Dual Infection of Sheep Aborted Foetus with Peste des Petits Ruminants Virus and *Brucella melitensis*

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Abstract: In this study, we investigated the potential roles of *Brucella melitensis* and PPR virus (PPRV) infections in a case of sheep abortion. Samples were collected from PPR-suspected ewe and its aborted foetus from a sheep flock in the Antalya Province in the Mediterranean region of Turkey in 2016. The presence of *Brucella* spp. directly assessed by bacterial isolation and detection of PPRV was carried by real time RT-PCR. Genetic characterization of the PPRV field isolates was conducted by sequencing the fusion (F) gene of PPRV. *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. PPRV RNA was detected in samples of PPR-suspected ewe and its foetus. Phylogenetic analysis showed that the field isolate of PPRV obtained in this study was clustered within lineage IV. To the best of our knowledge, this is the first report on the dual infection of aborted sheep foetus with PPRV and *Brucella melitensis*.

Key words: Sheep foetus, Peste des petits ruminants virus, Genetic characterization, F gene, *Brucella melitensis*

Abort Olmuş Koyun Fötusunun Peste des Petits Ruminants Virus ve *Brucella melitensis* ile İkili Enfeksiyonu

Özet: Bu çalışmada bir koyun abort vakasında, koyun ve keçi vebası virusu (PPRV) ile *Brucella melitensis*'in potansiyel rolleri araştırılmıştır. Çalışmada kullanılan örnekler, 2016 yılında Türkiye'nin Akdeniz Bölgesinde yer alan Antalya İlindeki bir koyun işletmesindeki PPR şüpheli bir koyun ve bu koyunun fötusundan elde edilmiştir. *Brucella* spp. varlığı bakteriyel izolasyon, PPRV ise real time RT-PCR yöntemi ile araştırılmıştır. PPRV'unun genetik karakterizasyonu, PPRV'unun füzyon (F) geninin sekans analizi ile gerçekleştirilmiştir. Abort koyun fötusundan izole edilen *Brucella* suşu biyokimyasal karakteri ve monospesifik A ve M serumları ile aglütinasyonuna bağlı olarak *Brucella melitensis* olarak identifiye edilmiştir. PPR şüpheli koyun ve fötusunda PPRV tespit edilmiştir. Filogenetik analiz sonucu, bu çalışmada izole edilen PPRV saha suşunun lineage IV'de yer aldığı belirlenmiştir. Bizim bildiğimiz kadarıyla, bu çalışma koyun fötusunun PPRV ve *Brucella melitensis* ile ikili enfeksiyonu hakkındaki ilk rapordur.

Anahtar Kelimler: Koyun fötus, Koyun ve keçi vebası virusu, Genetik karakterizasyon, F gen, *Brucella melitensis*

Introduction

Peste des petits ruminants (PPR) is a highly contagious disease of small ruminants, which is characterised by high fever, pneumonia and enteritis. The causative agent, peste des petits ruminants virus (PPRV) belongs to the *Morbillivirus* genus of the *Paramyxoviridae* family [6]. Transmission of PPRV mainly occurs during close contact [3]. However, vertical transmission of PPRV has been reported [9,16].

B. melitensis, the main etiological agent of brucellosis in small ruminants, is the most important and pathogenic *Brucella* spp. with a worldwide distribution [14]. *B. melitensis* is usually transmitted both vertically and horizontally. It can cause abortions and stillbirths [11]. Furthermore, border disease virus (BDV) can be one of the causes of abortion in small ruminants [12]. The current study was conducted to investigate the potential roles of *B. melitensis*, PPRV and BDV in a case of sheep 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 Antalya Province in the Mediterranean region of Turkey in 2016. According to farmer' report, fever, ocular and nasal discharge and nodular lesions around the mouth were observed in the ewe before

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abortion, and abortions occurred at 2 months of gestation. The rate of abortion in this flock was 25% (20/80). Foetal stomach contents and liver of the aborted foetus were collected. Furthermore, nodular lesions of ewe that had aborted and internal organ specimens (spleen, lung and liver) of aborted foetus were collected for PPRV 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% CO_2 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 [11].

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 real-time RT-PCR

Viral RNA extraction was carried out from the nodular lesions of ewe that had aborted and organ specimens of aborted foetus using a QIAamp Cador Pathogen Mini Kit (Qiagen, Hilden, Germany) in a QIAcube (Qiagen, Hilden, Germany). Real-time RT-PCR was performed using PPRV nucleocapsid protein (N) gene specific primers and probe designed by Batten et al. [1].

Furthermore, aborted foetus samples were also tested by real-time RT-PCR for detection of BDV. The protocol described by La Rocca and Sandvik [10] was used for detection of BDV RNA.

RT-PCR and sequencing of PCR products

One-step RT-PCR was performed with primers that amplified 448 bp of the fusion (F) protein gene of PPRV [5]. PCR products were purified from gels and sequenced. Sequence analysis was performed by using ChromasPro software (Version 1.7.5, Technolysium Ltd.). Phylogenetic tree was constructed for the F gene of PPRV with additional sequences from GenBank.

Results

Bacteriological isolation

Brucella was isolated from stomach contents and liver of the aborted 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 PPRV

PPRV RNA was detected in spleen, lung and liver samples from foetus and nodular lesions of ewe that had aborted. However, BDV RNA was not detected in the investigated foetus samples.

Sequence analyses

Analysis of the PPRV F gene sequences revealed the homology between the two isolates in the present study was 100%, whereas the similarity among the field isolate in this study and previously characterized Turkish isolates ranged from 87.6% to 100%. The deduced amino acid homology among the field isolate and previously characterised PPRV isolates ranged between 96% and 100%.

