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BOVINE PESTIVIRUSES AS CONTAMINANTS OF BIOLOGICAL PREPARATIONS

(review)

A.G. GLOTOV, T.I. GLOTOVA✉, S.V. KOTENEVA, A.V. NEFEDCHENKO, O.V. SEMENOVA

Siberian Federal Scientific Center of Agro-BioTechnologies RAS, Institute of Experimental Veterinary Science of Siberia and the Far East, r.p. Krasnoobsk, PO box 463, Novosibirskii Region, Novosibirsk Province, 630501 Russia, e-mail glotov_vet@mail.ru, t-glotova@mail.ru (✉ corresponding author), koteneva-sv@mail.ru, homeovet@narod.ru, k-olga-83@mail.ru

ORCID:
Glotov A.G. orcid.org/0000-0002-2006-0196
Glotova T.I. orcid.org/0000-0003-3538-8749
Koteneva S.V. orcid.org/0000-0003-2649-7505
Nefedchenko A.V. orcid.org/0000-0002-4181-4268
Semenova O.V. orcid.org/0000-0002-1165-5243

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Abstract

Bovine pestiviruses are causative agents of bovine viral diarrhea-mucosal disease, a widespread and economically significant infection (J.F. Ridpath, 2010; C.A. Evans et al., 2019). These viruses include the prototype species *Pestivirus A* (bovine viral diarrhea virus type 1; BVDV-1), *Pestivirus B* (bovine viral diarrhea virus type 2; BVDV-2) and *Pestivirus H* (Hobi-like pestivirus, HoBiPeV; bovine viral diarrhea virus type 3; BVDV-3) (P. Simmonds et al., 2017; ICTV, 2019). All agents are represented by cytopathogenic (CP) and non-cytopathogenic (NCP) biotypes. The NCP biotype, unlike CP, does not cause visible morphological destruction of cell cultures and represents more 90 % of virus population (P.H. Walz et al., 2020). The number of known subtypes of BVDV-1 is 22 (a to v), BVDV-2 4 (a-d) and BVDV-3 4 (a-d) (N. Su et al., 2023). In Russia, circulation of 12 subtypes of BVDV-1, three subtypes of BVDV-2 and one subtype of BVDV-3 has been established (A.G. Glotov et al., 2022). One of the ways of spreading pathogens in cattle populations may be biological products produced using contaminated fetal sera (L.V. Uryvaev et al., 2012; A.G. Glotov et al., 2018), cell cultures and trypsin (O . Lung et al., 2021), namely veterinary vaccines and interferons. Agents can be distributed through embryos and sires' semen (J.A. Gard et al., 2007; K. Gregg et al., 2010). Contaminated medical vaccines (M. Giangaspero et al., 2004), as well as biotechnological materials (L. Djemal et al., 2021), and stem cells (S. Viau et al., 2019) can pose a significant problem. Contamination of vaccines occurs during their production with NCP strains of all types of pestiviruses which are randomly introduced into cell cultures from untested fetal serum (B. Makoschey et al., 2003; P.P. Pastoret, 2010). Existing decontamination methods cannot always ensure complete inactivation of agents (W.P. Paim et al., 2021). An additional challenge is the increasing number of virus species and subtypes (C. Luzzago et al., 2021). Contamination of mammalian cell cultures can lead to false diagnostic test results, contamination of biological products and their transfer to recipients. Antibodies to bovine pestiviruses were found in 40 % of serum samples from twins with schizophrenia (M. Giangaspero, 2013), and their antigens were found in 23.6 % of fecal samples from children with gastroenteritis (R. Yolken et al., 1989). Only careful routine monitoring and culling animals used to obtain fetal serum or organs for cell cultures, all batches of serum, cell cultures and biological products based on these components, can prevent potentially dangerous contamination with pestiviruses. It is necessary to take into account the plasticity of viruses and the emergence of new species and subtypes.

