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RESEARCH ARTICLE

High Prevalence of Bovine Viral Diarrhea Virus-1 in Sheep Abortion Samples with Pestivirus Infection in Turkey

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ABSTRACT

The aim of this study is the detection of prevalence of bovine viral diarrhea virus-1 (BVDV-1) in sheep abortion samples in Turkey. For this purpose, initially, the detection of pestiviruses, bluetongue virus (BTV) and akabane virus (AKAV) was performed by reverse transcription-polymerase chain reaction (RT-PCR) from the aborted sheep fetus specimens. Then, for differentiation of pestiviruses, further investigations were carried out from the pestivirus positive samples. Pestiviruses RNAs were detected from 98 (24.74%) of 396 aborted fetus samples collected from 13 provinces in Turkey. By using border disease virus (BDV) and bovine viral diarrhea virus (BVDV) specific primers, 58 (59.18%) of pestivirus positive 98 samples were detected as BDV and the remaining 40 (40.81%) were found as BVDV with RT-PCR. According to the results, BVDV RNAs were detected from 10.10% (40/396) of aborted fetus specimens. By sequencing and phylogenetic analysis of BVDV positive four samples, BVDV-1 was identified as the genotype of BVDV. The BTV and AKAV genome were not detected from the 396 aborted samples tested in this study. In conclusion, there's a high ratio of BVDV-1 among sheep abortions in Turkey. According to this study results, we think that the high prevalence of BVDV in sheep abortion cases may be important for the control programme against persistent BVDV infections.

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INTRODUCTION

Abortion is one of the main causes of reproduction losses in sheep. The abortions in sheep can be caused by metabolic and hormonal disorders, nutritional deficiencies, trauma, poisoning and infectious agents. Therefore, it is very difficult to determine the aetiology of abortion cases in sheep (Givens and Marley, 2008; Menzies, 2011). Although the aetiology of abortions in sheep is not fully elucidated, the abortions caused by viral agents constitute a significant part of the cases identified (Menzies, 2011). The viruses that are the subject of this study, pestiviruses, bluetongue virus (BTV) and akabane virus (AKAV) are the primary viral agents of the abortions in sheep and cattle (Nettleton *et al*., 1998; Menzies, 2011).

Pestiviruses, located in the *Pestivirus* genus of the *Flaviviridae* family, make intrauterine or transplacental infection at different stages of gestation and cause a number of disorders with embryonic deaths, mummification, congenital anomalies and disorders, premature birth, the birth of weak calves, and the birth of persistently infected animals. The transplacental infections caused by pestiviruses also affect the overall herd health as they may cause persistently infected animal births (García-Pérez *et al*., 2009).

Classical swine fever virus (CSFV), bovine viral diarrhea virus 1 (BVDV-1), bovine viral diarrhea virus 2 (BVDV-2) and border disease virus (BDV) are antigenically similar viruses, which are found in the pestivirus genus and cause important diseases in pigs and ruminants (Vilcek and Paton, 2000). For years, the naming of these viruses has been made according to the animal species that is virus-affected, detected or isolated. Pestiviruses affecting sheep and goats were evaluated as BDV, cattle pestiviruses as BVDV and pigs pestiviruses as CSFV. However, in the light of recent studies, it has been revealed that these assessments are not really convincing and that each of the pestiviruses may cause

similar clinical manifestations with heterologous species in the heterologous species (Paton *et al.,* 1995; García-Pérez *et al.,* 2009; Passler and Walz, 2010). BDV may infect both its own specific hosts, sheep and goat, as well as cattle, and pigs and wild ruminants (Paton *et al.,* 1995; García-Pérez *et al.,* 2009). A similar situation was also identified for BVDV (Carlsson, 1991; Passler and Walz, 2010). Heterologous infections of these closely related viruses are problems in diagnosis and control of pestiviruses. There are mandatory and voluntary eradication programs for cattle for BVDV in some countries of Europe (Greiser-Wilke *et al.,* 2003). There is no BDV eradication program system for sheep in Europe. In Turkey, there is no mandatory eradication and control program for pestiviruses for both species. However, although not very common, it has been reported that vaccination, selection and removal programs have been carried out on some cattle herds.

