

Co-infection with Border Disease Virus and *Brucella melitensis* in an Aborted Sheep Foetus

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Abstract: In this study, we investigated the potential roles of BDV and *Brucella melitensis* infections in a case of sheep abortion. Internal organ specimens from aborted sheep foetus and EDTA whole blood sample from mother of the foetus were collected from a sheep flock in the Konya Province in the Central Anatolia region of Turkey in 2017. The presence of *Brucella* spp. directly assessed by bacterial isolation and detection of BDV was carried by real time RT-PCR. Genetic characterization of the BDV field isolate was conducted by sequencing the 5'-end untranslated region (UTR) region of BDV. *Brucella* strain was isolated from the samples of aborted sheep foetus, and it was identified as *Brucella melitensis* by biochemical characteristics, agglutination with monospecific A and M sera. BDV RNA was detected in EDTA whole blood sample and aborted sheep foetus. Phylogenetic analysis in 5'-UTR region allocated the field isolate of BDV obtained in this study into BDV-7 genotype. To the best of our knowledge, this is the first report on the dual infection of aborted sheep foetus with BDV and *Brucella melitensis*.

Key words: Abortion, Border disease virus, Genetic characterization, *Brucella melitensis*, Sheep

Bir Koyun Abort Fötusunun Border Disease Virusu ve *Brucella melitensis* ile Koenfeksiyonu

Özet: Bu çalışmada, bir koyun abort vakasında border disease virusu (BDV) ve *Brucella melitensis* enfeksiyonlarının potansiyel rolleri araştırılmıştır. İki bin on yedi yılında Türkiye'nin İç Anadolu Bölgesinde yer alan Konya İlindeki bir koyun işletmesinden, bir abort koyun fötüsüne ait iç organ örnekleri ve fötüsün annesinden EDTA'lı tam kan örneği elde edilmiştir. *Brucella* spp. varlığı bakteriyel izolasyon, BDV ise real time RT-PCR yöntemi ile araştırılmıştır. BDV'un genetik karakterizasyonu, 5'- translate olmayan bölge sonunun (UTR) sekans analizi ile gerçekleştirilmiştir. Abort koyun fötüsünden izole edilen *Brucella* suşu biyokimyasal karakteri ve monospesifik A ve M serumları ile aglütinasyonuna bağlı olarak *Brucella melitensis* olarak tanımlanmıştır. Koyun abort fötüsünde ve EDTA'lı kan örneğinde BDV tespit edilmiştir. Filogenetik analiz sonucu, bu çalışmada izole edilen BDV saha suşunun BDV-7 genotipinde olduğu belirlenmiştir. Bizim bildiğimiz kadarıyla, bu çalışma koyun fötüsünün BDV ve *Brucella melitensis* ile birlikte enfeksiyonu hakkındaki ilk rapordur.

Anahtar Kelimeler: Yavru atma, Border disease virus, Genetik karakterizasyon, *Brucella melitensis*, Koyun

Introduction

Border disease (BD) is a reproductive disease of sheep, and occasionally seen in goats. The clinical manifestations of the disease are infertility, abortion, mummified fetuses, stillbirths, and the birth of 'hairy-shaker' lambs and persistent infections of the offspring [9]. The causative agent of disease, border disease virus (BDV), classified in the genus *Pestivirus* of the *Flaviviridae* family, and is closely related to bovine virus diarrhea viruses (BVDV 1, 2) and classical swine fever virus (CSFV) [6]. BDV can also infect cattle, chamois and pigs [1,8]. Transmission of BDV mainly occurs by horizontal

and vertical routes, and weak lambs can be persistently infected (PI) [2].

Ovinebrucellosis is another economically important disease of small ruminants that causes reproductive problems such as infertility and abortions. *B. melitensis* is the main etiological agent of brucellosis in small ruminants. The main clinical signs of *B. melitensis* infection in small ruminants are abortion and stillbirths, which usually occur during the last two months of gestation following infection [3]. The current study was conducted to investigate occurrence of BDV and *B. melitensis* in the case of small ruminant abortion.