Keywords: pestiviruses, cattle, contamination, fetal serum, cell cultures, vaccines, biological products, sperm, embryos

The classification of the genus *Pestivirus* (*Flaviviridae*) has currently under-

gone changes. In 2017, the International Committee on Taxonomy of Viruses (ICTV, https://ictv.global/) added seven new species to the four previously existed. Names based on the *Pestivirus* X format were proposed, regardless of primary hosts. Species names have changed, but isolates are still referred to by their original names. The prototype species of the genus is bovine viral diarrhea virus type 1 (BVDV-1), renamed to *Pestivirus* A, bovine viral diarrhea virus type 2 (BVDV-2) to *Pestivirus* B, classical swine fever virus (CSFV) to *Pestivirus* C, border disease virus (BDV) to *Pestivirus* D. New species are *Pestivirus* E (pronghorn pestivirus), *Pestivirus* F (Bungowannah virus), *Pestivirus* G (giraffe pestivirus), *Pestivirus* H (Hobi-like pestivirus, HoBiPeV; bovine viral diarrhea virus type 3, bovine viral diarrhea virus 3, BVDV-3), *Pestivirus* I (Aydin-like pestivirus), Pestivirus J (rat pestivirus), and *Pestivirus* K (atypical porcine pestivirus) [1].

For cattle, three species are of primary importance, the *Pestivirus* A, *Pestivirus* B and *Pestivirus* H, represented by cytopathogenic (CP) and non-cytopathogenic (NCP) biotypes. NCP, unlike CP, does not cause visible morphological destruction of cell cultures and represents more than 90% of the virus population [2]. The viruses are causative agents of viral diarrhea-mucosal disease (VD-MD), a widespread and economically significant infection of cattle [2-6].

There are 22 currently known subtypes of BVDV-1 (from a to v), 4 subtypes of BVDV-2 (a, b, c, d) and 4 subtypes of BVDV-3 (a, b, c, d) [7, 8]. In Russia, the circulation of 12 BVDV-1 subtypes (from 1a to 1r), three BVDV-2 subtypes (2a, 2b, 2c) and one BVDV-3 subtype (3a) has been established [9]. The disease is global but more common in countries with high animal densities, where systematic control is not applied and pathogens persist for a long time in cattle populations [5].

The epizootic situation and the stationarity of foci are maintained mainly due to the circulation and constant evolution of enzootic strains or the introduction of new ones. In the second case, a wide range of symptoms described may occur, and the virus may be considered emergen [10-13].

In susceptible animals, all species of the genus Pestivirus cause a similar pathology, namely acute infections with immunosuppression, enteritis, embryo resorption, abortion at all stages of pregnancy, congenital fetal malformations, the birth of weak calves, infertility, respiratory pathology and disease of the mucous membranes [14-16].

A characteristic feature of the genus *Pestivirus* is a persistent infection (PI) when the fetus is infected only with the NCP biotype of the virus from day 40 to day 125 of intrauterine development when the fetal immune system is not yet formed [5]. This leads to the birth of immunotolerant calves which become constant sources of pathogens for non-immune animals. The infectious activity of the blood virus in such individuals, starting from prenatal development, is high (up to 10^6 TCD_{50}/ml), and they excrete it throughout their lives with all the secrets, excrements and blood. They do not produce specific antibodies [17-20]. The prevalence of persistent infection in the cattle population is in general within 2-3% [21, 22].

Pestiviruses can contaminate biological products, e.g., fetal serum, cell cultures, vaccines for humans and animals, interferons, trypsin, biotechnological drugs, stem cells, sperm of bulls, embryos, etc. [23-25].

Spreading pathogens in cattle populations through contaminated vaccines is of great concern. Contamination of the cell cultures and biological products with NCP strains of pestiviruses occurs accidentally through untested fetal serum used for propagation of vaccine strains [26, 27]. Therefore, testing for contaminating viruses is mandatory in the use of fetal serum to produce medicinal preparations [28].

In this review, we briefly analyze the prevalence of bovine pestiviruses in a number of biological products, including medical vaccines, and the risks of the use

of contaminated vaccines and biotechnological products.

Fetal bovine serum. Fetal bovine serum (FBS) is the most common and widely used additive to nutrient media for initiating and increasing the rate of growth of mammalian cell cultures [29]. FBS is a natural mixture of factors to stimulate cell attachment, growth and proliferation [30]. FBS is a product of the biotechnology industry and a by-product of the meat industry [31-33].

