Problems of cattle health improvement and protection of population against viral diarrhoea under current conditions of animal husbandry

Yulia Klepova*, Antonina Poryvaeva, Yana Lysova, Daria Belousova, and Venera Nurmieva

FGBNU UrFANIC Ural Branch of RAS, Yekaterinburg, Russia

Abstract. The article presents the dynamics of the prevalence of bovine viral diarrhoea virus (BVDV) in livestock enterprises of the Ural region in the period 2018-2023. Retrospective and operational analysis of monitoring data showed that the epizootic situation was consistently tense. The share of unfavourable livestock enterprises ranged from 18.2% to 33.3%. The cumulative infection rate in different age groups of cattle ranged from 2.3% to 23.3%. Clinical symptoms of acute course of BVDV-infection in examined cattle were registered in 10.9% of cases; persistent form of BVDV-infection - in 16.5% of cases; subclinical course of BVDV-infection and latent form - in 73.6% of cases. Phylogenetic analysis of BVDV isolates obtained from animal biosamples showed that 81.8% of isolates belong to the Cp-biotype of BVDV, 18.2% of isolates - to the Ncp-biotype of BVDV. It has been established that the intensity of pathogen eradication decreases in livestock enterprises when the regulations of specific prophylaxis are violated, as evidenced by the increase in the number of animals carrying BVDV by 4%; calves with diseases caused by BVDV infection - by 2.5%; cows and heifers with reproductive losses - by 5.7%.

1 Introduction

For veterinary science and practice, the issues of biological well-being of farm animals under conditions of technological processes intensification of livestock production are now extremely acute. According to both Russian and foreign researchers, industrial livestock breeding serves as one of the main reasons for violation of the evolutionary mechanisms balance of relationships between the farm animals environment and microorganisms of various taxonomic groups persistently circulating in this environment [4, 6, 8, 13, 15].

In the etiological structure of infectious diseases in cattle, the pathogen of diarrhoea (mucous membrane disease) Bovine Viral Diarrhea Virus (BVDV) occupies one of the leading positions along with the pathogens of infectious rhinotracheitis and parainfluenza - type 3 [3]. The incidence of Bovine Viral Diarrhea (BVD) has been reported in 63 to 85% of cattle populations in countries with developed livestock industries [9, 12]. In the Russian

* Corresponding author: uvk79@mail.ru

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Federation, financial losses of breeding and commercial livestock farms from viral diarrhoea in cattle amount to tens of millions of roubles.

The main strategic directions in solving the problems of viral diarrhoea in the conditions of modern animal husbandry are eradication of the causative agent and restraining the rate of its spread in the territory of the farm. The ways of realizing this direction are presented in Figure 1.

![Diagram of measures for health improvement and cattle protection from viral diarrhoea in the modern husbandry conditions](image)

**Fig. 1.** Algorithm of measures for health improvement and cattle protection from viral diarrhoea in the modern husbandry conditions [5].

However, despite the measures taken, the epizootic process of BVD infection is far from being successfully controlled and managed. In herds, which have been epizootically favourable for BVD infection for 3 or more years, reappearance of BVDV-infected and carrier animals is registered. What are the reasons for the "return" of the viral diarrhoea pathogen in "healthy improved" and "closed" cattle herds? In the scientific community, the reasons for the "return" of the pathogen are considered to be: 1) evolutionary changes in viruses; 2) modernisation and intensification of technological processes in the livestock industry; 3) external epizootic risks associated with the systematic development of cooperation between agricultural organisations in the field of animal imports [2, 7, 10, 11, 14].

**Purpose of the research:** to study the dynamics of BVDV prevalence in cattle populations under the conditions of vaccine prophylaxis in the Ural region.
2 Materials and Methods

The research was carried out in the department of monitoring and forecasting of infectious diseases, in the department of veterinary diagnostics with the testing laboratory of the Ural NIVI, a structural subdivision of FGBNU UrFANIC Ural Branch of RAS, under the state assignment "To study the structure of antigenic scene of causative agents of emergent infections in farm animals, biological features of mechanisms of their interaction with macroorganism" (№ 0532-2021-0007). Comprehensive clinical and laboratory studies were conducted in livestock breeding organisations (n=69) of the Ural region in 2018-2023.