In this study, it was aimed to determine the prevalence of pestiviruses from clinical samples taken during abortions in sheep and then to investigate the differentiation of these pestiviruses.

MATERIALS AND METHODS

Aborted fetal samples: The samples were collected from aborted fetal sheep specimens from Firat University and Pendik Veterinary Control Institute Diagnostic Laboratories between 2011-2015. Localisation and numbers of the samples were shown in Fig. 1 and Table 1, respectively. Each one of samples refers to abortion cases from different flocks. Some detailed information was given by veterinarians and the owner of the herds within the anamnesis received during the sample submission. Also, as a result of the anamnesis, it was reported that some abortions had malformations such as arthrogryposis and scoliosis in some newborn lambs.

Vira RNA extraction: Fetal tissue samples (Lenf nodes, liver, lungs, brain) were homogenized with pestle and mortar within one ml of PBS solution for each animal. RNA extractions were done with High Pure Viral RNA kit (Roche, Germany). The RNA samples were stored in -80°C until analysis.

Panpestivirus RT-PCR: The detection limit for RT-PCR analysis with primers specific with BVDV and panpesti virus specific primers was performed using BVDV NADL strain, before testing of clinical specimens. Panpestivirus detections were done with the method described by Vilcek *et al.* (2000). Briefly, RT-PCR analysis were done with p324 (5'-ATGCCCGTAGTAGGACTAGCA-3') and p326 (5'-TCAACTCCATGGCCATGTAC-3') with using OneStep RT-PCR kit (Qiagen, Germany). Pestivirus specific 288 bp RT-PCR amplifications were carried out 35 cycles at 94°C for 1 min, 56°C for 1 min and 72°C for 1 min and final extension was done 72 for 5 min. The size of amplified RT-PCR product was visualized on 1.5% agarose gel stained with ethidium bromide.

BVDV and BDV RT-PCR: RT-PCR detection of BVDV was done with primers 5'-UTR fwd (5'-CTA GCC ATG CCC TTA GTA GGA CTA-3') and STAR-Trev (5'-CAA CTC CAT GTG CCA TGT ACA GCA-3' using the method described previously (Dabak *et al.,* 2007). Differentiation of BDV was also performed with primers PBD1 (5'-TCG TGG TGA GAT CCC TGA G-3') and PBD2 (5'-GCA GAG ATT TTT TAT ACT AGC CTA TRC-3') (Vilcek *et al.,* 2000). The size of amplified RT-PCR product was visualized on 1.5% agarose gel stained with ethidium bromide.

Bluetongue virus and akabane virus RT-PCR: In addition to the detections of pestiviruses in all samples, RT-PCR analysis for bluetongue virus (BTV), akabane virus (AKAV) were carried out with 294 abortion samples collected from 10 provinces (Balıkesir, Çanakkale, Bursa, Kırklareli, Tekirdağ, Edirne, İstanbul, Kocaeli, Sakarya, Düzce) using the method described previously (Akashi *et al.,* 1997; Shaw *et al.,* 2007).