Material and Methods

Collection of samples

An aborted sheep foetus was submitted to the Konya Veterinary Control Institute from a sheep flock in the Konya Province in the Central Anatolia region of Turkey in 2017. According to farmer' report, flock had a history of barren ewes, birth of small weak lambs with hairy fleeces, and abortions occurred at 2 to 3 months of gestation. The rate of abortion in this flock was 15% (24/160). Foetal stomach contents and liver of the aborted foetus were collected. Furthermore, whole blood sample from mother of the foetus and internal organ specimens of aborted foetus were collected for BDV detection.

Bacteriological examinations

Samples from stomach contents and liver of the aborted foetus were inoculated onto Farrell's medium (5-10% v/v sterile inactivated horse serum) (Oxoid, SR0035) supplemented with *Brucella* selective supplement (Oxoid, SR083A). After incubation of the plate at 37°C and 5% CO₂ conditions for 7 days, the observed colonies were investigated and identified as *Brucella* spp. by morphological, cultural and characteristics. The strain was biotyped by agglutination with monospecific A and M antisera. Samples were also inoculated onto Campylobacter agar base with selective supplement (Oxoid, SR069E) and 7% defibrinated sheep blood and MacConkey agar for isolation of other bacterial agents.

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Viral RNA extracted from the buffy coat cells from whole blood sample and organ specimens of aborted foetus using a QIAamp Cador Pathogen Mini Kit (Qiagen, Hilden, Germany). A quantitative real-time RT-PCR described by La Rocca and Sandvik [7] was used to detect BDV RNA. Amplification of part of the 5'- end untranslated region (UTR) was carried out for samples in one step RT-PCR using primers 324 and 326 [11].

Sequence and phylogenetic analysis

PCR products were purified from gels with a High Pure PCR Product Purification Kit (Roche Diagnostics, Indianapolis, USA), and sequenced on

an ABI 3130xl DNA Analyser (Applied Biosystems, USA). Phylogenetic tree was constructed with the programme MEGA software version 6, based on the evolutionary distances between different sequences calculated by Kimura two-parameter model. The confidence of the neighbour-joining tree was assessed by bootstrapping, using 1000 replicates, and only values above 50% are reported.

Results

Bacteriological isolation

In this study, *Brucella* was isolated from aborted sheep foetus. *Brucella* strain was identified as *B. melitensis* by biochemical characteristics and agglutination with monospecific A and M antisera. Other bacterial agents were not detected in the investigated foetus.

Detection of BDV

BDV RNA was detected in the investigated foetus and EDTA whole blood sample from mother of the foetus

Sequence analyses

A 100% level of identity was observed between the deduced amino acid sequences of the two isolates, from foetus and its mother, in the present study, whereas the similarity with sequences from different regions ranged from 67.8% to 96%, lowest with United States isolate (890) highest with Turkish isolate (Aydin-04).

Discussion

In this study, *Brucella* was isolated from aborted sheep foetus, and identified as *B. melitensis*. The rate of abortion in *B. melitensis* positive flock was 15% (24/160). This rate is consistent with the findings of previous studies in which it has been reported that rate of abortion in *B. melitensis* positive flocks ranged between 6% and 45% [4,5].

Border disease virus can cause abortion in pregnant small ruminants [9]. In this study BDV RNA was detected in the investigated foetus. To the best of our knowledge, this is the first report on the dual infection of aborted sheep foetus with BDV and *B. melitensis*. It has been reported that foetal death may occur at any stage of gestation, but is

more common during the first 2 months of gestation [12]. In this study abortion occurred at 2 to 3 months of gestation. This situation can be explained by the period of infection, immune status of the host and the virulence of virus.

Phylogenetic analysis has been used to determine the subgenotypes of field isolates from different areas of the world. The most frequent genetic

classification is based on a comparison of nucleotide sequences from the 5'UTR [1,10]. The phylogenetic analysis of 5'UTR sequences typed the field isolate in this study as BDV and clustered within the BDV-7 isolates together previously characterized Turkish isolates (Figure 1). The circulation of BDV-7 genotype in Turkey was also reported in previous study [10]. Results show that BDV-7 genotype is in circulation in Turkey.

