to 7.7 ± 0.63; BPI3V — from 6.7 ± 0.63 to 7.5 ± 0.68. When the concentration factor was increased to 50-fold, viral activity increased accordingly: BVDV—to 8.3 ± 0.79; BRVA—to 8.2 ± 0.74; BCoV — to 8.3 ± 0.79; BoAHV-1 — to 8.4 ± 0.81; BPI3V — to 8.4 ± 0.75. The data obtained confirm the effectiveness of using ultrafiltration membranes for concentrating viral preparations. We also tested a method for precipitating viruses using high-speed ultracentrifugation. For this purpose, viruses from culture medium that had been pre-clarified by low-speed centrifugation were precipitated at a centrifugation force of 90,000 g for 1 hour. The results of this experiment are presented in Table 4. The data presented in Table 4 demonstrate that high-speed ultracentrifugation effectively precipitates viruses from a virus-containing suspension. Centrifugation for 1 hour at 90,000 g resulted in an increase in the infectious activity of BVDV from 6.9 ± 0.57 to 7.2 ± 0.68; BRVA virus—from 6.8 ± 0.58 to 7.4 ± 0.67; BCoV virus—from 6.6 ± 0.54 to 7.3 ± 0.65; BoAHV-1 virus — from 6.5 ± 0.63 to

7.4 ± 0.72; BPI3V virus — from 6.7 ± 0.62 to 7.1 ± 0.69. These results confirm the potential of using ultracentrifugation for the concentration of viral preparations.

Table 3. Concentration of viral particles with ultrafiltration.

Virus	Preparation	Volume (mL)	The infectious dose ($TCID_{50}$ mL^{-1})
BVDV	Supernatant	1000	6.9±0.56
	Concentrated	100	7.4±0.62
	Concentrated	20	8.3±0.79
BRVA	Supernatant	1000	6.8±0.52
	Concentrated	100	7.5±0.61
	Concentrated	20	8.2±0.74
bCOV	Supernatant	1000	6.6±0.61
	Concentrated	100	7.7±0.71
	Concentrated	20	8.3±0.79
BoAHV-1	Supernatant	1000	6.5±0.54
	Concentrated	100	7.7±0.63
	Concentrated	20	8.4±0.81
BPI3V	Supernatant	1000	6.7±0.63
	Concentrated	100	7.5±0.68
	Concentrated	20	8.4±0.75

Table 4. Concentration of viral particles with ultracentrifugation.

Virus	Preparation	Volume (mL)	The infectious dose ($TCID_{50}$ mL^{-1})
BVDV	Supernatant	5	6.9 ± 0.57
	Precipitant	1	7.2 ± 0.68
BRVA	Supernatant	5	6.8 ± 0.58
	Precipitant	1	7.4 ± 0.67
bCOV	Supernatant	5	6.6 ± 0.54
	Precipitant	1	7.3 ± 0.65
BoAHV-1	Supernatant	5	6.5 ± 0.63
	Precipitant	1	7.4 ± 0.72
BPI3V	Supernatant	5	6.7 ± 0.62
	Supernatant	1	7.1 ± 0.69

DISCUSSION

The primary method of preventing gastrointestinal diseases is the active immunization of pregnant cows, which leads to the formation of specific antibodies in colostrum, providing passive immune protection to neonatal calves. However, in some cases, calves do not receive a sufficient amount of immunoglobulins, which may be due to delayed or insufficient colostrum intake, as well as impaired absorption of antibodies in the intestine. In such situations, it is recommended to use polyvalent therapeutic and prophylactic hyperimmune sera and biogenic preparations for the prevention and treatment of gastrointestinal diseases (Mutushev 2025). Clinical manifestations of infection caused by the bovine viral diarrhea virus (BVDV) can vary significantly—from mild to severe forms—depending on the virus strain and the animal's immune status. Infection of pregnant cows with BVDV during gestation can lead to intrauterine developmental abnormalities, increased susceptibility of newborns to secondary infections, and increased calf mortality, with the severity of the consequences depending on the timing of infection (Bachofen *et al.* 2010; Brodersen 2014). The virus is transmitted both horizontally—through bodily fluids from persistently infected (PI) animals—and vertically—from mother to fetus during the intrauterine period. Rotavirus primarily replicates in mature enterocytes of the intestinal villi, destroying them, which leads to impaired absorption. The infection most commonly affects calves under one month of age, with a case rate reaching 90–100% and a mortality rate of up to 50%. The infection is accompanied by severe diarrhea and dehydration and often leads to death, causing significant economic losses in livestock farming (Díaz *et al.* 2022). Among the viral pathogens causing acute diarrhea in young cattle, the most significant enteropathogens are considered to be group A rotavirus (BRVA) and bovine coronavirus (BCoV), which cause significant economic losses in global livestock farming (Kostanić *et al.* 2024; Olsen *et al.* 2025). Bovine coronavirus (BCoV) is actively shed in feces, nasal mucus, as well as in respiratory and intestinal secretions, causing three main clinical syndromes: diarrhea in calves, hemorrhagic diarrhea (winter dysentery) in adult animals, and respiratory diseases