Serum is prepared from the blood of fetuses of pregnant beef cows intended for slaughter for meat and selected at random. In large herds, animals of both sexes graze freely together and, as a result, many cows are pregnant at slaughter. The fetuses aged 6 months are separated and their blood is aseptically collected. Each batch of commercial FBS is collected from many farms, so contamination with NCP viruses is possible due to mixing preparations from various sources, including the sera from persistently infected fetuses, into the common batch [34]. FBS is in greatest demand in the USA and Europe, while the main sources of raw materials are Brazil, Argentina, Australia, New Zealand and Central America [29]. The main exporters of cell culture products for manufacturing vaccines and drugs are the USA, New Zealand and Australia [34, 35].

In the 1960s, it was discovered that many viruses contaminate fetal serum, with the VD-VD virus being the most common [36]. As new types and subtypes of the virus were discovered, their presence in commercial batches of the drug was revealed. Given the risk of viral contamination, serum inactivation with validated methods was strongly recommended in addition to direct viral testing. Despite these procedures, the risk of serum contamination still remains [37, 38].

To ensure the quality of FBS, the samples from pooled lots are tested for sterility (bacteria, fungi), endotoxins, immunoglobulins, viruses, biochemical parameters and electrophoretic profiles. After this, the lots are sterilized by filtration and exposed to gamma radiation or high temperatures. These procedures, together with final freezing, provide additional anti-viral safety. Premium quality FBS must be low in immunoglobulins, free of viruses and endotoxins [36, 39, 40].

However, not all serum batches always undergo the necessary testing, or it turns out to be insufficiently effective. Thus, B. Makoschey et al. [26] showed that 4 of 7 batches of FBS were contaminated with infectious NCP BVDV-1 after treatment. H. Xia et al. [41] demonstrated for the first time that commercial FBS products of different geographic origins are contaminated not only with BVDV-1 and BVDV-2 but also with emergent BVDV-3. RT-PCR analysis of 33 FBS lots from 10 manufacturers identified BVDV-1 in 29 lots from 11 countries. BVDV-2 was detected in 11 shipments from South America. BVDV-3 was detected in 13 shipments from Australia, Brazil, Canada and Mexico. S.Q. Zhang et al. [42] in 2014 reported that Chinese bovine serum from different regions was contaminated with at least one species of pestivirus, including BVDV-1 and BVDV-2.

It is currently believed that *Pestivirus* H originated in Brazil was introduced to other countries and continents through contaminated fetal serum and vaccines. According to F.V. Bauermann et al. [43], more than 30% of FBS batches from South America tested in Europe contained the virus. M. Giammarioli et al. [44] tested 26 archival batches of the drug obtained in 1992-2013, which underwent a process of filtration and gamma irradiation. The PCR analysis detected at least one bovine pestivirus in all samples, 20 of the samples contained BVDV-1, 10 contained BVDV-2, and 15 contained BVDV-3 of the “Brazilian group”. Seven batches were from South America, one from Australia, and the origin of seven batches was not identified.

C. Luzzago et al. [45] believe that the spread of pestiviruses in Italy was largely due to contaminated fetal serum and vaccines. F.V. Bauermann et al. [46] after testing 90 batches of commercial serum produced in the United States but

packaged in Europe, did not detect BVDV-3 and concluded no BVDV-3 circulation in the country. However, another 19 lots contained BVDV-1, and one contained BVDV-2. T. Kozasa et al. [47] identified the BVDV-1 genome in 28 of 49 samples in Japan. O. Zabal et al. [36] detected BVDV-1 in two of 20 commercial serum pools in Argentina. In Russia, A.G. Glotov et al. [9] and S.V. Koteneva et al. [48] identified BVDV-3 of the Italian-Brazilian group (3a) in 7 lots of fetal serum used in several medical and veterinary research institutes.

