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Molecular Investigation of Bovine Viral Diarrhea Virus, Bovine Herpes Virus-1 and Bovine Herpes Virus-4 Infections in Abortion Cases of Cattle in Van District, Turkey

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ABSTRACT Abortions, fetal mummification, calf anomalies, and infertility problems constitute most of the reproductive problems in cattle. Viruses play a significant role in the cause of these cases. In cattle, these agents are known as primary abortion agents and the most common of these agents are Bovine Viral Diarrhea Virus (BVDV), Bovine Herpes Virus Type 1 (BoHV-1), and Bovine Herpes Virus Type 4 (BoHV-4). The objective of this research is to determine the potential role of BVDV, BoHV-1, and BoHV-4 as viral abortion agents in cattle housed in the Van district. For this, a total of 115 animal specimens (blood, serum, vaginal swab, vaginal fluid discharge, nasal swab, and abortion material) from 100 abortion, early embryonic deaths, and infertility cases in cattle over the age of 2-5 years old were collected. All samples for detection of BVDV, BoHV-1 and BoHV-4 genomes were tested by the Polymerase Chain Reaction (PCR) technique using specific primers encoding Panpesti 5'-UTR, Glycoprotein C (gC) and Glycoprotein B (gB) genes, respectively. Result out of the samples tested, 41.73% were positive for BVDV and all samples were negative for BoHV-1 and BoHV-4. In conclusion, the presence of BVDV in cattle in the Van region and its role in the occurrence of abortion cases was emphasized for the first time. It is necessary to the consideration of viral abortions and determine the etiology of abortion cases and genital system problems. According to this, we need to focus on the detection of persistently infected (PI) animals for prevention and control of infection and the most effective way of vaccinating susceptible populations.

Keywords: *Abortion, BoHV-1, BoHV-4, BVDV, Cattle, Van.*

ÖZ

Van Yöresi Sığırlarında Bovine Viral Diyare Virus, Bovine Herpes Virus-1 ve Bovine Herpes Virus-4 Nedenli Abort Olgularının Moleküler Olarak Araştırılması

Sığırlarda görülen reprodüktif problemlerin başında abortlar, fötal mumifikasyon, anomali yavru doğumları ve döl tutmama sorunları gelmektedir. Bu olguların sebeplerinin arasında viruslar önemli bir paya sahiptir. Viral enfeksiyonların en yaygın olanları ve sığırlarda primer abort etkenleri olarak bilinen; Bovine Viral Diarrhea Virus (BVDV), Bovine Herpes Virus Tip 1 (BoHV-1) ve Bovine Herpes Virus Tip 4 (BoHV-4) viruslarıdır. Van yöresinde yetiştirilen sığırlarda BVDV, BoHV-1 ve BoHV-4 enfeksiyonlarının viral abort etkenleri olarak olası rolünün ortaya çıkması bu araştırmanın öncelikli hedefi olarak belirlenmektedir. Bu amaçla, abort, erken embriyonik ölümler, anomalili yavru doğumları ve fertilite problemi olan 2-5 yaşları arasında 100 adet sığırdan toplam 115 adet örnek (Tam kan, kan serumu, vaginal swab, vaginal sıvı akıntısı, nazal swab ve abort materyali) alındı. Bu örnekler BVDV, BoHV-1 ve BoHV-4 nükleik asit varlığı yönünden sırasıyla Panpesti 5'-UTR, Glikoprotein C (gC) ve Glikoprotein B (gB) genini kodlayan spesifik primerler kullanılarak Polimeraz Zincir Reaksiyonu (PCR) tekniği ile test edildi. Söz konusu örneklerin %'41.73'ü BVDV nükleik asiti varlığı yönünden pozitif olarak değerlendirildi, BoHV-1 ve BoHV-4 nükleik asiti yönünden pozitif örneğe rastlanmadı. Sonuç olarak, Van yöresindeki sığırlarda BVDV enfeksiyonunun varlığı ve abort olguların oluşumundaki rolü ilk kez ortaya konulmuş; abortların ve genital kanal problemlerin etiyolojisinin belirlenmesine ve viral abortlara dikkat çekerek, enfeksiyonunun korunma-kontrol programında uygulanan en etkili yolun persiste enfekte (PI) hayvanların tespiti ve duyarlı popülasyonun aşılanması üzerinde durulması vurgulanmıştır.

