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Molecular and Histopathological Diagnosis of Bovine Viral Diarrhea Virus in a Dairy Cattle Farm Associated with an Abortion Storm

Ahmad Paidar [1], *Mona Hamedi* [2*]

1 Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

2 Department of Veterinary Pathobiology Sciences, SR.C., Islamic Azad University, Tehran, Iran.

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*Corresponding Authors: Mona Hamedi: Department of Immunopathology, Faculty of Veterinary Medicine, Islamic Azad University, Science and Research Branch, Tehran, Iran. Tel: +98-21-44868536, E-mail: mhamedi42@ut.ac.ir

ABSTRACT

Background: Bovine viral diarrhea (BVD) which caused by a pestivirus, results in huge economic losses through abortion, weight loss, drop in milk production and even death of the affected animal. Reproductive disorders due to the virus infection are common in unvaccinated herds which are predominant causes of dairy cattle exclusion. The present study diagnosed BVD infection associated with an abortion storm in a farm following vaccination cessation. In addition, the present report investigated a variety of the disease complications other than abortion and it emphasized on the elimination of all the risk factors before any interruption in vaccination plan.
Methods: The specimens were collected from 66 aborted fetuses and examined by real-time RT-PCR and histopathological methods for the presence of BVD virus.
Results: Real-time RT-PCR detected BVDV in 47.14% of the aborted fetuses. The lesions of BVD were visible in the samples obtained from aborted fetuses.
Conclusion: In the studied herd, we could see several complications of BVD such as infertility, abortion and agalactia. Each of the disorder has significant economic losses on dairy cattle herds, so it is necessary to implement control programs such as biosecurity and monitoring before any decision to stop vaccination.

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Introduction

Bovine viral diarrhea (BVD) which adversely effects on reproductive, respiratory and gastrointestinal tracts is one of the most challenging diseases of cattle in many parts of the world (1, 2, 3). The causative agent is a pestivirus of the family flaviviridae and the economic losses of the disease include abortion, neonatal death, calf abnormalities, the costs of replacing animals and treatment (4). BVD infection in pregnant animals is usually subclinical, but following viremia, transplacental spread of the virus is likely to occur (5). The affected fetus may encounter with three different consequences including abortion, malformation or the virus may establish persistent infection (PI) in the fetus. The latest results in the survival and transmission of the virus among cattle which is responsible for increasing the disease incidence (6).

The prevalence of BVD in different countries is approximately 40-80% (4, 7) and despite vaccination, seroprevalence of the virus has remained high (7). A meta-analysis study on the disease status in vaccinated cattle showed BVD virus (BVDV) in 85% of the fetuses and introduced it as the cause of abortion in 45% of vaccinated cattle (8). The prevalence rate of the disease in Australian farms was also estimated as 80% (9). Iran is among the countries with high rate of BVD infection and the disease is endemic. In a serological study in 2019, the distribution of BVDV was 28.6% in 10 dairy herds in Zanjan province (10). Kaveh et al. in 2017, evaluated abortion etiologies among cattle in Qazvin province and BVDV was detected in 20.31% of the fetuses (11).

As reproduction has a significant role in dairy farms incomes, various studies have been addressed abortion aspect of BVD (2, 11, 12, 13). Detection of the virus in abortion cases represents the agent circulation in a herd which is associated with defects in BVD control program (14). Primary control strategy is based on the simultaneous vaccination and elimination of PI calves which massively shed the virus. In the study of Rypula et al. in 2020 in Poland, non-vaccination was strongly associated with BVD seropositivity (15). However, other measures including biosecurity and prevention of introducing new infected replacements are important as well (16).

Since vaccination reduces the disease incidence, some of the herds decide to stop the procedure to implement an eradication plan. In such herds any exposure to the virus is prevented and new positive cases should be slaughtered. However, in endemic regions, vaccination cessation may contribute to the return of the disease with various signs in the affected herd. The present study diagnosed BVD infection associated with an abortion storm in a farm following vaccination cessation using molecular and histopathological examinations. In addition, the present report investigated a variety of the disease complications other than abortion and it emphasized on the elimination of all the risk factors before any interruption in vaccination plan.

Materials and Methods

The study design

A dairy herd in Qazvin province, Iran, decided to stop vaccination against BVDV for costs management since the results of ear notch test were all negative. Within a year after vaccination cessation, the herd had reproduction and production depression and experienced huge economic losses. BVD recurrence was suspected and 66 aborted fetuses were collected for the diagnostic procedures.

