Original Article

Molecular and Histopathological Identification of Bovine Viral Diarrhea Virus in Aborted Fetuses From a Vaccinated Herd Around Tehran Province

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ABSTRACT

Background: Bovine viral diarrhea virus (BVDV) is one of the most important economic diseases affecting many livestock-producing countries. The disease can lead to embryo resorption, abortion, and the birth of weak calves. BVDV control strategies include vaccination and implementation of effective biosecurity.

Objectives: The current research with the aim of highlighting the biosecurity principles, investigated the presence of the virus in cases of abortion in a vaccinated dairy herd using molecular and histopathological methods.

Methods: A total of 23 swab samples from abortion secretions, 7 aborted fetuses, and parenchymatous tissues from 2 slaughtered calves with neurological symptoms were sent to the laboratory for the detection of BVDV using real-time polymerase chain reaction (real-time PCR and histopathological examination.

Results: Four aborted fetuses (57.14%) and 15 swab samples (65.22%) were detected as positive for BVDV by the molecular test. The two slaughtered calves with neurological symptoms, including exophthalmos, ataxia and hydranencephaly were both BVDV positive. The positive samples in the molecular test, showed mild to severe changes related to BVDV infection except for the heart samples. The specific findings included lymphoid tissue depletion in the spleen, multifocal gliosis in the cerebrum, vesicular degeneration in the hepatocytes, multifocal purulent interstitial nephritis, interstitial pneumonia, and multifocal necrotic cryptitis in the histopathological examination.

Conclusion: The results of the present research indicate that vaccination alone is not enough to control BVD and strict adherence to biosecurity protocols is as important as vaccination in the prevention and control of the disease.

Keywords: Abortion, Bovine viral diarrhea, Dairy cattle, Histopathology, Real-time polymerase chain reaction (real-time PCR)

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Introduction

Bovine viral diarrhea (BVD) is recognized as one of the most economically significant diseases of cattle, which affects many cattle-producing countries (Givens et al., 2012). The causative agent is a member of the genus Pestivirus, a group of small, enveloped RNA viruses in the family of Flaviviridae (Yildirim et al., 2011). The disease can lead to embryo resorption, abortion, the birth of weak calves, increased treatment costs, prolonged calving intervals, and reduced milk production (Abbasi et al., 2016).

Various infectious pathogens negatively impact the reproductive system, among which the BVD virus (BVDV) is a significant agent affecting both production and reproduction in dairy cattle. Fetal infection with non-cytopathic BVDV before the development of immune competence can result in early embryonic death, abortion in the last months of pregnancy, or the birth of an immunotolerant calf that is persistently infected (PI). This situation can later necessitate culling to eliminate the virus from the herd (Wathes et al., 2019).

Epidemiological studies indicate that the virus is widely distributed across various regions and can persist in herds through multiple transmission routes, including direct contact and vertical transmission from dam to calf (Ghaemmaghami et al., 2013; Khodakaram-Tafti & Farjanikish, 2017; Noaman et al., 2020). In a study conducted by Abbasi et al. (2016) 123 cattle (68.33%) were proven seropositive in Sistan and Baluchistan, Iran. Similarly, in another investigation, from 803 collected serum samples belonged to non-vaccinated herds of Arak, 436(54.3%) were seropositive for the virus infection (Ghaemmaghami et al., 2013).

Research has shown that demographic factors, including herd size and density, play a key role in predicting the prevalence of infection in populations where BVD is recurrent (Khodakaram-Tafti & Farjanikish, 2017). Basic principles of infectious disease control rely on two main strategies: eliminating the pathogen's reservoir and preventing or limiting transmission from infected individuals to susceptible animals, with BVDV being no exception (Walz et al., 2018). Vaccination against BVDV is a common practice in many developed countries (Newcomer et al., 2017). Vaccination can boost immunity in vulnerable cattle populations, helping to prevent clinical disease after exposure to BVDV and reducing the risk of viremia that could result in transplacental infection and the birth of PI offspring (Newcomer et al., 2017). However, vaccination alone does not necessarily lead to complete immunity to infection and abortion. In the study conducted by Wathes et al. in 2019, vaccinated cattle experienced a significant reduction in both abortion and fetal infection rates by nearly 45% and 85%, respectively, but did not achieve complete immunity (Wathes et al., 2019).

The current study aimed to detect BVDV in aborted fetuses from a vaccinated dairy cattle farm using molecular and histopathological methods and to describe other risk factors that should be considered in a susceptible farm despite vaccination.