Anahtar Kelimeler: *Abort, BoHV-1, BoHV-4, BVDV, Sığır, Van.*

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INTRODUCTION

The potential impact of viral infections on fertility and reproduction is frequently ignored, despite the fact that they continue to be a significant source of economic loss for the modern cattle industry. Although most deaths in cattle occur around birth and one week of age, the rate of embryonic loss is generally higher than perinatal loss. Most abortions occur sporadically, and just <5% of pregnant animals have abortions (Wathes et al. 2020). Average abortion rates in cattle herds are 1.5–2%, and a higher abortion rate might result in financial losses. Therefore, abortions are considered a significant loss in milk and meat production, so it is crucial to identify the agents and implement effective control measures to prevent them (Yildirim et al. 2011).

The causative agents of abortion are classified as infectious and non-infectious. Non-infectious causes include genetic, hormonal disorders, breeder-link (such as care-feeding), climatic conditions (such as heat stress), iatrogenic, and toxic factors. Infectious agents include microorganisms such as bacteria, fungi, protozoa, and viruses. Among the infectious agents that cause abortions, the role of viruses is considerable. The viral agents that infect the genital system, infertility and abortions in cattle are listed in Table 1. Bovine Viral Diarrhea Virus (BVDV), Bovine Herpes Virus Type 1 (BoHV-1), and Bovine Herpes Virus Type 4 (BoHV-4) are known as the viral agents commonly seen in cases of reproductive disorders in cattle such as abortion, neonatal death, and births with congenital abnormalities (Doğan and Bilge Dağalp 2017; Bilge Dağalp et al. 2020).

Table 1. The list of important viral agents causing abortions in cattle (Doğan and Bilge Dağalp 2017).

Virus	Family	Subfamily/Genus	Species/ Agent
BoHV-1*	*Herpesviridae*	*Alfaherpesvirinea/ Varicellavirus*	BoHV-1.1 BoHV-1.2a
BoHV-4*	*Herpesviridae*	*Gammaherpesvirine/ Rhadinovirus*	BoHV-4
BVDV*	*Flaviviridae*	*Pestivirus*	BVDV-1, BVDV-2
BTV*	*Reoviridae*	*Orbivirus*	BTV (1-27)
AKAV*	*Bunyaviridae*	*Orthobunyavirus/ Simbu serogrup*	AKAV
SBV*	*Bunyaviridae*	*Orthobunyavirus/ Simbu serogrup*	SBV
RVFV	*Bunyaviridae*	*Phlebovirus*	RVFV
Aino virus	*Bunyaviridae*	*Orthobunyavirus*	Aino virus
EHDV	*Reoviridae*	*Orbivirus*	EHDV (1-10)
Wesselborn virus	*Flaviviridae*	*Flavivirus*	WSL
LSD	*Poxviridae*	*Chordopoxvirinae*	Capripoxvirus
Bovine parvovirus	*Parvoviridae*	*Bovine parvovirus*	BoPV (1-3)

BoHV-1 and BoHV-4 infections affect the respiratory and genital systems, and BVDV infection can occur with various clinical manifestations in the respiratory, genital, and gastrointestinal systems. These viruses can also cause deadly fetal infections by crossing the maternal and fetal barrier following viremia (Ackerman and Engels 2006). In viral infections, molecular diagnostic techniques such as polymerase chain reaction (PCR) for direct detection of the viral genome have been determined as a specific and sensitive alternative method, since PCR is 2-100 times more sensitive and provides results faster than compared to virus isolation (Van Engelenburg et al. 1993).

BVDV infection: BVDV causes diarrhea and acute infection in cattle known as contagious viral diarrhea (VD) (Denise Goens 2002). The fatal form of BVDV was defined as Mucosal Disease (MD). BVDV belongs to the *Pestivirus* genus of the *Flaviviridae* family; it is enveloped, approximately 12.3-12.5 kilobases (kb) in length, single-strand, positive sense RNA genome, and surrounded by a 5'-3' untranslated region (UTR) (Baker 1995). Two different genotypes, BVDV-1 and BVDV-2, have been defined due to variation in the nucleotide sequence of the 5' UTR, envelope glycoprotein (E2) and autoprotease (Npro) coding regions of the BVDV genome (Vilcek et al. 1994). The first live BVDV vaccine was administered in 1961, and immunotolerance persistent infection (PI) was described in animals in 1973 (Denise Goens 2002). There are two different biotypes of BVDV, including cytopathogenic (cp) and non-cytopathogenic (ncp) strains. BVDV-cp strains were isolated from cattle diagnosed with MD, while ncp strains are common in nature and cause persistent infected (PI) in calves (Schweizer et al. 2006).