Preparation of the samples

Samples including spleen, heart, liver, kidney, lung, small intestine, cerebrum, and cerebellum were taken from the fetuses. The samples were divided into two parts; one part was collected in sterile urine tube for the molecular test and was transmitted to the laboratory with ice pack all surrounded. The other part of the samples was transmitted into another urine tube with 10% formalin solution for the pathological examinations.

Real-time RT-PCR

RNA was extracted from the homogenized tissues using CinnaGen RNA extraction kit. RNA was reverse-transcribed using Superscript II (Invitrogen) and random primers (Amersham Pharmacia Biotech). Real-time RT-PCR was conducted according to the recommended method of world organization for animal health (17). Each reaction included an internal amplification control. Forward and reverse primers and 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.

Nuclease free water was considered as negative control in all the stages and the standards of commercial kit were used as positive control. Real-time PCR was performed using 2X TaqMan universal PCR Master Mix, 1µ concentration of each primer/probe mix, 2 µ tRNA (40 ng/µl), 1.5µ 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 25µ including 20µ master mix and 5µ of sample.

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

Histopathological examinations

The pieces of 6-8 cm- thick of the formalin tissues were cut and stained using hematoxylin and eosin method (H&E). Then the samples were examined for the pathological changes by light microscopy.

Results

The herd status

During the study course, the herd showed a significant decline in the reproduction programs and a half of the pregnant cattle aborted their fetuses following vaccination cessation which indicated an abortion storm in the herd. The fetuses had no macroscopic lesions. Nervous signs and hydrocephalus were observed in 7.42% of the newborn calves. A total of 21.98% of calves were excluded with nervous signs and other disorders such as pneumonia and heart failure. Reproductive disorders were the cause of 29.86% of the dairy cattle exclusion. Fertility rate had also a considerable reduction from 71.83% to 52%. The milk production plunged from 37 kg/day to 32.73 kg/day (Fig. 1.).

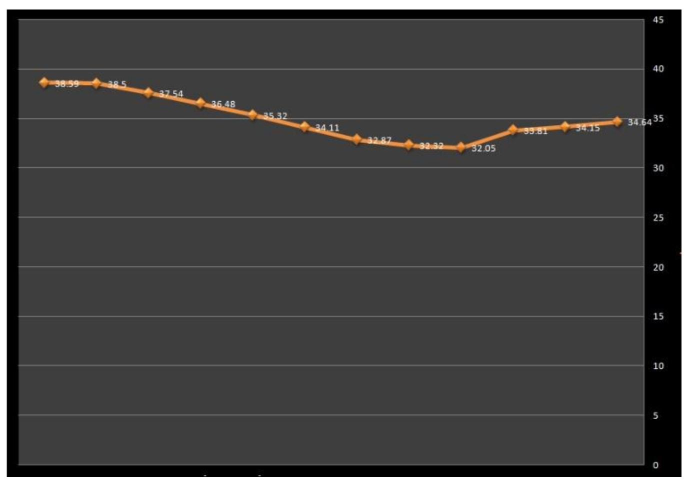

Fig 1. The milk production plunged from 37 kg/day to 32.73 kg/day following BVDV infection.

Real time RT-PCR

The samples that had a cycle threshold (Ct) value less than 40 was considered positive. Real-time RT-PCR detected BVDV in 47.14% of the aborted fetuses.

Pathological findings

The lesions of bovine viral diarrhea were visible in all the real-time positive samples obtained from spleen, cerebrum, lung, small intestine, liver and kidney. Lymphoid tissue depletion was severe in spleen. Cerebrum showed multifocal gliosis but the tissues of cerebellum were normal. There was a mild vesicular degeneration in hepatocytes and chronic multifocal purulent interstitial nephritis was visible in kidney. There was a very mild interstitial pneumonia and in the small intestine samples, multifocal necrotic cryptitis was found. The crypts were dilated and mucus, epithelial debris, and leukocytes were accumulated. The tissues of the heart samples were normal. The pathological findings typically demonstrated the presence of BVDV in the tissues.