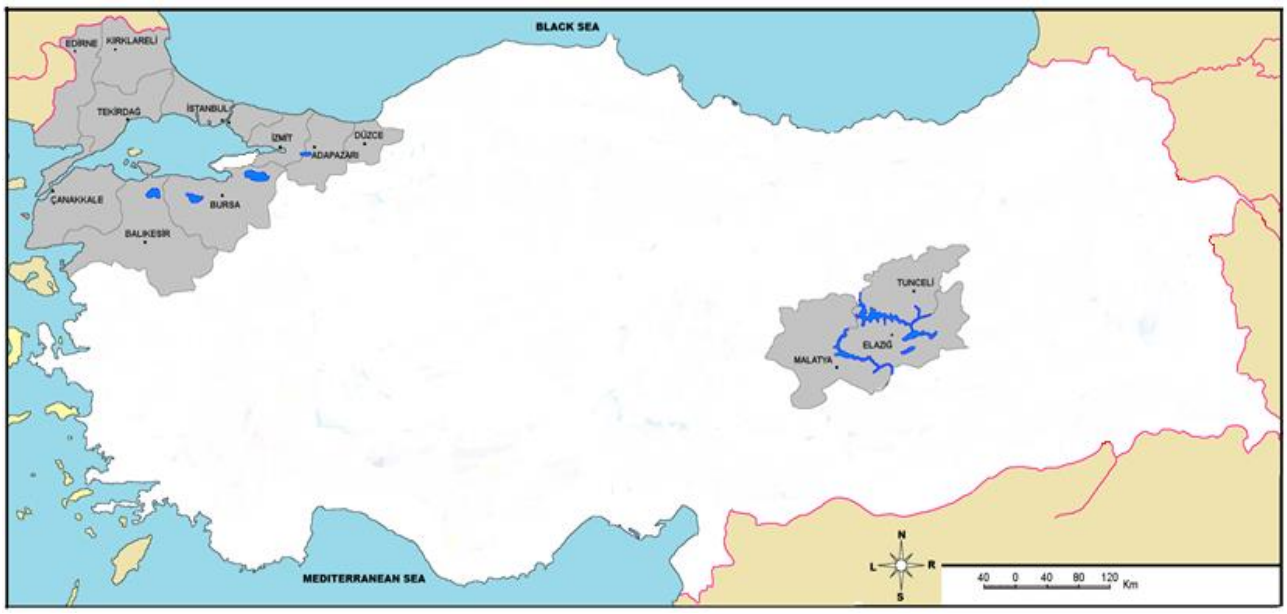

Fig. 1: The provinces collected of the abortion samples.

Table 1: The provinces collected of the abortion samples and panpestivirus, BDV and BVDV positive rates

Provinces collected of the abortion samples	Panpesti RT-PCR +/n (%)	BDV RT-PCR +/n (%)	BVDV RT-PCR +/n (%)
Balıkesir	9/42	6/42	3/42
Çanakkale	8/24	4/24	4/24
Bursa	6/25	4/25	2/25
Kırklareli	7/32	4/32	3/32
Edirne	8/41	5/41	3/41
İstanbul	3/24	3/24	0/24
Kocaeli	6/34	4/34	2/34
Sakarya	4/16	3/16	1/16
Düzce	3/24	2/24	1/24
Tekirdağ	14/32	6/32	8/14
Elazığ	14/42	8/42	6/42
Malayta	8/24	5/24	3/24
Tunceli	8/36	4/36	4/36
Total	98/396 (24.74%)	58/396 (14.64%)	40/396 (10.10%)

Sequencing: Some of the RT-PCR positive amplification products were sequenced for confirmation of RT-PCR results and obtaining more detailed epidemiological data. For this purpose, 5'UTR regions of four BDV positive and four BVDV positive samples were sequenced. Total eight panpestivirus RT-PCR products were purified by using DNA purification kit QIA quick gel purification kit (Qiagen Co., Hilden, Almanya). Purified products were then sequenced in both directions (Applied Biosystems 3130xl Genetic Analyzers; Hitachi Appliance Inc., Japan). Sequences were assembled by using ClustalW software and sequence analysis and alignments were done with the previously submitted pestivirus sequences in GenBank. Phylogenetic analysis was carried out with neighbour-joining method using PHYLIP multiple alignment software.

RESULTS

The detection limit of RT-PCR performed using BVDV NADL was about 50 virion per each reaction. RT-PCR analysis were carried out for detecting of pestiviruses from 396 aborted sheep specimens collected between 2011-2015 from 13 different provinces of Turkey. As a result of the RT-PCR performed with panpestivirus primers, pestivirus positivity was detected from 98 (24.74%) of 396 aborted specimens. Then, for differentiation of pestiviruses, the pestivirus positive 98 samples were studied by RT-PCR analysis using BDV and BVDV specific primers. Of 98 pestivirus positive samples, 58 (59.18%) were found as BDV and 40 (40.81%) found as BVDV. According to these results, the presence of BDV and BVDV were detected from 58 (14.64%) and 40 (10.10%) of the total 396 abortion cases, respectively. No co-infection of BDV and BVDV was identified in any of the cases of the RT-PCR positive for panpestivirus. The positive rates based on provinces are presented in Table 1.