BVDV infection is endemic in cattle populations in many districts of the world. According to serological studies conducted in various geographical regions, the incidence of BVDV infection in individual cattle ranges from 40-90% and in cattle herds from 28-66%, with 0.5-2.5% of calves detected as PI (Oguejiofor et al. 2019). PI animals have a significant role in infection transmission. These animals asymptomatically infect healthy animals in the herd with their body fluids. Furthermore, the use of infected bulls in natural copulation or artificial insemination procedures is a significant factor in virus spread (Kirkland et al. 1991). Acute BVDV infection not only causes clinical symptoms in the respiratory, gastrointestinal, reproductive, and central nervous system, but it also causes reproductive problems including abortion, infertility, early embryonic deaths, and congenital malformations. The severity of the infection varies based on the virus's biotype, virulence, and the period of pregnancy at the time of infection. PI calves are born as a result of BVDV infection after 30 days of pregnancy (Baker 1995; Oğuzoğlu et al. 2012). Therefore, studying the presence and possible effects of BVDV on cattle abortions and reproductive problems is critical for designing and developing effective control-eradication strategies, including vaccination.

BoHV-1 infection: BoHV-1 causes infection in cattle with a variety of clinical symptoms such as rhinotracheitis, pustular vulvovaginitis and balanoposthitis, abortion, infertility, conjunctivitis, and encephalitis. The respiratory form of this infection is known as "infectious bovine rhinotracheitis" (IBR), a severe and highly contagious respiratory system disease, whereas the genital form in cows is known as "infectious pustular vulvovaginitis" (IPV), which is abortion and lethal systemic disorders in newborns are the most serious effects of this form (Muylkens et al. 2007).

BoHV-1 is a significant pathogen in cattle and belongs to the *Herpesviridae* family, *Alphaherpesvirinae* subfamily, *Varicellovirus* genus. Viral genome with 135-140 kb in length and double-stranded DNA encodes nearly 70 proteins, 33 of which are structural proteins (SP) and 15 non-structural proteins (NSPs). According to genetic diversity and clinical symptom variety, BoHV-1 isolates are classified into three subtypes: BoHV-1.1, BoHV-1.2a, and BoHV-1.2b (Spilki et al. 2004; ICTV 2012).

BoHV-1 infection is transmitted between animals directly or indirectly. Direct transmission usually occurs in direct contact with an infected animal, while indirect

transmission occurs through nasal exudate, genital secretions, fetal fluids, fetal tissues, and semen. Transmission of infection also occurs during artificial insemination and embryo transfer applications with infected semen. This infection causes economic losses due to weight loss in animals, a decrease in milk production, and restrictions on animal trade (Van Schaik et al. 2001).

BoHV-1 affects all cattle populations worldwide, with a high prevalence of infection in Australia, New Zealand, Canada, the United States, Zaire, Italy, Belgium, India, and Turkey (Dağalp et al. 2012). The control of the infection with vaccination usually prevents the development of clinical signs and can significantly reduce the spread of the virus after infection. However, vaccination cannot completely prevent the disease. Since BoHV-1 is a latent infection and highly contagious virus, vaccination is usually recommended at 4-6 months of age after decreased passive immunity in calves (Kaur 2016).

BoHV-4 infection: BoHV-4 is a worldwide distributed virus that is characterized by symptoms such as reproductive and respiratory system disorders, mastitis, dermatitis, and conjunctivitis in cattle and causes considerable economic losses in cattle breeding due to the direct or indirect effects of these problems. The virus was first isolated from cattle with respiratory symptoms in Hungary in 1963 and later in the USA (Mohanty et al. 1971). Subsequently, the virus was isolated from cattle with clinical signs such as conjunctivitis, pneumonia and upper respiratory tract infections, skin lesions, mamillitis, enteritis, postpartum metritis, chronic metritis, and mastitis (Bilge Dağalp et al. 2020).