Materials and Methods

Study design and sample collection

A total of 23 pregnant cattle lost their fetuses at a gestational age of over four months. Additionally, 2 calves with neurological symptoms were born in the herd. All the aborting cattle and those that gave birth to abnormal calves had received vaccine against BVDV according to the herd vaccination protocol.

Vaginal swab and abortion discharges were collected from all the aborting cattle. Tissues, including spleen, heart, liver, kidney, lung, small intestine, cerebrum, and cerebellum were taken from 7 aborted fetuses and 2 culled calves with neurological signs. The tissue samples were divided into two parts; one part was collected in sterile urine container for the molecular test and the other part was placed into another urine container with 10% neutral buffered formalin solution for histopathological examinations.

Real-time polymerase chain reaction

RNA was extracted from the swabs and the homogenized tissues using the CinnaGen RNA extraction kit. RNA was reverse transcribed using Superscript II (Invitrogen) and random primers (Amersham Pharmacia Biotech). Real-time polymerase chain reaction (RT-PCR) was conducted according to the recommended method by the World health Organization for Animal Health, 2024. Each reaction included an internal amplification control. Forward and reverse primers and the TaqMan probe used included BVD 190-F 5'-GRA-GTC-GTC-ART-GGT-TCG-AC, V326 5'-TCA-ACT-CCA-TGT-GCC-ATG-TAC and TQ-pesti 5'-FAM-TGC-YAY-GTG-GAC-GAG-GGC-ATG-C-TAMRA-3', respectively.

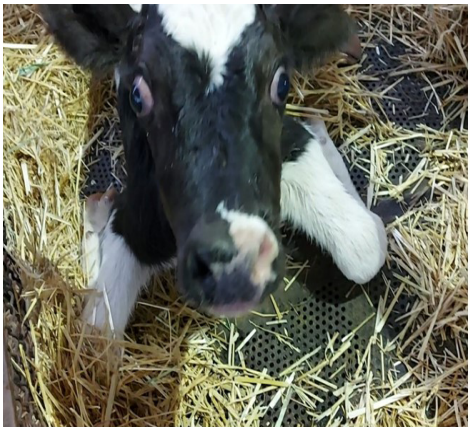

Figure 1. Exophthalmos in a calf with neurological symptoms that tested positive for BVDV in both molecular and histopathological examinations

Nuclease free water was considered a negative control in all the stages, and the standards of the commercial kit were used as positive controls. Real-time PCR was performed using 2× TaqMan Universal PCR Master Mix, 1 µ concentration of each primer/probe mix, 2 µ tRNA (40 ng/µL), 1.5 µ RNase/DNase-free water, 1 µ 25× enzyme mix, 12.5 µ 2× RT buffer, and sample (or controls). The final volume of the reaction mixture amounted to 25 µ, including 20 µ master mix and 5 µ of sample.

Amplification was carried out using a Rotor-Gene Q Series (QIAGEN) with the following cycling parameters: heating at 48 ˚C for 10 min, 95 ˚C for 10 min (single denaturation step), followed by 45 cycles of 95 ˚C for 15 s and 60 ˚C for 1 min (annealing and extension).

Histopathological examinations:

The tissue samples were isolated from each aborted fetus and the slaughtered calves and then, immediately were placed in 10% neutral buffered formalin. The specimens were dehydrated and embedded in paraffin and subsequently, were sectioned with a microtome to obtain 5 µm thick of each tissue. Following deparaffinization and rehydration, the sections were stained with hematoxylin and eosin (H&E) for microscopic analysis.

Results

The calves with neurological symptoms, including exophthalmos, ataxia, and hydranencephaly (Figure 1) were born in the herd and were found to be BVDV-positive in the molecular method. None of the fetuses showed gross pathological changes.

Real-time polymerase chain reaction results

The samples with a cycle threshold (CT) value less than 40 were considered positive. Four out of 7 fetuses (57.14%) and 15 swabs from aborted cattle (65.22%) sent to the laboratory were found positive for BVDV by the molecular test.

Histopathological results

Pathological lesions attributed to BVDV were visible in the real-time RT-PCR positive samples obtained from the spleen, cerebrum, lung, small intestine, liver, and kidney. Lymphoid tissue depletion was severe in the spleen sections, which was a diagnostic finding for the virus detection. Cerebrum showed multifocal gliosis, but cerebella were normal. Mild vesicular degeneration in the hepatocytes and chronic multifocal purulent interstitial nephritis were visible. The lung sections were indicative of a moderate interstitial pneumonia. Inflammatory response in the small intestine sections, was found by multifocal necrotic cryptitis along with the accumulation of mucus, epithelial debris, and leukocytes. Since BVDV infection leads to the lymphoid tissue depletion along with suppurative and non-suppurative inflammatory responses in the organs, including lung, kidney, intestine, spleen, heart, brain and liver (Montgomery et al., 2008), we strongly suggested that the pathological findings were attributed to BVDV.

