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REVIEW ARTICLE

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# Bovine viral diarrhea (BVD): A review emphasizing on Iran perspective

**Mohammad Khezri**

*Veterinary Research Department, Kurdistan Agricultural and Natural Resources Research Center, AREEO, Sanandaj, Iran.*
**Correspondence: khezri1836@yahoo.com*

## ABSTRACT

**Bovine viral diarrhea (BVD) is one of the most important diseases of cattle responsible for major economic losses in dairy industries of Iran. So far, no nationwide program has been taken in Iran to control and eradicate the disease. Moreover, until now, no vaccination program has been practiced against BVD in Iran, although the disease is prevailing in the country. For effective controlling of BVD, it is necessary to cull the affected animals, and new entry of BVD in the farm should be prevented. Focusing on biosecurity in systematic control programs of BVD can also reduce the risks of introduction and spread of other epizootic and zoonotic diseases, thereby improving both cattle health and welfare in general. In this review paper, an overview on BVD emphasizing on Iran perspective has been discussed focusing on clinical manifestations of BVD, routes of transmission of BVD virus (BVDV), its diagnostic methods and possible prevention strategies.**

## Keywords

Bovine Viral Diarrhea Virus, Cattle, Control, Diagnostic, Prevalence

## ARTICLE HISTORY

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## INTRODUCTION

Bovine viral diarrhea virus (BVDV) is the causal agent of Bovine viral diarrhea (BVD) and mucosal disease; both the diseases are economically important (Laureyns et al., 2010). The clinical manifestations of BVDV infection in cattle were first described by Olafson et al. (1946). In Iran, the disease was first described in 1970 by Mirshamsy et al. (1970). Pestivirus genus, a member of the family Flaviviridae, contains a single stranded RNA genome (Liu et al., 2009). In this genus, there are four species: BVDV-1 and 2, border disease virus, and classical swine fever virus (Heinz et al., 2000; Krametter-Froetscher et al., 2007; Yazici et al., 2012). In addition, phylogenetic analyses have shown that BVDV can be divided into at least three different genotypes, such as BVDV-1, 2 and 3 (Mudry et al., 2010). In many countries, all these species are endemic (Ridpath, 2012). Sheep, goats, cattle, pigs and wild animals, such as deer and wild boars, can be infected with BVDV, and these animals may act as an important source of infection (Liu et al., 2009; Krametter-Froetscher et al., 2010).

In cattle, reproductive, digestive, respiratory, and other body systems are affected by BVDV, but in calves, this virus can cause severe malformations (Al-Afaleq et al., 2007; Saa et al., 2012). The fetal stage and fetal stage after infection are the two phases of primary reasons for transmission and dissemination of BVDV in herds (Bazargani et al., 2008; Laureyns et al., 2010; Segura-Correa et al., 2010). The environmental factors and knowledge of herd management which augment the risks of BVDV infection would make better the ability to control and impede the transmission, minimizing the unfavorable effects of BVDV infection on herd health and productivity (Saa et al., 2012).

## CLINICAL MANIFESTATION OF BVDV

BVDV is an economically imporant pathogen causing gastrointestinal (GI), respiratory and reproductive diseases in cattle. Although acute infections with BVDV are often asymptomatic, clinical signs, such as depression, oral erosions, lack of appetite, decreased milk production, diarrhea, embryonic death, abortion, teratogenesis, respiratory problems, immune system dysfunction, and death may occur (Rodninga et al., 2012). BVDV virulence differs markedly, and due to a

transient immunosuppression, acute infections are often exacerbated by secondary infections (Baker, 1995).

The clinical offering of a BVDV infection is based on viral strain and the animal's immune and reproductive position at the time of infection (Givens et al., 2012; Rodninga et al., 2012). Acute infections of cattle happen especially in young animals, and may be clinically in apparent or linked with diarrhea (Baker, 1995; Givens et al., 2012). Severe cases are characterized by fever, anorexia, depression, erosions and hemorrhages of the GI tract, diarrhea, and dehydration. In mild cases, diarrhea may not be prominent. Most BVD infections are subclinical, and the course of the disease varies from 2-3 days up to 4 weeks; however, this results in measurable increases in antibody levels. Calves with clinical BVD as dull, depressed, anorectic, and mild bloat may occur. Temperatures are 40-41°C early, but usually return to normal or below in 1-2 days and before diarrhea occur (Brock, 2004). However, when a dam is infected with BVD for the time of pregnancy, trans-placental infection may happen, and as a result, fetal abortion, mummification or congenital defects may occur depending on the gestation stage (Kozasa et al., 2005).