Object of the research: cattle of Holstein breed - cows, calves.

Bioassays: blood and serum from cows and calves; scrapings from cervical canal and vagina of cows; nasopharyngeal washings from calves; faeces from cows and calves; placenta and tissue samples from aborted foetuses; tissue samples of parenchymatous organs from dead calves (liver, kidneys, lungs).

Serological screening of biosamples to determine the BVDV status of livestock enterprises was performed by solid-phase enzyme-linked immunosorbent assay (ELISA) using Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus (IDEXX Laboratories, Inc., USA). The results were recorded on a SUNRISE reader (Tecan, Austria) with interpretation of the results on the original xChek Assay Management System software (IDEXX Laboratories Ins., USA).

Determination of antigens of rotavirus, coronavirus and E. coli was performed in ELISA test system "Rota-Corona-K99 Antigen Test" (IDEXX Laboratories Ins., USA). The results were recorded on a photometer "iMarkTM" (Bio-RAD Korea).

Sero logical studies to assess the intensity of postvaccinal immunity were carried out in indirect haemagglutination reaction (RNHA) using "Diagnostic kit for serological diagnosis of viral diarrhoea-mucous membrane disease of cattle by indirect haemagglutination method (RNHA) produced by LLC "Agrovet", Moscow. RNHA results were recorded visually. Antibody titre was expressed in log values to base 2.

Molecular genetic identification and differentiation of BVDV isolates were performed using reagent kits for DNA isolation "Diatom DNA Prep 200" (IsoGen LLC, Moscow), "Test-systems for detection of cattle VD by PCR" (LLC "Vetbiochem", Moscow), "Test-systems for detection of BVDV RNA by PCR with GFL-detection" ("AmpliSens", Moscow), specific oligonucleotide primers for three main regions of the BVDV genome: 5'-UTR, glycoprotein E2 gene, non-structural protein Npro gene; BLAST system and NCBI GenBank database [1].

Molecular genetic studies of biosamples to detect the genome of pathogens of mycoplasmosis, chlamydia, clostridiosis, infectious rhinotracheitis of cattle (BHV1), parainfluenza type 3 were performed by Polymerase chain reaction (PCR). PCR was performed in accordance with the manufacturer's instructions for the use of test systems. The following diagnostic kits were used: for determination of species-specific mycoplasmas in cows Mycoplasma bovis and Mycoplasma bovigenitalium" (LLC "IDS", Moscow); for determination of Chlamydia spp. (LLC "FactorMed", Moscow); a set of reagents for identification of Chl. abortus, Chl. pecorum (LLC "IsoGen", Moscow); "RealBest-Vet DNA Cl.difficile/Cl.perfringens" (JSC "Vector-Best", Moscow); "GenPak DNA PSR Test BHV1" (Russia), "PCR-PARAGRIPP-3-CRS-Factor" (LLC "Vet Factor", Russia).

Molecular genetic studies were performed in the electrophoresis variant using agarose gel and Mini-Sub Cell GT (Bio-Rad. USA) with visualisation under ultraviolet light in the CHEMIDOCXRS+ chamber with interpretation of results using GelDoc XR+ gel documentation (Bio-Rad, USA) with visualisation under ultraviolet radiation in the CHEMIDOCXRS+ chamber with interpretation of results using GelDoc XR+ gel.
documentation (Bio-Rad, USA) on Rotor-Gene 3000 (Corbett Life Science, Australia), QuantStudio 5 (USA), Appliede Biosystems 2720 thermocycler (Singapore).