Thus, in fact, the FBS contamination with pestiviruses occurs that, together with the use of serum as a culture media additive, poses a risk of cell cultures infection even if levels of viruses are low. Therefore, it is necessary to constantly update the procedure for identifying bovine pestiviruses and improve the regulations for international trade in FBS and animals.

In recent years, given the potential diversity of pestiviruses in commercial FBS batches, veterinary professionals have tested several technologies aimed at reducing or inactivating the virus with the goal of eliminating it. In accordance with the directive of the European Medicines Agency (EMEA, https://www.ema.europa.eu/en/homepage), the list of tests for quality control of the inactivation procedure includes the virus [28]. Methods for inactivating the virus are being improved. In particular, pulsed ultraviolet irradiation of FBS with $\underline{\lambda}$ = 355 and $\underline{\lambda}$ = 266 nm is effective [31, 49].

Cell cultures. Cell and tissue cultures are an indispensable tool in veterinary, biomedical research, and biotechnology, where cell culture lines of animal origin are used in the manufacturing of biological products [32]. Cell and tissue cultures are widespread in many areas of veterinary medicine and medicine, for example, in laboratory diagnostics, in the production of biopharmaceuticals and vaccines, in study of oncological diseases, drug screening and development, in gene and cell therapy, tissue engineering, in artificial insemination, and in toxicity testing [50, 51].

Unlike contamination with bacteria and mycoplasmas, which are relatively easy to detect, viral contamination poses a serious threat due to the difficulty of detecting some viruses and the lack of effective methods for decontaminating infected cell cultures. Contamination occurs only with non-cytopathogenic strains of viruses the reproduction of which does not cause visible destruction of cells, but leads to a decrease in their energy potential. The virus propagated in such cell lines is potentially dangerous to other cell cultures in research laboratories due to cross-contamination, as well as to animals and humans in the case of the production of injectable biologicals. The only way to protect cell cultures for research, development and the biotechnology industry from viruses is to prevent such contamination. Cell cultures can become contaminated during the preparation of primary trypsinized cultures (because the source has already been contaminated, e.g., trypsin or fetal serum), due to the use of contaminated raw materials, or during passage through animals [52-54].

Both primary trypsinized and continuous cell culture lines derived from tissues of cattle or other animal species are highly susceptible to infection with a non-cytopathogenic virus biotype. Contamination of human stem cells has been described in the literature [55].

Testing of 41 cell culture lines from the American Type Culture Collection (ATCC) bank in 1994 showed a high degree of pestivirus contamination of cell cultures from sheep, goats, rabbits, cats, and cattle. Cell cultures of porcine origin were found to be virus-free [56, 57]. Monkey cell lines (LLC MK2) were variably susceptible, but the African green monkey kidney cell line (Vero) efficiently supported viral replication [26].

Due to the cell culture contamination by non-cytopathogenic pestiviruses, conclusions about virulence and other processes associated with interactions with the host at the cellular and molecular levels may be erroneous. Infection with non-cytopathogenic BVDV strains causes unreliable results in in vitro studies that is unacceptable when using cell cultures for commercial purposes, and also poses a serious risk in virological and diagnostic procedures and vaccine production [27]. According to D.A. Stringfellow et al. [58], 5 of 39 laboratory cell lines were infected with BVDV, and the source was found to be FBS.

In our country, L.V. Uryvaev et al. [59] for the first time discovered contamination with the viral diarrhea virus in 127 cell lines and inoculations used in medical virological studies, and in 37 commercial domestic and foreign fetal sera for culturing cells. An enzyme-linked immunosorbent assay (ELISA) of blood sera from veterinary workers from Russian livestock farms found antibodies to BVDV-1 in 42 samples. In another study, the same authors detected the virus in 25% of cell samples and in 75% of fetal sera. It was first shown that the virus replicates and persists at relatively high titers in many cell lines of humans, monkeys, pigs, sheep, rabbits, hamsters, dogs, cats, and other animal species [60].

S.V. Vangeli et al. [61] studied several continuous cell lines used to obtain bovine leukemia virus antigen and revealed a mixed chronic infection with leukemia and diarrhea viruses, which, according to the authors, is also due to contaminated fetal serum. Production of gp51 and p24 antigens with these lines requires mandatory test for positive serum using the diagnostic kits.