Discussion

The results of the current study indicated a significant abortion rate of 65.22% due to BVD in the cattle vaccinated against the disease, which was confirmed by both molecular and histopathological methods. Histopathological examination which was used on real-time PCR-

positive samples to find microscopic changes of BVDV in the fetuses, clearly showed the virus invasion to fetal tissues. Severe lymphoid depletion observed in the spleen, was consistent with the previous studies showing similar findings in peripheral lymphoid tissues due to BVDV-induced immunosuppression (Khodakaram-Tafti & Farjanikish, 2017). Another key histopathological finding noted by Khodakaram-Tafti and Farjanikish, (2017), was multifocal necrotic cryptitis in aborted fetus. Multifocal gliosis in the cerebrum, which was visible in the current study, may represent an early or milder response to BVDV infection, while pseudocyst formation and neuropile degeneration, as described by Montgomery et al. (2008), suggest more advanced lesions. Among all the mentioned histopathological changes, lymphoid depletion and epithelial necrosis of intestine are considered pathognomonic for BVD infections, which were demonstrated in our study.

It is plausible that the real number of positive cases was higher in the current evaluation, as not all the fetuses were accessible for testing due to the factors, such as accidental crushing by heavy machinery of the farm or scavenging by stray dogs. Our findings suggest that vaccination alone may not be sufficient in preventing BVD-induced abortions. Factors other than vaccination affecting the eradication of BVD include biosecurity and control of other diseases (Pinior et al., 2019).

The studied herd exhibited weaknesses in health and management practices despite vaccination. Stray dogs were observed at the farm, and parts of the fetuses' body, which had been eaten by dogs were found in the cattle's feed. These factors contributed to the spread of diseases and compromised herd health. Dogs serve as definitive hosts for *Neospora caninum*, facilitating its transmission and persistence within cattle herds. Studies have demonstrated a potential link between *N. caninum* infection and the occurrence of BVD (VanLeeuwen et al., 2021). In some cases, infection with the parasite may exacerbate BVD symptoms, including abortions. As reported by VanLeeuwen et al., (2021) co-infections with *N. caninum* and BVD can increase the severity of clinical signs, such as abortions. In the current study, antibody against *N. caninum* was detected in 7 of the serum samples and concurrent infection with BVDV was diagnosed in 4 of the aborted animals. As a result, controlling other diseases, especially neosporosis is another factor for eliminating BVD infection.

The overall hygiene conditions in the studied herd were unsatisfactory, as evidenced by the significant decline in production. Implementing effective hygiene management practices can substantially reduce the risk of BVD outbreaks. Accordingly, good management and biosecurity measures are equally important as vaccination for BVD eradication, and both should be considered components of a control strategy (Moennig & Yarnall, 2021).

Given the high prevalence of BVD in Iran, it is essential to use appropriate vaccine strains and minimize exposure to other pathogens. The significant abortion rate observed in this study shows the detrimental impact of BVD on cattle reproduction. The impact of an outbreak of BVD on reproductive performance and milk production was investigated in 28 Spanish dairy herds using an observational study design, and the group without vaccination showed a higher mean calving-to-conception interval (CCI) and mean number of inseminations per conception (AI/P) (Arnaiz et al., 2021). The reliance of dairy industry on both production and reproduction, lead BVD-induced abortions and neonatal abnormalities caused by the virus to have a substantial negative impact on the economic viability of dairy farms. This is particularly concerning in countries like Iran, where BVD remains endemic (Khezri, 2015).

Conclusion

To effectively monitor herd health and prevent BVD outbreaks, regular testing of calves is necessary. In addition to employing proper vaccination strategies, implementing comprehensive biosecurity measures is crucial. This includes controlling animal density, using disinfectants, limiting the presence of stray dogs, managing other diseases, and ensuring adequate nutrition. Despite vaccination, a substantial number of cases tested positive, underscoring the critical need for strict adherence to biosecurity protocols along with vaccination.

Ethical Considerations

Compliance with ethical guidelines

This article does not contain any studies with human or animal participants.

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Authors' contributions

Study design: Mona Hamedi; Data collection: Shayan Mofidi and Amir Mohammad Fallah; Writing: Saber Vahedi.

Conflict of interest

The authors declared no conflict of interest.

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