In non-pregnant susceptible animals, occurrence of BVD depends on genotype and strain of the virus (David et al., 1994; Pellerin et al., 1994; Hamers et al., 1999). However, in non-immune pregnant animals, the BVDV may infect the conceptus without considering the time of gestation (Duffell and Harkness, 1985; Lindberg, 2003). Persistently infected (PI) animal with BVDV occurs when a bovine fetus is infected with a non-cytopathic strain of BVDV before 125 days of gestation (Leyh et al., 2011). In general, PI cattle display varied clinical advent such as diarrhea, pneumonia (as a result of immunosuppressant), poor growth, some succumb to mucosal disease, and some PI cattle show no clinical manifestations.

The PI cattle on dairy farms are doubtful as the cause of milk production loss and/or increase in event of secondary or opportunistic infections (Baker, 1995). Thus BVD plays a main role in epidemiological aspects of other bacterial and fungal diseases. Studies showed that changes in placenta caused by BVDV could accept the other pathogens passing fetal placenta barrier (Murray, 1991). Seropositive rate of BVDV in older cattle was more in younger animals; it seems that because of the increment of exposure to respiratory viruses during life (Shirvani et al., 2012). However, the first outbreak of gastro-neuropathogenic BVDV infection occurs in Iran with the most important clinical signs in affected calves were fever (40-42°C), severe anorexia, hyperpnoea and coughing, muco-purulent nasal discharge, conjunctivitis, stomatitis with erosions in the palatine, gum, dorsal and ventral side of tongue, simple o bloody diarrhea with or without melena and progressive weight loss and severe depression, recumbence, incoordination, severe convulsion and death (Bazargani et al., 2008).

In dairy herds, high levels of milk production can raise metabolic disorders such as plasma concentrations of non-esterified fatty acid (NEFA) (Rezaei Saber et al., 2012). Increased NEFA levels are temporally correlated with immune function suppression and may contribute to disruption of the immune system in dairy cows (Rukkwamsuk et al., 1999). It is probably that metabolic disease in cow increases the susceptibility of the cow to evolution of infectious disease such as BVD. However, this is serious that relationships between the quantity of immune response and production traits of dairy cows are largely secret (Wagter et al., 2003).

## RESERVOIR AND TRANSMISSION OF BVDV

There are many factors in the epidemiology of BVDV infections. PI animals, uncontrolled animal movement and interspecies transfer are the main factors for the spread of infection. PI animals are a key cause of spreading the infection and hence, they represent a risk to the flock (Loken et al., 1991). During early gestation, the young calves remain closer to the breeding herd of cattle. As a result, PI suckling calves are considered to be the primary source of BVDV infection in breeding herds (McClurkin et al., 1984; Duffell and Harkness, 1985; Wittum et al., 2001; Larson et al., 2004). However, mortality of PI calves before weaning is high due to fatal congenital defects and secondary infections (McClurkin et al., 1979; McClurkin et al., 1984; Brock et al., 1991). A 17-50% of PI calves may reach breeding age (Binkhorst et al., 1983; Barber et al., 1985; Houe, 1993). PI females of reproduction age are not only an origin of horizontal transfer of BVD, but will always make a PI calf themselves (McClurkin et al., 1979; Houe, 1993; Houe et al., 1995). The prevalence of PI animals in the cattle population reported 0.13-2.0% (Bolin et al., 1985; Howard et al., 1986; Houe et al., 1995; Wittum et al., 2001). However, PI animals have immunotolerance to the strain or strains with which they have been infected and commonly shed large quantities of BVDV throughout life (Brock et al., 1998),

thus exposing herd mates and jeopardizing efforts to control and/or eradicate BVDV. Subsequent to immunosuppression associated with BVDV infection, PI calves also had a 50% higher death risk within the first year of life than uninfected calves (Duffell and Harkness, 1985). PI has a clustered distribution, which means most herds contain only non-PI cattle but a few herds may contain several PI cattle (Bolin, 1990; Larson et al., 2004).