S.V. Alekseenkova et al. [62] revealed the contamination of bovine and porcine cell cultures with bovine diarrhea virus. The source of the virus was probably cattle serum.

In a study of 117 cell series and 35 fetal serum samples for contamination with bovine viral diarrhea, the agent was detected in 25% of cell cultures and in 45% of serum samples. The authors note that in Russia a large number of batches of fetal serum from calves are contaminated with this pathogen. Cell cultures used in the production of live antiviral vaccines should not be used for more than 20 passages, and each passage should be tested for the presence of contaminating viruses [63].

A.G. Glotov et al. [64] studied 9 lines of continuous cell cultures of different origins, namely, MDBK, CRFK, RK13, TEB, L929, MF (mouse fibroblasts), KCT, BHK21, Vero. All lines contained the BVDV-1a genome. Additional studies revealed BVDV-1b in continuous cell lines Taurus and FLK, and BVDV-2 in FK-81 cell culture. L.G. Holinka-Patterson et al. [65] reported the detection of the BVDV-1a genome in the continuous cell line LFBK-$\alpha v\beta 6$ used for culturing bovine foot-and-mouth disease virus.

High temperature short-time technology (HTST) is a method to decontaminate fetal serum, trypsin, pepsin, etc. It is believed that the risk of viral contamination can be reduced by inactivating or eliminating viruses from cell culture media and nutrient solutions. Nanofiltration, ultraviolet irradiation, and gamma-ray technology have also been proposed [66, 67].

Vaccines. Many biotechnology products (vaccines, recombinant proteins, expressed proteins, diagnostic drugs) have been developed using various cell cultures to which fetal bovine serum has been added. Consequently, these products are potentially susceptible to pestivirus contamination and may pose a danger to animals and even humans in certain situations. During the production of vaccines, the contaminating virus can prevent the reproduction of the vaccine strain in an infected cell culture, reducing its titer which requires an increase in the infecting dose, and also replicate together with it in high titers. The consequences of vaccine contamination may vary [26, 27, 52].

Veterinary vaccines. The result of contamination depends on the type of vaccine (live or killed), the titer of the contaminating virus, virulence and the degree of its inactivation during production. The practical consequences of BVDV vaccine contamination may be infection, clinical or subclinical, of the recipient or a serological reaction to the contaminant.

Pestiviruses have strong immunosuppressive properties and interact synergistically with other pathogens [68], which can lead to the emergence of new infections in the herd. In addition, vaccination of pregnant seronegative heifers with NCP contaminated live vaccines leads to the birth of persistently infected calves, malformations, abortion [2, 69] and mucosal disease in calves [70], which increases the risk of spread of the virus throughout the herd.

C.J. Bruschke et al. [71] detected diarrhea virus in 7 of 82 lots of marker vaccine against infectious rhinotracheitis on the Dutch market in February 1999. Six batches of the drug contained BVDV-1 and one batch contained BVDV-2. The use of this batches resulted in an outbreak of disease in the vaccinated population. H.W. Barkema et al. [72] described an outbreak of viral diarrhea caused by the administration of another batch of the vaccine against infectious rhinotracheitis contaminated with NCP BVDV-2 to animals. Disease incidence in vaccinated animals was high on 11 of 12 farms, and on five farms, more than 70% of animals became ill. Sick animals were culled. A total of three outbreaks of viral diarrhea following the use of contaminated vaccine were reported in Holland and Italy. These series of the drug were withdrawn from the market.

E. Falcone et al. [73] experimentally reproduced severe signs of viral diarrhea with noncytopathogenic BVDV-2 isolated from a live vaccine against infectious rhinotracheitis in 3-month-old seronegative calves. The data obtained indicate the infectious nature of the contaminant and its ability to cause outbreaks of disease when susceptible animals are vaccinated.