BoHV-4 is a member of the *Herpesviridae* family, *Gammaherpesvirinae* subfamily, and *Rhadinovirus* genus (ICTV 2012). The virus contains an enveloped, 100 nm diameter icosahedral nucleocapsid and a proteinaceous tegument. The viral genome consists of 144 ± 6 kb of double-stranded linear DNA (Zimmermann et al. 2001). Primary replication of the virus occurs within epithelial cells in the mucosa. Then the virus infects blood mononuclear cells and has an affinity for vascular endothelium, mammary tissue, endometrium, and fetal tissues. This virus causes latent persistent infection in blood leukocytes, spleen macrophages, and endothelial cells that can be reactivated by corticosteroids or stress in various tissues (Dağalp et al. 2012; Chastant-Maillard 2015).

Control of the BoHV-4 infection can be achieved by applying good hygienic measures and removing seropositive animals from the herd. Infected cows should be isolated after calving since high amounts of viruses can be shed in uterine exudates in cases of metritis. Direct contact between seropositive and seronegative animals should be avoided, as the virus is generally spread through the respiratory route (Thiry et al. 1990).

Based on the above-mentioned data, determining the potential impact and etiology of BVDV, BoHV-1, and BoHV-4 infections on abortion is extremely important due to the intensive animal husbandry and extensive production of animal products in the Van region. Therefore, the aim of this study was to conduct a molecular investigation into the role of BVDV, BoHV-1, and BoHV-4 infections in cattle abortion cases in the Van province for the first time.

MATERIAL AND METHODS

The study was approved by the Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (Approval date: 26/03/2020, Decision no: 2020/03).

Study Area and Sampling

This study was carried out on cattle bred by the public in the province of Van, located in the Eastern Anatolia region of Turkey, from September 2019 until June 2021. One hundred cattle with abortions, past aborted and early embryonic deaths (69 abortions, 28 past aborted and 3 embryonic deaths) aged 2–5 years and unvaccinated against the above-mentioned infections were sampled. A total of 115 animal specimens were taken from 100 cattle, including 97 EDTA blood, three blood serum, four vaginal swabs, three vaginal fluid discharge, one nasal swab, and seven abortion materials.

DNA and RNA Extraction

Blood samples (4 mL) taken into tubes with anticoagulant (EDTA) (Vacuette, Austria) were centrifuged for 10 minutes at 3000 rpm, the upper plasma was discarded, and the leukocyte layer was transferred to sterile 1.5 mL tubes. After washing the leukocytes with the antibiotic PBS three times, they are stored at -80 °C for use in molecular studies. Abortion materials were homogenized in 1/10 antibiotic PBS in a homogenizer, then centrifuged for 10 minutes at 3000 rpm, and the supernatant was taken into a sterile stock tube. Swab and fluid samples were centrifuged for 10 minutes at 3000 rpm in a sterile tube after vortexing, and the supernatant was stored in a sterile stock tube at -80°C to be used in studies for virological control. Viral RNA isolation for detection of BVDV nucleic acid and viral DNA isolation for detection of BoHV-1 and BoHV-4 was performed on a total of 115 samples taken into tubes using a universal RNA extraction kit for purification of genomic RNA (EURx, Poland) and a universal DNA extraction kit for isolation of DNA (EURx, Poland) as indicated in the manufacturer's recommendation. The extracted genomic RNA (gRNA) and DNA samples were stored at -80°C until used in the following study phase.

BVDV, BoHV-1 and BoHV-4 Nucleic Acid Detection

To detect the presence of BVDV nucleic acid after RNA extraction, reverse transcription PCR (RT-PCR) assay was employed on all RNA samples, using the OneStep RT-PCR kit (Grisp, Portugal) as described in the manufacturer's recommendation. An RT-PCR assay was run using primers encoding the 5'-UTR Panpesti gene region as described previously with minor modification (Vilcek et al. 1994). Briefly, sense and antisense primers (5'-ATGCCCTTAGTAGGACTAGCA-3' and 5'-TCAACTCCATGTGCCATGTAC-3') were used to amplify a 288 bp length fragment of BVDV. In the RT-PCR assay, positive and negative controls were processed with all samples as a standard control. The thermocycler conditions in the RT-PCR reaction were set as one cycle for reverse transcription at 45 °C for 15 minutes, followed by a single cycle at 94 °C for 5 minutes and 35 cycles at 94 °C for 30 seconds and 56 °C for 45 seconds and 72 °C for 1 minute. Finally, extension is followed by a single cycle at 72 °C for 10 minutes.