Discussion

B. melitensis is the main aetiological agent of sheep and goat brucellosis in Turkey. Previous investigations of abortion cases in sheep in different regions of Turkey have shown that *B. melitensis* is responsible for about 20-31% of sheep abortions [2,8]. Stomach contents, spleen, liver, lung and foetal membranes are useful for diagnosis of *B. melitensis* in aborted foetuses [11]. In this study, *Brucella* was isolated from stomach contents and liver of the aborted foetus, and identified as *B. melitensis*. However, Ilhan et al. [8] reported that using stomach contents for diagnosis is better than using other foetal materials. The rate of abortion in *B. melitensis* positive flock was 25% (20/80). 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% [4,7].

PPRV can cause abortion in pregnant animals [16]. In this study PPRV RNA was detected in spleen, lung and liver samples from foetus. This finding in agreement with previous report that suggest lung, liver, spleen and mesenteric lymph node samples can equally be used for PPR virus detection [15]. To the best of our knowledge, this is the first report on the dual infection of aborted sheep foetus with PPRV and *B. melitensis*. The phylogenetic tree based on F gene sequences revealed that field isolate obtained from investigated foetus clustered within lineage IV (Figure 1). The circulation of PPRV lineage IV in Turkey was also reported in previous studies [13,16]. Results show that lineage IV is in circulation in Turkey since the disease was first reported.

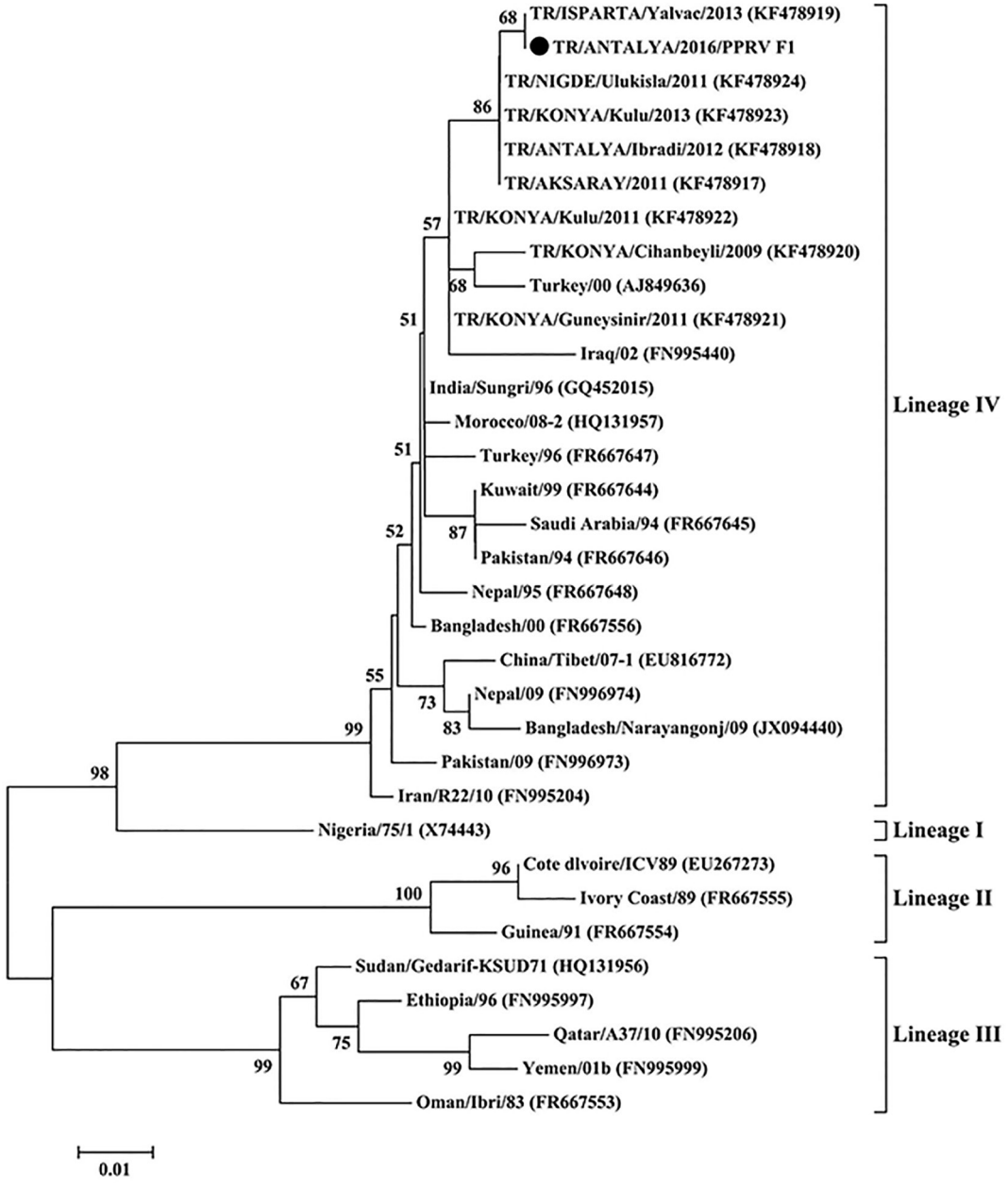

Figure 1. Phylogenetic tree constructed based on nucleotide sequences of the F gene (322 bp) showing the genetic relationships between PPRVs of Turkey and other virus isolates. The sequence obtained in this study is marked with round black spot (●).

The results of this study indicate that dual infection with PPRV and *B. melitensis* can occur in abortion cases. Therefore, PPRV should be taken into consideration in abortion cases in endemic areas.

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