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Border disease virus and *Chlamydophila abortus* co-infection in aborted sheep fetuses

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ABSTRACT: Abortion in sheep and goats is one of the most important problems that affect small ruminants breeding in Turkey. The aim of this study was to examine the abortion cases in two sheep flocks. A high rate of abortion (43.75%) was observed in two sheep flocks in Niğde Province in Turkey during the lambing season in 2016. To determine the reason of abortion, aborted sheep fetuses (n = 3) and EDTA whole blood samples (n = 3) from mother of the fetuses were collected from two sheep flocks. Aborted fetuses and buffy coat cells of the EDTA whole blood samples were used for total nucleic acid extraction. Extracted nucleic acids were analysed for akabane virus (AKAV), bluetongue virus (BTV), peste des petits ruminants virus (PPRV), pestiviruses, Schmallenberg virus (SBV), *Brucella* spp., *Chlamydophila abortus* (*C. abortus*), *Coxiella burnetii* and *Listeria monocytogenes*. Border disease virus (BDV) RNA and *C. abortus* DNA were detected in three aborted sheep fetuses, whereas other investigated infectious agents were not detected. Additionally, EDTA whole blood samples from mother of the fetuses were also found BDV positive. This present case report is the first report on BDV and *C. abortus* co-infection in aborted sheep fetuses.

Keywords: Border disease virus; *Chlamydophila abortus*; sheep; abortion; Turkey

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CASE HISTORY

Reproductive failures including abortion, stillbirth and neonatal losses are significant economic problems for small ruminant breeding (Alemayehu et al., 2021; Clune et al., 2021). Generally, infectious and non-infectious factors cause abortion in small ruminants (Menziés, 2007; Menziés, 2011; Alemayehu et al., 2021). Viral, bacterial, mycotic, parasitic agents are mostly diagnosed infectious agents of the abortion in sheep abortion cases (Menziés, 2011; Şevik, 2021). This paper aims to describe findings from two sheep flocks which had high level of abortions.

In January 2016, during the lambing season, an unusual high rate of abortion and stillbirths were observed in two sheep flocks in Niğde Province in Turkey. The total rate of abortion was 43.75% (35/80), of which 37.5% (18/48) was from one of the two flocks, whereas 53.12% (17/32) was from other flock. Breed of the sheep in these two flocks was Akkaraman. According to farmers' report, abortions (90%) mostly occurred in ewes on their first pregnancy, during the 2 to 5 months of gestation, and the main clinical signs were anorexia and fatigue. To determine the reason of abortion, aborted sheep fetuses ($n = 3$) and EDTA whole blood samples ($n = 3$) from mother of the fetuses were collected from two sheep flocks, and samples brought to laboratory under cold-chain conditions. Buffy coat cells were obtained from EDTA whole blood samples by centrifugation at $2000\times g$ at $4^{\circ}C$ for 10 min. A commercial extraction kit (QIAamp Cador Pathogen Mini Kit, Qiagen, Germany) was used for total nucleic acid extraction from organ specimens (lung, liver, kidney and spleen) of the aborted fetuses and buffy coat cells. Nucleic acid extracts of the samples were tested using molecular methods for

the presence of pestiviruses (Vilcek et al., 1994), akabane virus (AKAV) (Stram et al., 2004), bluetongue virus (BTV) (Hofmann et al., 2008), peste des petits ruminants virus (PPRV) (Batten et al. 2011), Schmallenberg virus (SBV) (Bilk et al., 2012), *Brucella* spp., (Bricker and Halling, 1994), *Listeria monocytogenes*, (Rossmann et al., 2006) *Coxiella burnetii* (Klee et al., 2006) and *Chlamydia abortus* (*C. abortus*) (Thiele et al., 1992). These infectious agents were chosen for analysing according to choose of the responsible veterinarian. Border disease virus (BDV) RNA and *C. abortus* DNA were detected in aborted sheep fetuses, whereas other investigated infectious agents were not detected (Figure 1). Additionally, mother of the fetuses were also found positive for BDV.

DISCUSSION

Pestiviruses have a worldwide distribution, and are one of the important viral agents that cause abortion in ruminants including sheep, goats and cattle (Nettleton et al., 1998, OIE, 2017). *C. abortus*, a gram-negative zoonotic bacterium, is considered as the main cause of abortion in sheep and goats worldwide (Brom et al., 2021). In this study, BDV and *C. abortus* co-infections were detected in aborted sheep fetuses. To the best of my knowledge, this is the first report on BDV and *C. abortus* co-infection in aborted sheep fetuses. Unfortunately, a detailed risk factor analysis was not performed in investigated flocks. However, owner of the flocks said that they bought new animals a few weeks before abortion events occurred. Therefore, it can be speculated that these agents could be introduced to flocks by purchased animals.