Bacteriological studies of biosamples from cattle were performed in accordance with the methodological recommendations "Methods of bacteriological study of opportunistic microorganisms in clinical microbiology - MUK 4.2.1890-04". The results of the study were recorded using an Axio Observer microscope (Zeiss, Germany).

Retrospective and operational analysis of data on cattle morbidity, on vaccine prophylaxis of pathogens of the group of acute respiratory viral infections was carried out on the materials of veterinary reports of agricultural organisations and statistical data of the Department of Veterinary Medicine of the Sverdlovsk region, for the period 2018-2023.

To process the obtained data, Microsoft Excel programme included in the Microsoft Office Pro software package was used.

3 Results and Discussion

The analysis of serological screening results to determine the status of "favorability for BVD infection" of livestock enterprises in the Sverdlovsk region in the period 2018-2023 is presented in Fig. 2.

![Fig. 2. Livestock enterprises epizootically unfavourable for viral bovine diarrhoea (as a % of the number of enterprises surveyed in 2018-2023).](image)

Retrospective and operational analysis of monitoring data in the surveyed livestock enterprises showed that the epizootic situation on viral diarrhoea in cattle had a consistently tense character. The total rate of BVD infection in different age groups of cattle varied from 2.3% to 23.3% (Table 1).

<table>
<thead>
<tr>
<th>Group of examined animals (age, physiological state)</th>
<th>Detection of BVD antigen in animal biosamples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2018</td>
</tr>
<tr>
<td>Newborn calves</td>
<td>23.2%</td>
</tr>
<tr>
<td>Calves, age 2-3 months</td>
<td>9.5%</td>
</tr>
<tr>
<td>Pregnant cows</td>
<td>2.8%</td>
</tr>
<tr>
<td>Cows and heifers with reproductive losses</td>
<td>19.7%</td>
</tr>
</tbody>
</table>

Table 1. - Dynamics of total BVD infection rate in different age groups of cattle in the Urals region
In 2020, a maximum decrease in the total BVD infection rate was observed in all examined animal groups compared to the indicators of 2018 and 2019. In 2021-2023, an increase in the rate of BVD infection in animals was registered: in "Newborn calves" by an average of 5.6%; in "Calves, age 2-3 months" - by 2.03%; in "Pregnant cows" - by 1.3%; in "Cows and heifers with reproductive losses" - by 5.1%.

In the animals examined in 2021-2023 (n=271), in which BVDV was diagnosed by laboratory methods, clinical symptoms of acute course of infection were registered only in young animals aged 2-3 months (Table 2).

Table 2. Clinical forms of BVD infection in the examined animals

<table>
<thead>
<tr>
<th>Group of examined animals (age, physiological state)</th>
<th>Results of laboratory diagnostics (% of &quot;positive samples&quot;)</th>
<th>Form of BVD infection in examined animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>detected BVDV$^1$ antigen</td>
<td>detected BVDV$^2$ gene</td>
</tr>
<tr>
<td>Newborn calves (n=67)</td>
<td>23.8%</td>
<td>no tests carried out</td>
</tr>
<tr>
<td>Calves, age 2-3 months (n=72)</td>
<td>15.3%</td>
<td>5.5%</td>
</tr>
<tr>
<td>Pregnant cows (n=74)</td>
<td>9.7%</td>
<td>2.8%</td>
</tr>
<tr>
<td>Cows and heifers with reproductive losses (n=74)</td>
<td>13.8%</td>
<td>6.9%</td>
</tr>
</tbody>
</table>

1 - BVDV antigen was detected in biosamples from cattle by ELISA, Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus test system (IDEXX Laboratories, Inc., USA);
2 - BVDV genome was detected in biosamples from cattle by PCR, "Test-system for detection of cattle VD by PCR" (LLC "Vetbiochem", Moscow)

In the analysed period (2022-2023), the total proportion of BVDV antigen detection in bio-samples from cattle by ELISA method was 15.5%, of BVDV genome detection by molecular genetic method (PCR) was 3.4% of the total number of examined biosamples.