BVDV is shed in most excretions and discharge from transiently infected and PI cattle. It is commonly accepted that transiently infected cattle shed considerably lower amounts of virus and for a time of only 1 to 7 days (Brownlie et al., 1987; Kelling et al., 2002; Hessman et al., 2009). The vertical transmission plays an important role in BVDV epidemiology and pathogenesis; however, the virus transmission is mainly caused by direct contact between animals. The secretion and excretion (milk, feces, vaginal secretions and urine) of the infected animals plays an important role in BVDV distribution (Safarpoor Dehkordi and Haghighi, 2012). In sheep and cattle, Pestiviruses by affecting the reproductive system can cause economic losses in the animal industry. Pestiviruses transmitted vertical transmission and PI animals in flocks and herds.

PI and healthy animals common use of pastures in the summer is the important reasons for Pestiviruses transmission within a population. Pestivirus may be passed of PI sheep to cattle (Yazici et al., 2012). In all bodily fluids of PI animals in large amounts shed virus endlessly, thus, PI animals are the source of infection (Brock et al., 1991). Because of immune system disorders, PI animals are susceptible to other diseases (Howard, 1990; Potgieter, 2004). The fetus present in the infected cow can be weak at birth, ill-thrifty, and therefore more susceptible to other infections (Moennig and Liess, 1995). However, the calves receiving colostrum achieve a passive immunity against BVDV (Bolin and Ridpath, 1995). The maternal antibodies in calve can be detected within few hours after the first meal, and the level is declined at a rate of one half their remaining antibody titers every 21 days (Bolin and Ridpath, 1995). More importantly, infection in the first trimester of pregnancy can result into the birth of immune tolerant calves that are PI with BVDV. Therefore, it is very important to identify and remove them from the cattle herd (Lindberg, 2003). There is significant change in the prevalence of antibody positive animals. Factors such housing system and management may outbreak of infection in dairy herds. For instance, in the Scandinavian countries, in northern region, the prevalence of infection is lower than regions located in the southern part because the herd sizes are small (Houe, 1999).

There is the risk of direct or indirect transmission of the disease. Therefore, Density of stocking farms and cattle population density are as risk factors for BVDV infection in farms (Talafha et al., 2009; Saa et al., 2012). Factors such as age, cow origin, meeting between field workers, and lack of separation of newly purchased cows from the herd, are the reasons for BVDV infection (Talafha et al., 2009).

## PREVALENCE OF BVDV

The prevalence of BVDV infection differs between different countries and even between different provinces within a single country; this may be related to the differences in management, environmental variation, size of herds, and existence of PI animals in these herds (Houe, 1999; Hemmatzadeh et al., 2001). The distribution of the virus has also been estimated in different parts of Iran as follows: The disease was first reported in 1970 in Iran by serum neutralization (NS) test which was estimated 16–69% positive in that study (Mirshamsy et al., 1970). However, Kargar Moakhar et al. (1995) showed that it was increased up to 100%. The sero-prevalence of 52.60% for BVDV in dairy herds that were shown related clinical symptoms and distributed throughout the country (Sedighi-nejad, 1996). In a sero-epidemiologic study carried out on 9968 sera collected from the whole country the rate of infection was estimated as 30.57% (Kargar Moakhar et al., 2001). In a survey, 23.36% of the all cattle in Chahar Mahal va Bakhtiary province had antibody against BVDV (Hemmatzadeh et al., 2001).

The sero-prevalence of the virus was estimated at 10% in buffalo herd kept for sperm production in Uremia (Kargar Moakhar et al., 2002). Out of 188 pair's milk and serum, 27.65% of milk and 31.38% of serum samples were ELISA positive in Uremia (Morshedi et al., 2004). Serological studies determined the rate of infection as 27.70% and 28.50% in Sanandaj and Ahvaz, respectively (Fakur and Hemmatzadeh, 2007; Haji HajiKolaei and Seyfi Abad Shapouri, 2007). BVDV-1 could be detected in bull semen by reverse transcription PCR (RT-PCR) and nested-PCR (Daliri et al., 2007). In small ruminants of Ahvaz at Khuzestan province of Iran, sero-neutralization was performed by NADL strain of BVDV genotype1. The results indicated the overall sero-prevalence of 46.62% in sheep and 32.87% in goats (Seyfiabad Shapouri et al., 2007). Evaluation of bulk milk samples in suburbs of