According to the RT-PCR, no positive results were detected from 294 samples tested for BTV and AKAV. For confirmation of RT-PCR differentiation of BVDV, four BVDV positive samples selected randomly into panpestivirus RT-PCR samples were sequenced and multiple sequence alignments and phylogenetic analysis were done. Phylogenetic analysis was validated as BVDV all of the four samples. The sequences of the four samples were found at 96-98% similarity. In addition, the similarity between the sequences of four samples and other BVDV sequences obtained from GenBank were found at 90-96%. The sequences belonging to one sample amplified with RT-RCR in this study had a compliance note of 96% with the sequences of BVDV-1 isolate previously reported in Iran (GenBank: EF2103479.1). Phylogenetic analysis result for the strain 2013/TR/EDRN.1 was shown in Fig. 2.

The results of the sequencing and phylogenetic analysis performed with BDV positive four samples were validated as BDV all of the four samples (Data not show).

DISCUSSION

Pestiviruses infections were reported from cattle, sheep, goat and pigs from different countries worldwide (Becher *et al.,* 2003; García-Pérez *et al.,* 2009). In previous studies, the presence of pestiviruses in aborted lamb samples was reported 10.4, 47.36 and 66.66% in Southern Turkey, Western Turkey and Northern Turkey, respectively (Hasircioglu *et al.,* 2009; Albayrak *et al.,* 2012; Goktuna *et al.,* 2016). In this study, pesitivirus presence was detected from 98 (24.74%) of 396 sheep abort samples. BTV and AKAV accepted as other important viral abortion agents of sheep were not detected. These results indicate that pestiviruses are common in the circulation in the sheep population of our country and an important etiological agent in abortion cases of sheep.

Pestiviruses cause significant economic losses in ruminants (Givens and Marley, 2008; Menzies, 2011). One of the most important problems in the struggle with pestiviruses is the persistence virus presence in infected animals and becoming as a virus reservoir in flocks. For this purpose, one of the most effective approaches to combat pestiviruses is the selection and removal of persistently infected animals with pestivirus. Some countries, especially in some European countries, have made important eradication decisions, especially in cattle fighting with BVDVs (Greiser-Wilke *et al.,* 2003). There is no application decision taken by the state in the fight against pestivirus in Turkey. It is known that some individual farms are carrying out the selection of pestivirus negative animals and vaccination. In Turkey, there are no official vaccination and suspension programs against pestiviruses in sheep. The results of this study indicate that some precautions must be taken for control of pestiviruses in the sheep in Turkey.

There are also studies reporting the presence of experimental and natural infections with BVDV of sheep and goats (Becher *et al.,* 2003). In Turkey, there is only one study reporting the presence of BVDV in sheep, and this pestivirus isolate was determined BVDV-2 (Yesilbag *et al.,* 2008). In this study, the BVDV-1 prevalence was determined as 10.10% in sheep abort cases. The important problem in control pestiviruses is that these viruses cause infection in heterologous species. In anamnesis of the cases, it was noted that many of the sheep with abortion were grazed in the pastures for about 7 months (April-October), and cattle were also grazed in similar pastures. Persistent BVDV infection is commonly reported in cattle in our country (Dabak *et al.,* 2007; Yesilbag *et al.,* 2008; Aslan *et al.,* 2011; Yesilbag, 2014). The presence of