Several articles have reported contamination problems with other veterinary vaccines. Thus, using RT-PCR, the presence of BVDV 1a, 1b, 1c and 1d was detected in live viral vaccines against Akabane disease, Ibaraki disease, infectious bovine rhinotracheitis, porcine parvovirus infection, and transmissive gastroenteritis. The source of the virus was fetal serum [74]. A virulent pestivirus which has caused outbreaks of viral diarrhea in vaccinated animals was present in vaccines against classical swine fever, respiratory syncytial infection, infectious rhinotracheitis, and bovine coronavirus infection in some European countries [27]. N. Gymez-Romero et al. [75] tested by RT-PCR six lots of live parainfluenza-3 and infectious rhinotracheitis vaccines and live rabies vaccine from different Mexican manufacturers, eight lots of MDCK, MDBK and BHK-21 cell cultures, and 10 lots of FBS subjected to gamma irradiation, 24 samples in tota). Fifteen of the 24 samples contained BVDV 1a, 1b and 2a.

BVDV-3 was detected in a batch of commercial vaccine against peste des petits ruminants, used in some administrative regions of the Republic of Tajikistan [76]. The role of BVDV-3a of the Italian-Brazilian group in the incidence of respiratory diseases of calves, abortions, systemic infections and enteritis in calves and adult animals in several farms in Siberia has been established. The source of infection was contaminated live vaccine [9].

Vaccines for humans. The plasticity of pestiviruses remains the question of their zooanthroponotic potential open. Facts of contamination of vaccine preparations for medicine have been reported since the late 1990s and early 2000s. M. Giangaspero et al. [77] studied 29 monovaccines against measles, mumps, rubella, polio, and eight polyvalent drugs containing measles, mumps, and rubella antigens, 24 drugs were produced in Europe, 10 in the USA, and 4 in Japan. As a result, 5 vaccines (13.1%) contained bovine pestivirus RNA. Three of them (two

vaccines against measles and one against rubella) came from Europe and two (against mumps and rubella) were made in Japan. Analysis of nucleotide sequences confirmed the presence of BVDV RNA in vaccines, with BVDV 1b detected in one measles vaccine, and BVDV 1a and 1c in Japanese mumps and rubella vaccines, respectively. BVDV-1d was present in two European measles and rubella vaccines. The viral genome has also been detected in a human influenza vaccine [78]. R. Harasawa et al. [79] tested live viral vaccines against measles, mumps, and rubella. The BVDV-1 genome was found in two combined measles-mumps-rubella vaccines and in two monovalent vaccines against mumps and rubella, as well as against Japanese encephalitis.

The consequences of contamination of cell cultures in which vaccine strains multiply depend on their origin. S.A. Audet et al. [80] analyzed 38 lots of viral vaccines and five lots of interferon-alpha produced in the United States. All drugs gave a negative result in RT-PCR, with the exception of the virus vaccine obtained in a rabbit kidney cell culture. In order to determine the permissiveness for replication of bovine pestiviruses, the cell lines used for the production of these vaccines were experimentally infected with the NADL strain of BVDV. Human MRC-5 and WI-38 cells did not support viral replication. Signs of pestivirus infection were found in Vero, CHO, CEF, etc. These data indicate that viral vaccines produced in human cell cultures have a low risk of contamination with pestiviruses, while those in cell lines of other animal species have a high risk.

E. Studer et al. [81] tested 36 batches of live human viral vaccines for contamination with viral diarrhea virus and detected BVDV-1a RNA in 33% of batches of mumps vaccine from one manufacturer. The authors concluded that the virus enters the diploid human cell culture MRC-5, in which the vaccine strain proliferates, from contaminated fetal serum, but cannot replicate. All attempts to detect viral antigen in this cell culture or to infect it with BVDV were unsuccessful. Thus, BVDV contamination of fetal sera used in the production of vaccines in MRC-5 cell culture does not pose an immediate threat to human health. In addition, gamma irradiation of the serum destroys virus particles, which prevents it from getting into vaccines.

M. Laassri et al. [82] used RT-PCR, immunofluorescenc, and sequencing methods to examine seven lots of trivalent (measles, mumps, and rubella) vaccine, one lot of measles monovaccine, 17 lots of FBS from different suppliers, four lots of horse serum, two lots of bovine trypsin, and five lots of pork trypsin. All batches of FBS and one batch of bovine trypsin contained the BVDV-1 genome, but vaccines and other samples did not. These data supported the view that BVDV nucleic acid may be present in bovine starting materials but not in finished vaccine products.