To detect the BoHV-1 genome, PCR assay was performed on all extracted DNA samples using the method described by Van Engelenburg et al. (1993) with some modifications. The detection of the BoHV-4 genome was performed using a PCR assay on all extracted DNA samples as described by Wellenberg et al. (2001) with minor modification. The

primer sequences for amplification of the gene regions encoding the glycoprotein C (gC) gene for BoHV-1 (gC1:5'-TGTGACTTGGTGCCCATGTCGC-3' and gC2:5'-GAGCAAAGCCCCGCCAAGGAG-3') and the glycoprotein B (gB) gene for BoHV-4 (gB1:5'-CCCTTCTTTACCACCACCTACA-3' and gB2:5'-TGCCATAGCAGAGAAACAATGA-3') were used to amplify a 389 bp and 615 bp length PCR product, respectively. PCR reaction mixture containing 3 µl of extracted DNA, 0.5 µM each primer, 2.5 µM 10X Taq buffer and 1.5 mM MgCl2, 0.5 µl dNTP mix (10 mM), and 0.5 µl (5u/µl) of Taq DNA polymerase in a total volume of 25 µl. In the PCR assay, positive and negative controls were processed with all samples as a standard control. The thermocycler conditions in the PCR assay were set as one cycle at 94 °C for 5 minutes and 35 cycles at 94 °C for 30 seconds and 56 °C for 45 seconds and 72 °C for 1 minute. Finally, extension is followed by single cycle at 72 °C for 10 minutes.

Statistical Analysis

In this study, SPSS (IBM SPSS for Windows ver. 22) statistical package program was used for the analysis of all data.

RESULTS

The expected size of amplicons following RT-PCR and PCR processes were run on a 1.5% agarose gel accompanied by positive and negative controls, visualized in a UV transilluminator (Figure 1). In an RT-PCR assay using panpesti primers, it was determined that 48 samples out of 115 samples (34/100 animals) were positive for BVDV nucleic acid. Based on sample types, 39 leukocytes (39/97), one blood serum (1/3), three vaginal swabs (3/4), one vaginal fluid (1/3), and four abortion materials (4/7) were evaluated positively for BVDV nucleic acid. Thus, the BVDV positivity rate was determined as 41.73% in total samples and 34% on an animal basis. In order to detect the presence of BoHV-1 and BoHV-4 DNAs, the PCR products obtained following the PCR process were run on a 1% agarose gel and visualized on a UV transilluminator. In 115 samples tested by PCR, no fragment was amplified at 389 bp or 615 bp length, and there was no positivity for BoHV-1 and BoHV-4. Data for sample types and PCR results for BVDV, BoHV-1 and BoHV-4 were summarized in Table 2.

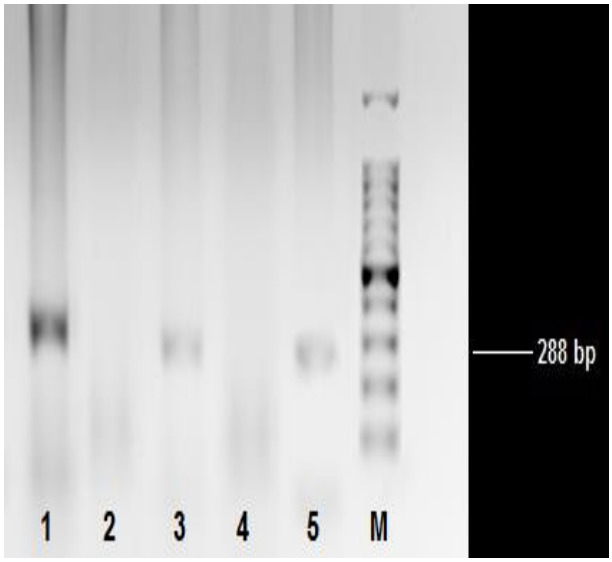

Figure 1. The result of BVDV in the RT-PCR assay. Line M: 100bp DNA ladder, Line 1: Positive control, Line 2: Negative control, Line 3 and 5: positive animal samples, Line 4: Negative animal sample.

Table 2. Sample types, number of material and viral nucleic acid detection in PCR assay in the tested animals.