in cattle of all ages, including “sea fever” and respiratory syndrome in feedlots (Furlan *et al.* 2025b). Viral infection is accompanied by severe consequences—weight loss, dehydration, reduced milk production, depression, and, in severe cases, death. All of this significantly increases the economic burden on the global meat and dairy industry. Seroprevalence data indicate the widespread prevalence of BCoV: the detection rate of the virus in diarrhea-containing feces ranges from 3.4% to 69%, and in animals with respiratory signs—from 11.8% to 74.6%, underscoring the seriousness of the threat to global livestock farming (Calderón *et al.* 2023). BoAHV-1 can cause a wide range of clinical forms: infectious rhinotracheitis, conjunctivitis, abortions, as well as generalized infections (Liu *et al.* 2024). In addition, the virus establishes latent (hidden) forms of infection, ensuring its lifelong presence in the animal’s body and the possibility of reactivation followed by further spread. The primary site of viral replication is the respiratory tract epithelium, from which it can spread to other tissues and organs (Karimi *et al.* 2022). The use of effective immunostimulatory components allows for a significant increase in the level of specific immunity, providing a reliable preventive and therapeutic effect (Chauhan *et al.* 2017). During the study, several methods of virus concentration were evaluated, including polyethylene glycol (PEG) precipitation, ammonium sulfate salting, ultrafiltration, and high-speed ultracentrifugation. All methods demonstrated effectiveness in increasing the concentration of viral particles and enhancing their infectious activity, but with varying degrees of efficacy.

Polyethylene glycol (PEG) precipitation

The use of PEG with a molecular weight of 6,000 daltons at a concentration of 7.5% yielded the best results compared to other options. The virus concentration increased significantly, and infectious activity rose by more than 1.5–2 logarithmic titer units. This method is simple and effective, making it attractive for the preparation of viral preparations.

Ammonium sulfate salting (40% solution)

Precipitation of viruses using a 40% ammonium sulfate solution also led to an increase in viral activity, but to a lesser extent than with PEG. At a tenfold concentration, infectious activity increased by approximately 1 unit, indicating the more moderate effectiveness of this method. Salting is a reliable method but requires complete removal of the precipitant.

Ultrafiltration

The ultrafiltration method allowed for the concentration of viruses up to 10 to 50 times, accompanied by a significant increase in infectious activity, particularly at higher concentration ratios. Maximum activity values reached 8.3–8.4 log units, indicating that viral viability was preserved and even enhanced when using membranes with a molecular weight cutoff of 10 kDa. The ultrafiltration method not only concentrates but also purifies viral preparations by removing minor impurities.

High-speed ultracentrifugation

Ultracentrifugation at 90,000 g for 1 hour effectively precipitated the viruses, increasing their infectivity by approximately 0.5–0.7 log units. Despite a less significant increase in activity compared to other methods, ultracentrifugation provides a high degree of purification of viral particles, which is important for obtaining high-quality preparations.

Comparison with previous studies

Previous studies have demonstrated that PEG precipitation is an efficient and inexpensive method for concentrating enveloped and non-enveloped viruses. Similar to our findings, PEG 6000 concentrations between 6–10% have been reported to provide high recovery rates while preserving viral infectivity. Ultrafiltration has also been widely recognized as a scalable technology suitable for industrial production, since it combines concentration with partial removal of low-molecular-weight contaminants. In contrast, ultracentrifugation generally provides superior purity but lower recovery efficiency and higher operational costs.

Why PEG 6000 was optimal

The superior performance of PEG 6000 may be associated with its optimal balance between polymer chain length and osmotic exclusion effects, promoting efficient aggregation of viral particles while minimizing structural damage and loss of infectivity.

Industrial relevance

The obtained results indicate that PEG precipitation and ultrafiltration may be integrated into industrial manufacturing workflows for the production of polyvalent immunological agents intended for passive immunization of neonatal calves.

Limitations of the study

This study has several limitations. Purity indicators such as residual host-cell proteins, nucleic acid content, endotoxin levels, and antigen-specific protein profiles were not evaluated. In addition, the immunogenicity of concentrated antigens was not assessed in target animals. Future studies should investigate purification efficiency using SDS-PAGE, ELISA, and electron microscopy and evaluate the *in vivo* immunogenicity of the obtained preparations.

CONCLUSION

All investigated concentration methods effectively increased viral titers and preserved biological activity. PEG 6000 precipitation (7.5%) and ultrafiltration demonstrated the highest concentration efficiency and therefore represent the most suitable approaches for large-scale production of polyvalent immunological preparations. Ultracentrifugation provided the highest expected level of purification and may be recommended for applications requiring highly purified viral antigens. A combined strategy involving PEG precipitation or ultrafiltration followed by ultracentrifugation may provide the optimal balance between viral recovery, purity, and production cost for veterinary biopharmaceutical manufacturing. All of the concentration methods studied demonstrated their effectiveness; however, the choice of the optimal method depends on the specific objectives. To maximize virus titer and preserve their activity, ultrafiltration and PEG precipitation are the most promising methods. Ultracentrifugation, despite a less pronounced increase in titer, provides high purity of viral preparations, which is important for further research and production. The use of a comprehensive approach combining several methods can optimize the quality and activity of viral preparations for diagnostic and therapeutic purposes.

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