The previously performed studies reveal that both non-cytopathogenic and cytopathogenic biotypes of BVDV circulated equally in the Sverdlovsk region (2016-2020) [1, 5, 8]. To determine the biotype of the pathogen circulating in cattle herds in 2022-2023, molecular genetic identification and differentiation of 11 isolates of BVDV was performed according to the method of Bezborodova N.A. et al [1]. The isolates were clinical samples of placenta (4 biomaterials), parenchymatous organ tissue homogenates of aborted foetuses (4 biomaterials), blood cell fraction (3 biomaterials) that contained BVDV genetic material. The performed studies show that 9 isolates belonged to the cytopathogenic biotype of BVDV, and 2 isolates (placenta, tissues of parenchymatous organs of aborted foetuses) belonged to the non-cytopathogenic biotype of BVDV.

When analyzing the etiological structure of diseases of young cattle in livestock enterprises unfavourable for viral diarrhoea, it was shown that the contribution of BVDV to the antigenic scene was 13.5%. Staphylococcus spp. pathogens (32.1%) dominated in the antigenic scene in respiratory and intestinal diseases of calves at the surveyed enterprises. Also, a significant part (up to 17.3%) in the antigenic structure was occupied by pathogens of Chlamydia spp. Infectious rhinotracheitis (IRT) and Mycoplasma spp. were diagnosed in almost equal proportions. The minor group of causative agents in the antigenic scene of these diseases of young animals consisted of parainfluenza-3 (PI3), clostridia, E. coli, rotavirus and coronavirus (Fig. 3).
Fig. 3. Spectrum of pathogenic agents in respiratory and intestinal diseases of young cattle in the surveyed agricultural enterprises

In BVD unfavourable herds reproductive disorders in cows associated with embryonic losses up to 45 days of gestation period made up to 20.2%; the total average level of abortions in cows and heifers was 6.1%, the level of pregnant animals abandonment - 9.3 %, which exceeded the average regional indicator by 2 times. The main share of perinatal losses was the birth of non-viable calves, the stillbirth rate was 14.1%, which is 3.5 times higher than the regional average [6]. In reproductive disorders, the first place in the antigenic structure was occupied by BVDV, 23%, the second place – by Chlamydia spp., 18.3%. Neospora caninum, Mycoplasma spp., Staphylococcus spp. and IRT were diagnosed in almost equal proportions (Fig. 4).

Fig. 4. Spectrum of pathogens in reproductive disorders in cattle in the surveyed livestock enterprises

One of the factors that contain and limit the spread of the viral diarrhoea pathogen in cattle populations is vaccine prophylaxis. The intensity and duration of specific immunity to BVD directly depends on the age-specific physiological characteristics of the animals and the immunogenicity of the BVDV strains that are included in the vaccines. With active and systematic vaccination of livestock, specific immunity is formed in 80-95% of animals, which provides population protection and contributes to the eradication of field strains of the BVDV [3, 5]. Only one of the biologicals for BVD vaccine prophylaxis registered and used in the Russian Federation is single-component, "BOVILIS® BVD", produced by Intervet International B.V., the Netherlands. Other biologicals are multicomponent vaccines...
that include antigens of other viruses and microorganisms in addition to the BVDV antigen. In 92% of cattle enterprises in the Sverdlovsk region, combined vaccines containing BVDV antigens are used to protect cattle from acute respiratory viral infections (ARVI) [6].

Over 80% of the livestock are vaccinated annually in the region. Over the study period, the highest cattle vaccination coverage rate against diarrhoea virus was recorded in 2021 at 85.9%, 1.8% higher than in 2023 and 3.5% higher than in 2019. For certain reasons, approximately 1/6th of the region's cattle population remained unvaccinated (Fig. 5).