Sample Type	The number Of materials	BVDV RT-PCR positive (%)	BoHV-1 PCR positive	BoHV-4 PCR positive
Leukocyte	97	39	-	
Serum	3	1	-	
Vaginal swab	4	3	-	
Vaginal discharge	3	1	-	
Nasal swab	1	-	-	
Abortion material	7	4	-	
Total	115	48 (41.73%)	-	

DISCUSSION AND CONCLUSION

In this study, the roles of BoHV-1, BoHV-4 and BVDV in the abortion cases were investigated virologically in cattle housed in Van province. BVDV is widespread all over the world and causes significant economic losses in cattle breeding due to its effects on reproduction and health. The seropositivity rates varied in different regions and were reported at between 12% and 89% (Cowley et al. 2014).

BVDV infection was first detected and diagnosed based on clinical disease findings in cows in Turkey in 1964 (Öncül et al. 1964). According to studies on BVDV infection carried out in various districts of Turkey, the seropositivity has been determined to be 50% on average, and it was reported that the infection is widespread, persistently infected animal births, and abortion can occur at any stage of the infection (Burgu et al. 2003; Yildirim et al. 2011; Oğuzoğlu et al. 2012; Yilmaz 2015; Bilgili and Mamak 2019; Timurkan and Aydın, 2019). In the last 5 years, studies focused on BVDV infection in different regions of Turkey reported that, the average seropositivity rate was 56.97%, the average rate of PI animals was 2.8%, and the average rate of BVDV nucleic acid presence in cattle with abortion problems was reported as 44.68% (Yılmaz et al. 2016; Özgünlük and Yıldırım 2017; Aldemir and Başbuğ, 2019; Bilgili and Mamak, 2019; İnce 2020; Demirsoy and Mamak 2020; Gürçay et al. 2020).

In this study, the presence of BVDV nucleic acid was detected at 41.73% (48/115) on a sample basis and 34% (34/100) on an animal basis in samples collected from cattle with reproductive disorders or abortion problems in the RT-PCR assay. It is clear that the results of the study carried out to support the findings of the studies (Yılmaz et al. 2016; Gürçay et al. 2020) carried out in areas close to the Van province and in other provinces of Turkey. It is thought that the high rate of animals found positive for BVDV infection was due to the fact that the majority of the sampled animals were managed in animal husbandry with traditional methods, PI animals were not detected and removed, and vaccination programs are not implemented. As a result of the information received from animal farm owners, it was concluded that the hygiene of the enterprise was insufficient and there was a lack of information about the control and prevention of disease.

BoHV-1 infection is recognized as an acute and latent viral infection with variable prevalence and incidence rates depending on geographic location and breeding factors in different countries (Van Schaik et al. 2001). Numerous studies regarding viral agents in cases of cattle abortion and seroprevalence of BoHV-1 in Turkey indicate the importance of BoHV-1 infection (Alkan et al. 2005; Dağalp et al. 2012; Tuncer-Göktuna et al. 2016; Doğan and Bilge

Dağalp 2017; Yilmaz et al. 2017; Yilmaz et al. 2020). The average seroprevalence rate for BoHV-1 as determined by serological research in various regions of Turkey in recent years was 36.22% (Özgünlük and Yıldırım 2017; Altun et al. 2019; Gür et al. 2019; Kadiroğlu et al. 2020). Altun et al. (2019) investigated the presence of BoHV-1 using the q-real time-PCR method in Erzurum and it was reported that the presence of BoHV-1 nucleic acid was 13.33%. In a study conducted in the provinces of Ankara, Çorum, Kırıkkale, and Yozgat, a positivity rate of 0.39% was found in PCR assay in blood samples found to be BoHV-1 seropositive in cattle with abortion and infertility problems (Aslan et al. 2015).

In this study, no positivity for BoHV-1 nucleic acid was found among 115 samples collected from 100 cattle in PCR assay. It can be noted that the results are identical with those of a study conducted in the province of Kars, which is close to the sampled Van province (Yilmaz et al. 2016), as well as with those of a study including the provinces of Konya, Aksaray, and Niğde (Şevik and Avcı 2015). It is believed that the inconsistency between these results and those of studies done on other districts of Turkey may be due to a wide range of factors such as geographic location, virus circulation, and growth conditions.

BoHV-4, which causes reproductive problems in many animal species, particularly cattle, was identified in both healthy and aborted cattle in the world, including in Turkey. This virus is a contributing factor in cases of cattle abortion along with other pathogens such as viruses, bacteria, and protozoans (Bilge Dağalp et al. 2020).

