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Assessment of Role of Epizootic Hemorrhagic Disease Virus in Abortion in Cattle and Small Ruminants in Türkiye

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ABSTRACT

Epizootic hemorrhagic disease (EHD) is a vector-borne viral disease of ruminants that can cause significant economic losses due to reduced production and trade restrictions. Furthermore, epizootic hemorrhagic disease virus (EHDV) can cause abortion. However, role of EHDV in abortion in cattle and small ruminants remains uncertain. Therefore, this research aimed to assess the frequency of EHDV in aborted foetuses. In this research, a total of 2029 aborted foetuses (from 553 cattle, 1388 sheep, and 88 goats) were collected from different herds and flocks in the Aegean, Mediterranean and Central Anatolian regions of Türkiye during the period of 2012 and 2017. A real-time reverse transcription polymerase chain reaction (RT-PCR) assay was used to detect EHDV specific RNA in aborted foetuses. EHDV specific RNA was not detected within aborted foetuses. The results of the study suggest that EHDV does not play a role in abortion in cattle and small ruminants in the studied regions of Türkiye. Further research is needed to determine the role of EHDV in abortion in domestic ruminants.

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INTRODUCTION

Epizootic hemorrhagic disease (EHD), included in the notifiable diseases list of the World Organization for Animal Health (WOAH), is a vector-borne viral disease of domestic and wild ruminants, including cattle, sheep, goats, bison, mountain goats, elk, pronghorn antelope, and white-tailed and black-tailed deer (Nol et al., 2010; Favero et al., 2013; WOAH, 2021; Roug et al., 2022; Jiménez-Cabello et al., 2023).

Although EHD causes high mortality in white-tailed deer (*Odocoileus virginianus*), cattle can suffer severe disease from epizootic hemorrhagic disease virus (EHDV) infection depending on virus serotype, virulence of the strain, and host-immune status (EFSA, 2009). Sheep and goats can also be infected with EHDV, yet they do not present clinical symptoms of the disease (Mahmoud et al., 2021; Duan et al., 2022). The morbidity and mortality rates can reach 90% in white tailed deer; however, morbidity rates in cattle range from 1 to 18%, and mortality rate is lower than 1% (Yadin et al., 2008; Temizel et al., 2009).

The infectious agent, EHDV, is a non-enveloped segmented double-stranded RNA virus of the genus *Orbivirus* within the family *Sedoreoviridae*, along with African horse sickness virus and bluetongue virus (Mertens et al., 2005). It has four nonstructural proteins (NS1, NS2, NS3/NS3a, and NS4) and seven different structural proteins (VP1-VP7) (Anthony et al., 2009). Up to now, eight EHDV serotypes and two new putative serotypes have been identified based on cross neutralization tests and genetic analyses of the segment 2 nucleotide sequences of the virus (WOAH, 2021; Thabet et al, 2023). The EHDV serotypes are mainly transmitted by biological vectors, usually biting midges of the genus *Culicoides*, especially *C. obsoletus*, *C. oxystoma*, *C. imicola*, *C. mohave*, *C. brevitarsis*, and *C. sonorensis* (McGregor et al., 2019; Mendiola et al., 2019; McGregor et al., 2022; Mendiola et al., 2022; Jiménez-Cabello et al., 2023).

EHDV infection was first reported in white-tailed deer in 1955 in the United States of America (Shope et al., 1955). After that time, EHD has been reported in South and North America, Asia, the Middle East, Africa, and Türkiye (Temizel et al., 2009; Ruder et al., 2017; Qi et al., 2019; Noronha et al., 2021). In Türkiye, EHDV outbreaks caused by serotype 6 in cattle were observed in 2007 (Temizel et al., 2009). Furthermore, EHDV was detected in sheep in Türkiye (Yavru et al., 2014). A previous serological study also reported presence of EHDV specific antibodies in cattle and G*azella subgutturosa* in Türkiye (Albayrak et al., 2010).

The major clinical signs of the disease in white-tailed deer are fever, excessive salivation, swelling of the tongue, neck and head, lameness, oral ulcerative lesions, serosal haemorrhages, and sudden death (Nol et al., 2010), whereas oral ulcerations, excessive ocular and nasal secretion, swelling of the tongue, lameness, and reduced milk production are the clinical signs mostly observed in other infected ruminants (Yadin et al., 2008; Temizel et al., 2009; Savini et al., 2011; Maclachlan et al., 2015). It has been also reported that EHDV can cause abortion in ruminants (Ohashi et al., 1999; Golender et al., 2019). EHDV related abortion in cattle was reported in few studies (Golender et al., 2017; Golender et al., 2021). However, the role of EHDV in abortion in cattle and small ruminants remains uncertain. Therefore, this research aimed to assess the frequency of EHDV in aborted foetuses.