Mashhad revealed that 93.98% of the herds had antibody against BVDV (Garoussi et al., 2008), and 37% in Karaj (Afshari et al., 2008). In a study, by commercial ELISA kits in Kerman province illustrated that sero-prevalence of BVDV was 30.39% (Sakhaee et al., 2009). In another study, 137 serum samples were collected from camels of Tehran and examined using SNT and found that 19.70% camels were positive to BVDV (Raoofi et al., 2010). Serological studies on BVDV in dairy cattle herds indicated the sero-prevalence of 59.46% in Shiraz (Badiei et al., 2010), and 33.90% in slaughtered buffaloes in Ahvaz (Haji Hajikolaei et al., 2010). In another study, 84 sera samples of pregnant cattle were used for the detection of antibody against BVDV by ELISA, and found that 29.80% was positive (Rezaeisaber et al., 2011).

Safarpoor Dehkordi (2011) conductted a study using ELISA and RT-PCR methods, and could determine BVDV in aborted Ovine, Bovine, Buffalo, Caprine and Camel fetuses. Besides, Hashemi Tabar et al. (2011) examined aborted cows by indirect ELISA, and found a high prevalence (74.16%) of BVDV. Sero-prevalence of BVDV infection in cows over 1-year old during the period surveyed was estimated at about 74.50% in Qazvin Province (Bahonar et al., 2011).

In dairy cattle in Esfahan province of Iran, sero-prevalence of BVDV was 49.20% (Shirvani et al., 2012). A total of 157 blood samples were taken from individual cows from 18 Holstein dairy cattle herds in suburb of Mashhad, and analyzed for the presence of BVDV using *Pestivirus* antigen-capture ELISA, and found that 16.17% herds contained antigen-positive cows (Garoussi et al., 2011). A total of 4500 (2400 cows and 2100 buffalo) milk samples were tested for conventional and RT-PCR. Results showed, in cows that the percentage of positive samples for BVDR was 28.37 and 28.75%; and in buffalo milk samples, 18 and 18.23% samples were found to be positive for conventional and real-time PCR respectively (Safarpoor Dehkordi and Haghighi, 2012). In a study, serological investigations were performed using the ELISA method on 176 serum samples to estimate the prevalence of BVDV infection in Sarabian and Holstein dairy cows, to assess to what extent it may affect abortion rates in two herds.

The overall BVDV sero-prevalence in Sarabian dairy cows was estimated at 30%, and in Holstein, sero-prevalence was 52% (Rezaeisaber et al., 2013). A total of 803 serum samples from 12 non-vaccinated herds were collected and evaluated for BVDV antibody using commercially available ELISA kits. Antibody was detected against BVDV in all herds. The prevalence rate of 54.30% was estimated for BVDV (Ghaemmaghami et al., 2013). The rate of active infections of BVDV in dairy farms in Isfahan and Chaharmahal va Bakhtiari provinces were 1.06% using I-ELISA test (Mokhtari and Mahzounieh, 2014). A total of five hundred suspected serum samples were taken from one hundred and twenty-eight individual cows in industrial centers of Qazvin province to detect the presence of antibodies against BVDV (P80) antigen, using Indirect ELISA test, positive, suspected and negative cases were reported 59.80-74%, 1.57-7.08% and 24.21-42.18%, respectively (Sadri, 2014), and in southwest region of Iran was 67% by PCR (Tajbakhsh et al., 2014). The results of studies indicate an increase in the rate of sero-prevalence with increasing ages of cattle, sheep and goats (Hemmatzadeh et al., 2001; Haji HajiKolaei and Seyfi Abad Shapouri, 2007; Seyfiabad Shapouri et al., 2007). Reported that, the prevalence of BVDV infection between female and male cattle was not significant, but this difference in other study was significant and was also related to the age of animals (Hemmatzadeh et al., 2001; Haji HajiKolaei and Seyfi Abad Shapouri, 2007).