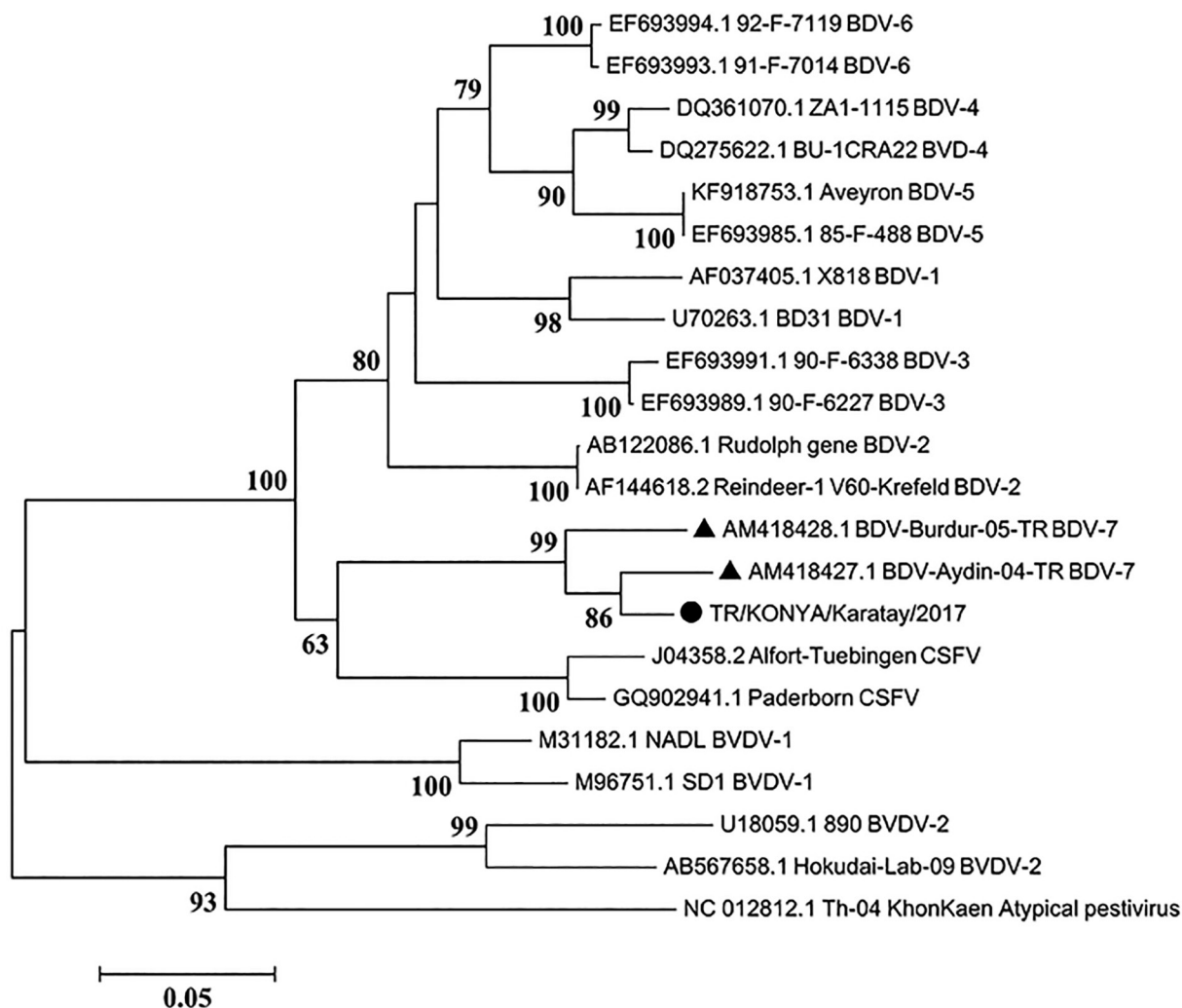


Figure 1. Phylogenetic tree constructed based on nucleotide sequences of the 5'UTR region (246 bp) showing the genetic relationships between BDVs of Turkey and other virus isolates. The sequence obtained in this study is marked with round black spot (●) and previous Turkish isolates are marked with black triangle (▲).

The results of this study indicate that dual infection with BDV and *B. melitensis* can occur in small ruminant abortion cases. BDV and *B. melitensis* infections cause important economic losses

due to reproductive failure in affected animals. Therefore, abortion cases should be examined for these two diseases.

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