MATERIALS AND METHODS

Field Samples

In this study, a total of 2029 aborted foetuses (from 553 cattle, 1388 sheep, and 88 goats), from different herds and flocks in the Mediterranean region (Isparta, Burdur, and Antalya Provinces), Aegean region (Afyonkarahisar Province) and Central Anatolian region (Niğde, Aksaray, Karaman, and Konya Provinces), were submitted during the period of 2012 and 2017 for detection of abortifacient infectious agents to the Konya Veterinary Control Institute, were included in this study.

At necropsy, foetal tissues were collected from each foetus separately under aseptic conditions to prevent the contamination. Samples from each animal were placed into separate sterile labelled tube and were kept at -85°C until RNA extraction.

RNA Extraction from Aborted Foetuses

Foetal tissues of each foetus, weighing 20-30 mg, were placed into 2.0 ml sterile microcentrifuge tubes containing RNase and DNase free water, and were homogenized using the TissueRuptor (Qiagen, Germany). RNA was extracted from homogenates (200 µl) of foetal tissues with QIAamp Cador Pathogen Mini Kit (Qiagen, Germany), using the protocol described by manufacturer. To verify the absence of contamination, RNase and DNase free water was used during RNA extraction.

Detection of EHDV in Foetal Tissues Using Real-time RT-PCR Assay

Firstly, the extracted RNA was denatured for 5 min at 95°C, and then used for real-time RT-PCR assay. The PCR reaction mix was prepared with a RealTime ready RNA Virus Master kit (Roche Diagnostics, Indiana, USA) in a final volume of 25 µl, containing 0.12 µM of forward primer, 0.8 µM of reverse primer, 0.2 µM of probe, and 3.4 µl of sample RNA. The primers and probe used in this study are shown in Table 1. Amplification of the assay was performed using the LightCycler 2.0 Instrument (Roche Diagnostics, Indiana, USA) with the following amplification conditions: 50 °C for 8 min, 95 °C for 30 sec, and 45 cycles of 95 °C for 1 sec, 60 °C for 20 sec, and 72 °C for 1 sec. In this study, samples with cycle threshold (Ct) values < 35 were considered as positive (Maan et al., 2017).

Table 1. Primers and probe sequences used in real-time RT-PCR assay in this study

Primer	Sequence (5'-3')	Genomic target	Reference
EHDV-15-32 F	ATGTCAGCTGCGGTYTTG	Seg-9	Maan et al. (2017)
EHDV-112-85 R	TCCCAATCAACTAARTGRATYTGVATCT	Seg-9	
EHDV-69-48 P	CCTCGGTCGAACGTTGGATCAC	Seg-9	

EHDV RNA obtained from the Central Veterinary Control and Research Institute (Etlik, Ankara, Türkiye) was used as positive control, whereas RNase and DNase free water was used as negative control in real-time RT-PCR assay.

RESULTS

EHDV RNA Detection in Foetal Tissues

In the present study, 127, 87, 74, 69, 81, and 115 aborted bovine foetuses were examined in 2012, 2013, 2014, 2015, 2016, and 2017, respectively. The ages of the foetuses range from 2 to 6 months.

All tested aborted bovine foetuses were found EHDV-negative by real-time RT-PCR, and samples had Ct values ranging between 42.01 and 44.35.

In this study, 198, 217, 336, 383, 157, 97 aborted ovine foetuses were examined in 2012, 2013, 2014, 2015, 2016, and 2017, respectively. The ages of the foetuses range from 1 to 5 months. All tested aborted ovine foetuses were found EHDV-negative by real-time RT-PCR, and samples had Ct values ranging between 41.90 and 43.89.

In the current study, 3, 4, 4, 36, 27, 14 aborted caprine foetuses were examined in 2012, 2013, 2014, 2015, 2016, and 2017, respectively. The ages of the foetuses range from 1 to 5 months. All tested aborted caprine foetuses were found EHDV-negative by real-time RT-PCR, and samples had Ct values ranging between 41.79 and 44.19.