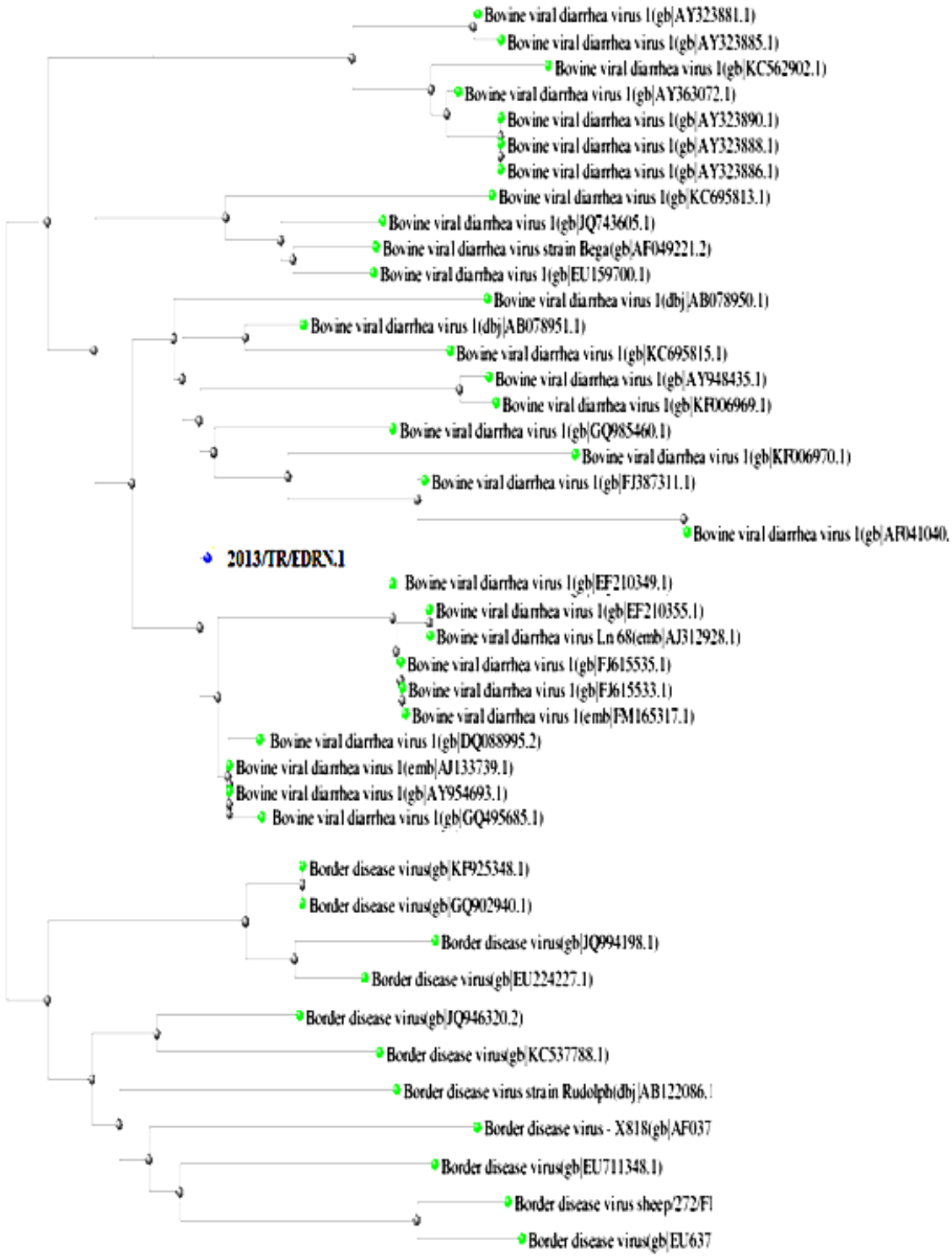

Fig. 2: Phylogenetic analysis belonging to the genomic sequence of one sample (2013/TR/EDRN.1) obtained from this study.

BVDV-1 as a genotype was reported in these studies in cattle. Although the source of the virus identified in this study has not been known, we thought that the cause of BVDV infection in sheep may be pasture which contaminated with nasal drifts and saliva of persistently infected cattle.