Numerous studies conducted on BoHV-4 in many countries, including Turkey, revealed varying seropositivity rates in cattle. The presence of BoHV-4 infection was first demonstrated serologically in Turkey in 2007, when 877 bovine serum samples were tested by ELISA in the study, and the seropositivity rate was reported as 54.3% (Bilge Dağalp et al. 2007). Another study (Bilge Dağalp et al. 2010) investigated the BoHV-4 in a dairy cattle herd with metritis problems serologically and molecularly; seropositivity was 69.6%, and BoHV-4 nucleic acid detection was 29%. A study on the potential role of BoHV-4 in cow infertility determined that the seropositivity rate was 69% in animals with fertility problems and 44% in healthy-looking animals in the same herd (Gür and Doğan 2010). In a study conducted on the presence of BoHV4 in the abortion of dairy cattle using ELISA, it was determined that 29.3% of samples had BoHV-4 specific neutralizing antibodies (Yildirim et al. 2011). Dağalp et al. (2012) in a study on the herpesviruses on the occurrence of reproductive disorders in dairy cattle herds in Turkey, detected BoHV-4 nucleic acid in the samples of leukocytes, vaginal swabs, and abortion materials by PCR technique as 26.1% and 33.6% in vaginal swab and leukocyte samples, respectively. In a study conducted in Kars province, 48 abortion materials were tested for the presence of BoHV-4 nucleic acid, and as a result, BoHV-4 could not be detected in any sample (Yılmaz et al. 2016). Similarly, in a study conducted to investigate the role of herpesviruses and pestiviruses in ruminant abortion cases in the Marmara region of Turkey using a PCR assay, no BoHV-4 was found in any of the tested samples from 81 aborted fetuses (Tuncer Göktuna et al. 2016). Furthermore, in another study (Aslan et al. 2015) of BVDV, BoHV-1, BoHV-4, and BoHV-5 infections in cows in Ankara, Çorum, Kırıkkale, and Yozgat provinces, the seropositivity rate for BoHV-4 was 28.78% and BoHV-4 genome was not detected by PCR in seropositive blood samples. In a recent study, Bilge Dağalp et al. (2020) molecularly investigated the etiological role of BoHV-4 in cows with fertility problems, and the positivity rate was reported as 65.7%.

In the current study, the presence of BoHV-4 infection on 115 animal specimens was analyzed by PCR and no positivity was found. The results of this study are similar to those of studies by Aslan et al. (2015), Tuncer Göktuna et al. (2016), and Yilmaz et al. (2016). According to study reports, the age of the animal considerably affects the seroprevalence, it increases after 2-3 years of age. In a study in Turkey related to it, while the seroprevalence of infection was determined as 42% in animals before the age of 2 years, it was reported that it was 62% in animals older than 2 years (Bilge Dağalp et al. 2007). The majority of the animals sampled within the study are 2-3 years old.

As a result, no co-infection was identified among the animal samples of the above-mentioned infections. It is clear that our findings support the above-mentioned similar studies describing the presence of BoHV-1, BoHV-4 and BVDV nucleic acids in cattle in Turkey. The most important outcome of our study has been to demonstrate the role of BVDV infection in abortion cases in cattle in Van province for the first time. In addition to the high prevalence of the BVDV genome in abortion cases, this indicates a causal relation between BVDV and fertility problems such as abortion, birth with unviable calves in cattle and the existence of persistently infected (PI) animals in Van province.

In conclusion, we consider that BVDV alone or in association with other pathogens such as bacteria, fungi, parasites, and metabolic factors contributed to the development of abortion problems. Based on these data; cases of viral abortions caused or contributed by BVDV, BoHV-1, and BoHV-4 infections are common in Turkey, and sanitation measures are difficult to implement in publicly owned enterprises in rural areas. Therefore, the most effective way to control viral persistent infections such as pestiviruses and herpesviruses may be the detection of PI animals and vaccination of the susceptible population. It is necessary to conduct additional studies using epidemiological data from BVDV, BoHV-1, and BoHV-4 infections as well as molecular characterization of field viruses in various clinical cases in order to understand their responsibility for the economic losses in cattle husbandry in Turkey.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Idea / Concept: ARB
Supervision / Consultancy: ARB
Data Collection and / or Processing: HY
Analysis and / or Interpretation: HY
Writing the Article: ARB, HY
Critical Review: ARB

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