DISCUSSION

Abortions in cattle and small ruminants cause significant economic losses in the livestock industry. Mostly infectious agents (viruses, bacteria, fungi, and protozoa) cause abortion in domestic ruminants. Among the infectious agents, viruses play an important role in the occurrence of abortion and congenital malformations (Golender et al., 2017; Maclachlan and Osburn, 2017; Sick et al., 2019). Mostly, pestiviruses (border disease virus and bovine viral diarrhoea virus), bovine herpesvirus type 1, bluetongue virus, akabane virus, and Schmallenberg virus are responsible for abortions and congenital malformations in domestic ruminants (Inaba et al., 1975; Nettleton, 1990; Osburn, 1994; Graham, 2013; Endalew et al., 2019; Şevik, 2021). Furthermore, some of the EHDV field strains can also induce abortion in pregnant ruminants without clinical signs (Golender et al., 2021). The role of EHDV in abortion is still unknown. Therefore, in this research, role of EHDV in abortion cases was investigated. To the best of my knowledge, this study is the longest research that assessed role of the EHDV in abortion cases of cattle and small ruminants.

Virus isolation is a gold standard method for EHDV detection, yet it is expensive and time consuming (WOAH, 2021). Molecular diagnostic methods can detect viral nucleic acids in a short time with high sensitivity and specificity (Aslanlar et al., 2023; Görkem et al., 2020; Guclu and Ayan, 2023; Karaselek et al., 2023; Şahin et al., 2023). Furthermore, it has been reported that real time RT-PCR has high specificity and sensitivity for detection of EHDV RNA than other diagnostic methods (Viarouge et al., 2015). Therefore, in this research real time RT-PCR was used for detection of EHDV in aborted foetuses.

Until now, eight EHDV serotypes and two new putative serotypes have been identified (WOAH, 2021; Thabet et al., 2023). EHDV serotype 1 has been reported in North America, Australia, China, Egypt, Nigeria, Ecuador and Israel, serotype 2 in North America, Australia, Japan and Oman, serotype 4 in Nigeria, serotype 5 in Australia, Japan, China, Sudan, serotype 6 in North America, Australia, China, Morocco, Algeria, Libya, Türkiye, Tunisia, Oman, Sudan, French Guiana, Trinidad and Israel, serotype 7 in Australia, Japan, China, French Guiana and Israel, and serotype 8 in Italy (Ahmed et al., 2019; Yang et al., 2020; Lorusso et al., 2022; Jiménez-Cabello et al., 2023).

In this study, EHDV was not detected in the samples tested. This situation can be explained by the geographical locations of the samples used in this study. To date, only EHDV serotype 6 has been detected in Türkiye, and EHDV outbreaks were observed in cattle in Muğla Province and in sheep in Aydın Province (Temizel et al., 2009; Yavru et al., 2014). These provinces are located in western part of the Türkiye, which are suitable for reproduction of *Culicoides* spp. which are the primary vector of

EHDV. However, in this study, except Antalya Province, other provinces do not have suitable conditions for reproduction of *Culicoides* spp.

In this study, EHDV RNA was not detected in aborted foetuses. Previous studies carried out by Golender and Bumbarov (2019), Kamomae et al. (2018) in Israel and Japan respectively did not find EHDV in aborted foetuses. However, different studies from Israel reported that they detected EHDV in aborted foetuses with the detection rates ranged from 22.4 to 36.7%. (Golender et al., 2017; Golender et al., 2021). This difference in results of studies may be related to the immune status of sampled animals, the differences in farm management, the sampling strategies, and the serotype of virus. It has been reported that strains of serotype 2 and serotype 6 of EHDV can cause abortion (Ohashi et al., 1999; Golender et al., 2017). Besides, Golender et al. (2017) and Golender et al. (2021) found EHDV specific RNA only in aborted cattle foetuses, and similar to the results of this study, they could not detect the virus in aborted sheep and goats. It seems that EHDV causes abortion in cattle.

CONCLUSION

The results of the research suggest that EHDV does not play a role in abortion in cattle and small ruminants in studied regions of Türkiye. EHD mostly exists in tropical and temperate regions which support vector populations. However, genetic evolution of the virus and climate change increase the risk of introduction of the EHDV in new regions (Jiménez-Cabello et al., 2023). Additional research is needed to determine the role of EHDV in abortion in domestic ruminants.

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Ethics Approval
This research was undertaken with the permission of the General Directorate of Food and Control dated 27.12.2017 and numbered E.3335546.

Conflict of Interest
The author declares that there is no conflict of interest. Conceptualization, methodology, formal analysis, writing-review and editing by Murat ŞEVİK.

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