![Fig. 5. Dynamics of specific prophylaxis of cattle ARVI (including BVD) in cattle herds of the Sverdlovsk region (2019-2023).](image)

The performed studies on the assessment of population antiviral immunity have shown that in a number of livestock enterprises the prolonged epizootic well-being both for BVD and cattle ARVI in general has led to superficial implementation of vaccine prophylaxis measures. The analysis revealed that the total share of farms vaccinating livestock against ARVI in full did not exceed 75% and tended to decrease (2019-2023). There was a steady increase in the total share of farms with violations of vaccination regulations and zoohygienic requirements of cattle keeping. In 2023, the share of such farms was 22.7% (Fig. 6).

![Fig. 6. Dynamics of specific prophylaxis of cattle ARVI (BVDV; BHV1, 2; PI-3; BRSV) in the surveyed livestock breeding organisations of the Ural region (2019-2023).](image)
The consequences of such "violations" had a negative impact on population antiviral immunity. The proportion of animals without protective postvaccinal immunity to BVDV increased to 10.9%, mainly in the group of young animals aged 3-4 months. An increase in the proportion of animals with minimal protective antibody titre against BVDV in the heifer and cow groups was also observed (Fig. 7).

Fig. 7. Distribution of cattle population by concentration of postvaccinal antibodies to BVDV under vaccine prophylaxis conditions. "Low antibody titre to BVDV" - < 3 log₂; "Min protective antibody titre to BVDV" - 3 log₂; "Seroconversion" - > 3 log₂

In fact, the cattle population with 53.6% positive seroconversion to BVDV was a target for field strains of the pathogens. Furthermore, mother cows with low and minimally protective levels of postvaccine antibodies to BVDV are unable to protect their offsprings from the pathogen through colostral immunity.

4 Conclusions

The epizootic situation with bovine viral diarrhoea in the Urals region is consistently tense. In 2021-2023, an increase in the rate of BVD infection in animals was registered: in "Newborn calves" by an average of 5.6%; in "Calves, age 2-3 months" - by 2.03%; in "Heifer cows" - by 1.3%; in "Cows and heifers with reproductive losses" - by 5.1%. In 2023, the BVD infection rate of newborn calves in the surveyed livestock enterprises was 22.7%, of young calves -10.1%, of pregnant cows - 3.2%, of cows and heifers with reproductive losses - 20.5%.

Clinical symptoms of acute course of BVD-infection in examined cattle were registered in 10.9% of cases; persistent form of BVD-infection - in 16.5% of cases; subclinical course of BVD-infection and latent form - in 73.6% of cases. In livestock enterprises unfavourable for viral diarrhoea, Staphylococcus spp. (32.1%), Chlamydia spp. (17.3%) and Mycoplasma spp. (9.5%) were the dominant causative agents in respiratory and gastrointestinal tract diseases in young animals. In cows and heifers with reproductive losses, BVD infection was diagnosed in association with Chlamydia spp. (18.3%), Mycoplasma spp. (11.4%) and Staphylococcus spp. (10.7%).

According to the results of phylogenetic analysis of BVDV isolates obtained from tissue samples of placenta, parenchymatous organs of aborted foetuses (lung, liver, kidney) and blood cell fraction, it was found that 81.8% of isolates belong to Cp-biotype BVDV (cytopathogenic biotype), 18.2% of isolates - to Ncp-biotype BVDV (non-cytopathogenic...
biotype); their role in the development of pregnancy and foetal pathologies in cattle was confirmed.

Eradication of the BVD pathogen and containment of its spread on the territory of an economic entity to a large extent depend on population antiviral immunity. When the regulations of specific prophylaxis of BVD in livestock enterprises were violated, the herd population antiviral immunity stress was 53-62%. There was an increase in the number of BVDV carrier animals by an average of 4%; of calves with BVD-infection-related diseases - by 2.5%; and cows and heifers with BVD-infection-related reproductive losses - by 5.7%.

Conflict of interest

The authors confirm that there is no conflict of financial/non-financial interests related to the writing of the article.

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References