It was also noted that some farms where abort specimens had come were also raised with sheep and cattle. In addition to these, sheep and cattle sales can be done together in local animal markets in Turkey. This may also be important in heterologous transitions. However, in this study, the results were evaluated on samples from the laboratory, information not available to all herds or information requested by all herds was not taken in a healthy way. For this reason, although the present study results do not rule out certain, it points to the possibility of pestiviruses spreading among heterologous species in countries such as Turkey where heterologous species occasionally or for a long time come together.

Another possible source of BVDV in sheep could be modified live vaccines contaminated with pestiviruses

(Thabti *et al.,* 2005). Animal modified live vaccines commonly prepared by using cell lines derived from cattle, small ruminant and pigs or by using serum taken from these type of animals. It is important to use pestivirus antigen and/or antibody negative cell lines and serum (Xue *et al.,* 1996). In some of the herds where the cases were identified, small ruminants were vaccinated for live attenuated sheep pox and peste de petit ruminants. Both vaccines were cell culture derived live vaccines. For this reason, the possibility pestivirus transmission source in these cases is considered as vaccination with a weaker possibility than pasture contamination. However, since the same batch of vaccines used in these cases could not be reached, the vaccine-related transmission results were not confirmed.

As mentioned above, pestivirus infections have been reported for many years in the studies performed from Turkey (Dabak *et al.,* 2007; Yesilbag *et al.,* 2008; Azkur *et al.,* 2011; Yesilbag, 2014). Most of the pestiviruses from cattle in Turkey were found as BVDV-1 with genotypic characterization (Dabak *et al.,* 2007; Yesilbag *et al.,* 2008; Yesilbag *et al.,* 2014; Aslan *et al.,* 2015). However, there are the studies in which BVDV 2 was also reported (Sarikaya *et al.,* 2012; Yilmaz *et al.,* 2012). According to the data of this study, the presence of BVDV-1 in sheep abortion specimens was determined. It's proved that BVDV-1 is most prevalent pestivirus genotype in Turkey with the findings of this study and previous reports. Furthermore, the phylogenetic analysis results performed from this study also showed that Turkish BVDV samples were closely related with Iran BVDV isolates. According to the results, we think that circulation of similar BVDV genotypes in Turkey and Iran may be possible.

As a result of the study, it was determined that BVDV 1 had a considerable prevalence in the sheep abort cases in Turkey. For this reason, we recommend that diagnostic approaches be applied at the detection point of BVDV as well as BDV in cases of suspected abortion of pestivirus in sheep and goats. In addition, we believe that the prevalence of BVDV should be studied more frequently in cases of abortion in sheep. In light of these results, it should be taken into consideration that heterologous virus transmission may be even more important in countries where sheep and cattle are occasionally or prolonged coexistence in breeding like Turkey. In conclusion, we believe that the evaluation of pestiviruses in sheep and cattle species together, and the exact detection of virus transmission sources in both species may be very important for success in combating pestiviruses.

Ethical approval: All animal studies were approved by the Animal Ethics Committee of Pendik Veterinary Control and Research Institute (Protocol number: 2011/47).

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Authors contribution: HB and IS; study design, data collection and manuscript preparation. ZP and HA; study design, sample collection, laboratory analysis. AS and AC; sample collection, laboratory analysis.

REFERENCES

Albayrak H, Gumusova SO, Ozan E, *et al*., 2012. Molecular detection of pestiviruses in aborted foetuses from provinces in northern Turkey. Trop Anim Health Prod 44:677-80.

Azkur AK, Gazyagci S, Aslan ME, *et al*., 2011. Molecular and serological characterization of pestivirus infection among sheep in Kırıkkale, Turkey. J Faculty Vet Med Kafkas Univ 17:83-92.

Akashi H, Nakamura K and Murakami T, 1997. Restriction fragment length polymorphism analysis of Akabane virus nucleoprotein gene. J Vet Med Sci 59:837-40.