Over a 23-year period in the northwestern United States from 1980 to 2000, there was a shift in the disease prolife associated with BVDV infection and in the age of animals at the onset of disease (Everman and Ridpath, 2002). Previous cross sectional studies have reported a broad range in prevalence of BVDV antibody in different countries. BVDV is thought to be present in most cattle-raising countries, and 60-90% of adult animals are seropositive (Sakhaee et al., 2009). In the USA, the prevalence of antibody to BVDV was reported to be 0.55% in 4530 animals distributed in 30 unvaccinated cow-calf operations (Fulton et al., 2009). The prevalence of BVDV in cows was 97.20% in Argentina (Carbonero et al., 2011), 14% in Greek (Billinis et al., 2005), 16.85% in Pakistan (Gohar et al., 2013), 1.1% in Sweden (Zimmerli et al., 2009), 17.1% in India (Behera et al., 2011), 32.1% in Turkey (Yesilbag and Gungor, 2009), 41.44% in Romania (Anita et al., 2013), 43% in Western China (Zhong et al., 2011), 64% in Brazil (Brito et al., 2010), 29.63% in Egypt (Refaat et al., 2010), and 31.6% in Jordan (Talafha et al., 2009). Recently, BVDV has been detected in pneumonic samples of cattle in Sudan (Saeed et al., 2015).

Several factors may contribute to cause difference of sero-prevalence of BVDV such as immunosuppressive stress condition, dehydration, early weaning, control of environmental factors, low or high temperatures, and set up of biosecurity (Bolin, 1990; Luzzago et al., 2010; Brodersen, 2010). One of the other important factors in

the spread of infection is that sheep and cattle are bred on the same farms and also share the same pasture. This situation may lead to interspecies transmission and may increase the incidence of Pestivirus infections (Larson et al., 2004; Gur, 2009). There are several predisposing factor that predispose respiratory diseases such as high stocking density, large group size, stress, and mixing of animals of different age group that are originated from different sources, (Svensson and Liberg, 2006).

## DIAGNOSIS OF BVDV

PI cattle with BVDV can be identified by virus isolation from whole blood (buffy coat) or other tissues, micro titer virus isolation (Immune Peroxidase Monolayer Assay; IPMA) from serum, immunohistochemistry (IHC) staining of viral antigen in skin biopsies, antigen-capture enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) methods (Dubovi, 1996). The virus may be isolated in a number of bovine monolayer cell cultures (e.g. kidney, lung, testis or turbinate). Growth of both biotypes is usually satisfactory. Non-cytopathogenic BVDV is a common contaminant of fresh bovine tissue, and cell cultures must be checked for freedom from adventitious virus by regular testing (Edwards, 1993; Bolin et al., 1994). The infected animals produce large number of BVDV particles that may infect the new animals (Kelling et al., 1990; Palfi et al., 1993; Brock et al., 1998).

Skin biopsy based IHC test can identify PI animals (Njaa et al., 2000). PCR-based identification of BVDV infection is more rapid as compared to virus isolation and detection by conventional methods (Brock et al., 1998; Kleiboeker et al., 2003). Several methods for the enzyme-linked immunosorbent assay (ELISA) for antigen detection have been reported (Entrican et al., 1995; Brock et al., 1998). Residual maternal antibodies present in the serum of young PI calves can cause a false-negative diagnosis (Bolin et al., 1985; Houe et al., 1995; Wittum et al., 2001). Antibody to BVDV can be detected in cattle sera by a standard virus neutralization (VN) test or by ELISA (Katz and Hanson, 1987; Edwards, 1990; Howard, 1990; Paton et al., 1991). Because VN makes the test easier to read, most laboratories use highly cytopathogenic, laboratory-adapted strains of BVDV (Edwards, 1990).

## PREVENTION AND CONTROL OF BVDV

The implementation of a program to control the infection must be based on, first, the identification of animals and herds either free of infection or presence of active infection, secondly, the clearance of virus shedder(s) from the infected herds, or thirdly, control measures to prevent the transmission of the virus within and between herds (Bitsch and Ronsholt, 1995). For prevalence studies at the animal level ELISAs applied to samples of serum or milk (Mars and Van Maanen, 2005), are widely used, because they are easy to apply to large numbers. Bulk milk testing for detection of antibodies is a cost-effective and non-invasive method that is easy to apply and valuable in the control programs for BVDV in dairy cattle herds (Lindberg and Alenius, 1999). It can be used as the first step in a control strategy to evaluate the possibly infected and non-infected dairy herds. The presence of one or a few lactating individual milking cows with high antibody titers to BVDV within a herd of otherwise sero-negative cows will give rise to positive bulk milk test (Niskanen, 1993). In BVDV infected areas, naive herds are very few (Houe, 1999). Grooms et al. (2014) reported that cattle acted as the primary source of BVDV transmission to other susceptible animal during trucking, marketing, and while in feeding pens and pastures.