At present, it is not completely known if cattle pestiviruses can cause disease in humans. The special literature describes cases of detection of virus-neutralizing antibodies to BVDV-1 in blood of people who had contact with infected livestock [59, 60]. Antibodies to BVDV were detected in 40% of sera collected from twins with schizophrenia [83]. In another study, BVDV antigen was detected in 23.6% of fecal samples from children with gastroenteritis [84].

Analysis of brain tissue samples from fetuses with microcephaly during the 2015 Zika disease outbreak in Brazil suggested the presence of peptides from a BVDV-like virus polyprotein [85]. On this basis, it has been hypothesized that Zika virus may act together with BVDV to cause fetal microcephaly in humans [86]. However, evidence that BVDV plays a role in this pathology is still insufficient [87].

In Russia, such work has not been carried out, despite the relevance and scientific significance of the problem and the need to carry out such research.

Sperm of stud bulls. One of the main sources of spreading many viral diseases is the sperm of stud bulls, but in viral diarrhea this route is not considered

significant. Semen is dangerous when it is obtained from seronegative persistently infected (PI) bulls which excrete the virus throughout their lives in secretions and excreta, from transitively infected bulls that secrete the virus for 2-3 weeks before the appearance of specific antibodies, and from seropositive bulls with testicular infection that secrete the virus for a long time [88-91].

A.V. Nefedchenko et al. [92] detected the first type virus in 0.4% of series from 4.1% of bulls at a breeding farm in semen collected from a PI bull. The fertilizing ability of infected sperm is reduced, and the consequences of insemination of seronegative individuals are reduced fertility, early embryonic mortality, abortion and the birth of PI calves [88].

Embryos. Currently, embryo transfer, along with artificial insemination, is widely practiced in dairy farming. Embryos can be contaminated with BVDV via infected oocytes or follicular fluid from persistently or transitively infected cattle. Embryos obtained from PI donors and transplanted into seropositive recipients do not always transmit the virus to their offspring [88].

The primary source of infection of donor cells during embryo production may also be fetal serum. The risk of virus transmission through contaminated oocytes from the slaughterhouse is relatively low and can be minimized by removal of cumulus cells and appropriate oocyte washing procedures [93].

Therefore, the risk of embryonic transmission of BVDV is considered negligible for BVDV, and trypsinization of embryos and transfer to seronegative recipients reduces the risk of infection [94].

To summarize, we note that the development of biological products (vaccines and others) for veterinary and especially medical use must strictly adhere to the requirements of the World Organization for Animal Health [95], Russian and European Pharmacopoeia [96-97]. A possible way to solve the problem, along with the control of raw materials, may be serum-free cell culture media in biotechnological production of vaccine preparations and prohibition of animal materials [98] with appropriate controls.

Thus, contamination of mammalian cell cultures with bovine pestiviruses poses a serious threat and can lead to false results of diagnostic tests, viral contamination of biological preparations produced using infected cell cultures, and, finally, to infection of recipients. Only careful routine monitoring and culling of animals used to collect fetal serum or organs for cell culture, all serum series, cell cultures and biological products derived from them, can prevent potentially dangerous contamination with pestiviruses. It is necessary to take into account the plasticity of viruses and the emergence of new species and subtypes. In globalization, the rapid development of cellular biotechnologies, and modern trends in veterinary and medicine, the demand for fetal blood serum (FBS) from cattle increases every year. The problem of FBS contamination remains relevant due to increased demand, the presence of unscrupulous manufacturers and sellers, non-compliance of product labeling with the declared one and the lack of unified control methods. With the expansion of the fetal serum market, the pestiviruses requires special attention. They were isolated from commercial blood serum pools used for cell culture and production of biologicals, and pose a risk due to potential spread to new regions. The lack of production of such biologicals in our country creates the risk of appearing dubious quality products from various manufacturers on the market.

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