Aslan ME, Azkur AK and Gazyagci S, 2015. Epidemiology and genetic characterization of BVDV, BHV-1, BHV-4, BHV-5 and Brucella spp. infections in cattle in Turkey. J Vet Med Sci 77:1371-7.

Becher P, Avalos RR, Orlich M, *et al*., 2003. Genetic and antigenic characterization of novel pestivirus genotypes: implications for classification. Virology 311:96-104.

Carlsson U, 1991. Border disease in sheep caused by transmission of virus from cattle persistently infected with bovine virus diarrhoea virus. Vet Rec 128:145-7.

Dabak M, Karapinar T, Gulacti I, *et al*., 2007. Hemorrhagic syndrome-like disease in calves with bovine viral diarrhea and mucosal disease complex. J Vet Intern Med 21:514-8.

García-Pérez AL, Minguijón E, Barandika JF, *et al*., 2009. Detection of border disease virus in fetuses, stillbirths and newborn lambs from natural and experimental infections. J Vet Diagn Invest 21:331-7.

Givens MD and Marley MS, 2008. Infectious causes of embryonic and fetal mortality. Theriogenology 70:270-85.

Goktuna TP, Alpay G, Oner EB *et al*., 2016. The role of herpesviruses (BoHV-1 and BoHV-4) and pestiviruses (BVDV and BDV) in ruminant abortion cases in western Turkey. Trop Anim Health Prod 48:1021-7.

Greiser-Wilke I, Grummer B and Volker M, 2003. Bovine viral diarrhoea eradication and control programme in Europe. Biologicals 31:113-8.

Hasircioglu S, Kale M and Acar A, 2009. Investigation of pestivirus infections in aborted sheep and goats in Burdur region. J Faculty Vet Med Kafkas Univ 15:163-7.

Menzies PI, 2011. Control of important causes of infectious abortion in sheep and goats. Vet Clin North Am Food Anim Pract 27:81-93.

Nettleton PF, Gilray JA, Russo P *et al*., 1998. Border disease of sheep and goats. Vet Res 29:327-40.

Passler T and Walz PH, 2010. Bovine viral diarrhea virus infections in heterologous species. Anim Health Res Rev 11:191-205.

Paton DJ, Sands JJ, Lowings JP, *et al*., 1995. A proposed division of the pestivirus genus using monoclonal antibodies, supported by cross neutralization assays and genetic sequencing. Vet Res 26:92-109.

Sarikaya B, Azkur AK, Gazyagci S *et al*., 2012. Genetic variability of bovine viral diarrhea virus in the 5'-UTR in the central Anatolia of Turkey. Acta Sci Vet 40:1013-8.

Shaw AE, Monaghan P, Alpar HO, *et al*., 2007. Development and initial evaluation of a real-time RT-PCR assay to detect bluetongue virus genome segment 1. J Virol Methods 145:115-26.

Thabti F, Leellier C, Hammami S, *et al*., 2005. Detection of a novel border disease virus subgroup in Tunisian sheep. Arch Virol 150:215-29.

Vilcek S and Paton DJ, 2000. A RT-PCR assay for the rapid recognition of border disease virus. Vet Res 31:437-45.

Xue W and Minocha HC, 1996. Identification of bovine viral diarrhea virus receptor in different cell types. Vet Microbiol 49:67-9.

Yesilbag K, Förster C, Bank-Wolf B, *et al*., 2008. Genetic heterogenity of bovine viral diarrhoea virus 11 (BVDV) isolates from Turkey: identification of a new subgroup in BVDV-1. Vet Microbiol 130:258-67.

Yesilbag K, Förster C, Ozyigit MO, *et al*., 2014. Characterisation of bovine viral diarrhoea virus (BVDV) isolates from an outbreak with haemorrhagic enteritis and severe pneumonia. Vet Microbiol 169:42-9.

Yilmaz H, Atan E, Ridpath J, *et al*., 2012. Genetic diversity and frequency of bovine viral diarrhea virus (BVDV) detected in cattle in Turkey. Comp Immunol Microbiol Infect Dis 35:411-6.