In this study, rates of abortion in two BDV pos-

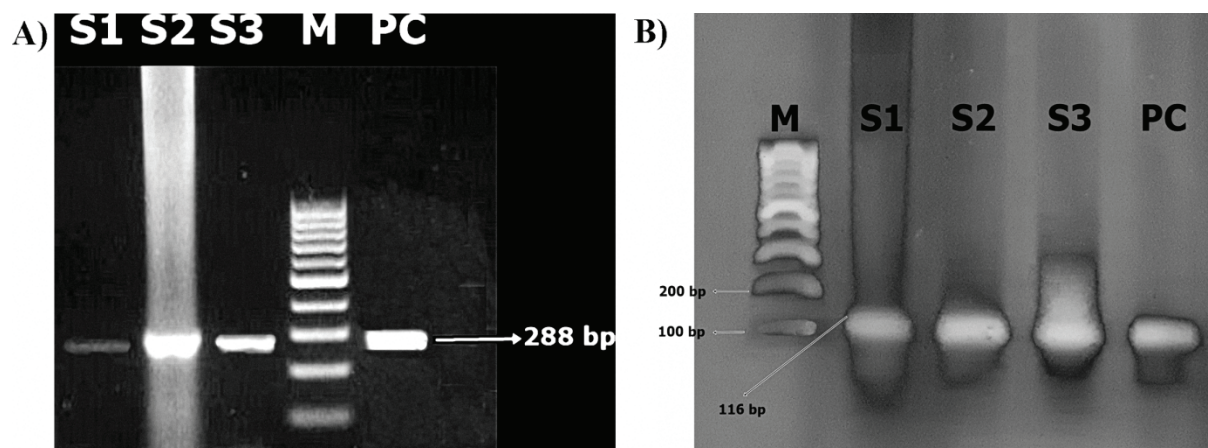


Figure 1. Results of the one step RT-PCR of the pestivirus (A) and PCR of the *C. abortus* (B). M: 100 bp DNA ladder, PC: Positive control, S1-S3: Samples of aborted sheep fetuses.

itive flocks were 37.5% and 53.12%, respectively. This finding is consistent with the findings of previous studies from Turkey that reported abortion rates in BDV positive flocks ranged between 47.36% and 66.66% (Albayrak et al., 2012; Tuncer-Göktuna et al., 2016). However, detected rate was higher than that found in different regions of Turkey, ranged between 0.93% and 12.6% (Cokcaliskan, 2002; Hasircioglu et al., 2009; Şevik, 2018). These differences in rates between this study and previous studies may be related to the strain of the BDV, host immune response, management practices and detection methods.

In this study, BDV positive flocks had abortion at 2 to 5 months of gestation. This finding is consistent with the reports of previous studies that reported abortion mostly occurred due to BDV infection at 2 to 3 months of gestation (OIE, 2017). Furthermore, if ewes infected with BDV late in pregnancy, abortion also occurs after 85 days of gestation (Givens and Marley, 2008).

In this study, rate of abortion in *C. abortus* positive flocks was 43.75%. This finding is in agreement with previous reports. Abortion rates could be as high as 40% in *C. abortus* positive flocks (GalVmed, 2022). Barati et al. (2022) found that rate of *C. abortus* was 34.25% in aborted sheep fetuses in Iran. However, detected rate in this study was higher than that found in different regions of Turkey, ranged between 3.49% and 6% (Güler et al., 2006; Kalender et al., 2013). The variations in abortion rates in *C. abortus* positive

flocks may be associated with number of sampled animals and flocks, detection methods and management practices.

Abortions due to *C. abortus* infection mostly occur last 4 weeks of gestation (OIE, 2018). However, in this study *C. abortus* positive fetuses were from 2 to 5 months of gestation. Similar result also found in a previous study (Longbottom et al., 2013). Longbottom et al. (2013) also reported that *C. abortus* infected ewes can abort at 74 to 138 days of gestation. This situation can be explained by host immune response, presence of other viral or bacterial infections and non-infectious conditions.

In conclusion, results of this study showed that high abortion rates in sheep can occur as a result of co-infection with BDV and *C. abortus*. Therefore, abortion cases should be tested for BDV and *C. abortus*, and prevention measures should be taken against these two diseases.

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CONFLICT OF INTEREST

The author stated that there are no conflicts of interest.

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