Discussion

The results of the current study identified a BVD occurrence with all the signs manifestations including abortion (47.14%), neonatal abnormalities of nervous system, decline in reproduction rate, reduction in milk yield (Fig. 1) and higher rate of susceptibility of the cattle to other diseases. Molecular and histopathological results of the fetal samples confirmed that high BVDV infection contributed to the herd situation. Abortion storm due to the virus has been reported in other studies especially in herds without vaccination implementation. In a study in 2017 in Iran, 20.31% of abortion of dairy herds was attributed to BVD (11). In another investigation in southwest of Iran in 2012, BVD was introduced as one of the most important causes of abortion by allocating 51.85% of cases (18). Lee et al. in 2018 identified BVDV in 350 aborted fetuses which belonged to 314 cattle farms in Korea (19).

BVD infection may also lead to other reproductive problems such as fertilization failure, dystocia, and early embryonic death (20). In the present study, 29.86% of dairy cattle had reproductive disorders and only 52% which received interstitial insemination were pregnant. The rest of the animals were recorded as infertile. Burgstaller et al. in 2016 showed that BVD had a significant effect on calving interval and first service conception. In the study, the calving interval in the affected herds was 7 days longer than the unaffected ones and first service conception in the healthy cattle was 1.3 times more than the affected animals (21). It is evident that the virus replicates in ovarian follicles and causes ovarian dysfunction (22). In addition, the secretion of ovarian hormone is affected by acute BVD infection which can lead to long-term infertility (Moennig et al., 2021). A dramatic decline in fertility rate with 19.83% reduction in the current study, shows the virus association with either failure in fertilization or early embryonic death.

The present pathological findings were indicative of predominant BVDV lesions in different fetal tissues. Exposure of fetuses younger than 150 days to the virus results in pathological changes in various organs (Casaro et al., 1971). However, BVDV has tropism to the neurological system (Fernandez et al., 1989) and in our study, multifocal gliosis in the cerebrum was visible. Moreover, there were 7.42% newborns with nervous signs such as hydrocephaly in the herd and a total of 21.98% of calves were slaughtered due to neurological disorders and other problems including pneumonia and heart failure.

The other target organs of the virus are respiratory and intestinal systems (Moennig et al., 2021). In addition, infection with the virus may lead to mononuclear inflammatory cell infiltration of peribronchiolar and intra- alveolar tissues in the lung of infected fetuses (Murray, 1991). Fulton in

2013, reported incomplete pulmonary development in BVD positive fetuses (Fulton, 2013). We also found interstitial pneumonia in the samples from the lung tissue and multifocal necrotic cryptitis in small intestine.

Abortion storm and other reproductive depression in the current BVD occurrence inflicted a huge economic loss to the herd. However, drop in milk production which is another common feature of BVD (Daves et al., 2016) was also damaging (Fig. 1). Analyzing clinical signs of BVD infected herds compared to healthy ones indicates that sudden drop in milk yield is a frequent complication of the disease in dairy cattle (15).

According to the present herd history, the disease was recurrent within a year after vaccination cessation. Since BVD showed downward trend and there wasn't any new positive result in ear notch test for 6 months, vaccination program was stopped to manage extra costs. However, the current results showed recurrence of the disease as the consequence of lack of vaccination and improper biosecurity.

The most important aspect of BVD control is based on vaccination (4, Moennig and Becher, 2018; Pinior, 2019,). Moreover, implementation of biosecurity and monitoring of the herd are essential as the other parts of the control program. In another word, vaccination should not be stopped until reducing the risk of exposure. Arnoux et al., in 2021 in France concluded that it is better not to stop vaccinating as long as every measure is not completely implemented to reduce the risk of BVDV introduction (16). In addition, antibodies produced by vaccination don't have long life so in an endemic region, the possibility of the disease recurrence is high (16). In the current studied herd, infection with Neospora caninum was substantial. It has been shown that prevention of other diseases specifically neosporosis effectively reduces BVD infection (Ghazi et al., 2007; 5). In the study of Bjorkman et al. in 2000 in Sweden, the relation between the presence of antibodies against N. caninum and BVDV in aborted fetuses was statistically significant (Bjorkman et al., 2000). In the research of Namavari et al. in 2012 in Iran, all the Neospora positive fetuses were also infected with BVD virus (18). This shows prevention of neosporosis should be considered as a part of BVD control plan.

Conclusion

The present study highlighted simultaneous vaccination, identification of risk factors and elimination of PI animals to reduce the virus circulation and for gradual eradication of BVD.

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Ethics approval and consent to participate

None.

Conflict of interest

None delared.

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