Vaccination is considered as the primary control method for BVDV (Loneragan et al., 2002). The cattle that were infected with BVDV after birth and recovered are generally protected from subsequent exposure to BDVD (Ridpath et al., 2003). The sero-positive animals caused by natural exposure to BVD may have a level of protection from subsequent transmission of BVDV; however, the level of protection may not be sufficient. Thus, control of BVDV can be achieved by a combination of vaccination of animals, culling of PI cattle, and ensuring a strict biosecurity system (Kelling et al., 2002). In industrial dairy cattle herds in Iran, cows are kept in open-shed intensive system and movement into or within the industrial dairy cattle are not common. However, dairy farmers purchase cows and heifers for replacement or increasing the herd size. Thus, the high rate of antibody titer may be due to the presence of a high population density of PI animals or positive BVD virus cows. On the other side, the high positive antibody may be due to the other ways of transmission of BVDV to the herds (Nettleton et al., 1992; Houe, 1999).

Although detecting animals carrying virus is essential for identification and removal of PI animals from an infected herd, screening herds for antibody carriers is also important to identify PI animals (usually sero-negative) and to determine the herd's infection status and susceptibility (Mainar-Jaime et al., 2001). Sero-prevalence in non-vaccinated herds differs among

areas or countries, ranging between 20 and 90% (Loken et al., 1991). Area differences could in part be explained by factors such as cattle density, herd size or livestock trade (Houe et al., 1995). However, central to an efficient BVDV control strategy is the prevention of infection of the fetus before it is immunocompetent. This is because infection before 120 days of pregnancy may lead to the birth of an immunotolerant, PI animal. As these 'carrier' animals are persistently viremic, they shed virus throughout their life and can become the main means by which BVDV infection is spread and maintained in the herd (Lindberg, 2003; Laureyns et al., 2010). In areas with a high cattle density and a high BVD prevalence, BVD management policy should be based on the four principles of BVDV control, of which (1) strict biosecurity, (2) disposal of PI animals and (3) permanent monitoring are indispensable, whilst (4) vaccination is recommended as a supplementary tool to bolster protection against reinfection. In an adequate control program all four of these principles will be implemented at the same time and in a meticulous way.

For the detection of PI animals with BVDV, it requires precise administration and continuous efforts (Laureyns et al., 2010). Therefore, preventing BVDV from entering a herd via biosecurity and providing immunity against BVDV via vaccination is preferable to avoid potentially adverse health effects. Accurate diagnostic tests are available for detection of PI animals prior to introduction of new animals into a herd (Givens et al., 2012), and vaccine-induced immunity to BVDV can significantly reduce and/or eliminate the development of PI calves (Leyh et al., 2011; Xue et al., 2011; Givens et al., 2012; Rodninga et al., 2012).

Considering the level of protection provided by natural exposure and vaccine-induced BVDV immunity, combined with the accurate diagnostic tests available to identify PI animals, BVDV can be safely and quickly eliminated from the majority of cow-calf farms (Rodninga et al., 2012). Vaccination against BVDV was not practiced in Ian; therefore, serological response reflected natural infection (Garoussi et al., 2009).

## CONCLUSION

Presence of genetic variation and capability of BVDV to persist in carriers limit the success of its control in cattle population. However, a precisely designed systematic control program focusing on regular diagnosis and selection of BVD infected cattle, vaccination against BVDV, and maintenance of strict biosecurity can be effective in controlling BVD. This review shows that a high prevalence of BVDV antibodies in cattle is present in some areas in Iran, and the disease may spread to all other areas of the country. The risks of spreading the disease can be influenced by- (a) high density of animal population, particularly cattle, (b) high susceptibility of cattle to BVDV infection, (c) lack of restriction of animal movement, (d) lack of vaccination program, and (e) lack of early warning and eradication program. Finally, this review warns the severity of BVD in Iran, and shows the possible ways of controlling the disease to save the cattle population in Iran, which can be followed in other countries as well.

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