The absence of the Bluetongue virus in abortion cases in cattle, sheep, and goats in Türkiye: 2012-2017

Research Article

ABSTRACT

Bluetongue (BT) is a disease that affects domestic and wild ruminants, and it is caused by a virus called bluetongue virus (BTV) that is transmitted by *Culicoides* midges. Although clinical signs of BT are most apparent in sheep, BTV could induce abortion and birth defects in cattle, sheep and goats. BTV infection has been reported in Türkiye, but the role of BTV in cattle and small ruminant abortion cases in Türkiye remains uncertain. Therefore, this research aimed to fill this research gap by investigating the prevalence of BTV in cattle and small ruminant abortion cases. To investigate the frequency of BTV in ovine, caprine, and bovine foetuses, a total of 1718 foetuses were collected from different farms between 2012 and 2017. A one-step real-time reverse transcription polymerase chain reaction (RT-PCR) assay was used to detect BTV RNA in aborted foetuses. BTV specific RNA was not detected in the analysed foetuses. To the best of my knowledge, this is the longest study that has investigated whether BTV infection has a role in cattle and small ruminant abortion cases in Türkiye. The results of this study are limited only to the regions studied. Therefore, further epidemiological studies are needed to confirm the findings of this study.

Keywords: Abortion, bluetongue virus, cattle, goats, sheep.

Murat Şevik[1a]

[1] Department of Virology, Veterinary Faculty, Necmettin Erbakan University, Konya, Türkiye

ORCİD-

[a] 0000-0002-9604-3341

Correspondence
Murat Şevik
murat.sevik@erbakan.edu.tr

INTRODUCTION

Bluetongue (BT), one of the reportable diseases by the World Organization for Animal Health (WOAH), is an arboviral disease of sheep, goats, cattle, and wild ruminants, including pronghorn antelope, bighorn sheep, and white-tailed deer (Backx et al., 2007; Falconi et al., 2011; Johnson et al., 2006; Maclachlan, 1994; Niedbalski, 2015). Furthermore, bluetongue virus (BTV) infection has been reported in camelids (Schulz et al., 2012), and BTV was isolated from aborted foetuses of dogs (Dubovi et al., 2013).

The causative agent of the disease, BTV, is a non-enveloped RNA virus of the genus *Orbivirus* within the family *Sedoreoviridae* (ICTV, 2022). The viral genome consists of 10 segments that encode five non-structural proteins (NS5, NS4, NS3/NS3A, NS2, and NS1) and seven structural proteins (VP-1 to VP-7) (Belhouchet et al., 2011; Mertens et al., 1984; Ratinier et al., 2011; Roy, 1992). Until now, a total of 29 serotypes of BTV have been recognised worldwide based on sequence analysis of the segment 2 nucleotide sequences and serum neutralisation tests (Bumbarov et al., 2020; Thota et al., 2020; Yang et al., 2021). Serotypes 1, 2, 3, 4, 6, and 10 have the potential to cause epidemics (Dungu et al., 2004). Furthermore, pathogenicity may change within a serotype due to antigenic drift (i.e. point mutations) and antigenic shift (i.e. reassortment of BTV gene segments) in BTV (Bonneau et al., 2001; Van Schalkwyk et al., 2023).

Article info
Submission: 02-04-2024
Accepted: 07-11-2024
Online First: 16-12-2024
Publication: 27-12-2024

e-ISSN: 2548-1150
doi prefix: 10.31797/vetbio
http://dergipark.org.tr/vetbio

How to cite this article

Şevik M., (2024). The absence of the Bluetongue virus in abortion cases in cattle, sheep, and goats in Türkiye: 2012-2017. *Journal of Advances in VetBio Science and Techniques, 9*(3), 233-241. https://doi.org/10.31797/vetbio.1463932

The signs of BT vary from mild to severe; the disease is more severe in sheep than in cattle and goats (Maclachlan, 1994). Although fever, cyanosis of the tongue, oedema of the face and lips, mucopurulent nasal discharge, excessive salivation, and coronitis are the main clinical signs of the disease (Darpel et al., 2007; Elbers et al., 2008), BTV infection may lead to abortion and teratogenic defects in vertically infected foetuses, depending on the foetal age at infection (Maclachlan and Osburn, 2017). The morbidity rate of the disease ranges from 2 to 48% (Calistri et al., 2004; Conraths et al., 2009), whereas the mortality rate ranges from 1 to 67% (Conraths et al., 2009; Gambles, 1949).

BTV is mainly transmitted by species of *Culicoides* midges, especially *C. imicola*, *C. pulicaris*, *C. chiopterus*, *C. dewulfi*, and *C. obsoletus* (Goffredo et al., 2015; Mellor et al., 2000; Purse et al., 2015). Furthermore, direct contact, vertical transmission of BTV (Wouda et al., 2009; Bréard et al., 2018) and its transmission through semen have been reported (De clercq et al., 2021).

BTV infection was first reported in southern Africa in the late 18th century, and then it was reported in other regions of Africa, Asia, Australia, the Americas, the Indian subcontinent, the Middle East, and Europe (Gestier et al., 2023; Khanal et al., 2016; Mellor and Wittmann, 2002; Sreenivasulu et al., 2004; Wilson and Mellor, 2009). In Türkiye, the first BT cases were reported in 1944, and later outbreaks caused by serotypes 4, 8, 9, and 16 were observed at different times (Rajko-nenow et al., 2020; Saegerman et al., 2008; Taylor and Mellor, 1994). Although previous serological and molecular studies have reported the presence of BTV infection in Türkiye (Ertürk et al., 2004; Ozkul et al., 2009), the role of BTV in cattle and small ruminant abortion cases in Türkiye remains uncertain. Therefore, this research aimed to fill this research gap by investigating the prevalence of BTV in cattle and small ruminant abortion cases.

MATERIALS AND METHODS

Study location

A total of 1718 foetuses (from 1144 sheep, 82 goats, and 492 cattle) were submitted to the Veterinary Control Institute (Konya, Türkiye) from different farms between 2012 and 2017 (Table 1). Farms included in this study were located in three geographical regions of Türkiye, including the Mediterranean (Isparta, Burdur, and Antalya Provinces), Aegean (Afyonkarahisar Province), and Central Anatolian (Konya, Karaman, Aksaray, and Niğde Provinces) regions with elevations of 30-1229 m (Figure 1).

Table 1. Distribution of the foetal samples by provinces and years.

Province	Years																	
	2012			2013			2014			2015			2016			2017		
	O	C	B	O	C	B	O	C	B	O	C	B	O	C	B	O	C	B
Afyonkarahisar	9	-	7	15	-	11	14	-	10	15	-	11	14	-	6	6	-	7
Aksaray	6	-	7	17	-	8	16	2	9	15	21	-	10	2	3	3	2	3
Karaman	6	-	2	4	-	-	17	-	1	4	-	-	7	-	-	1	-	3
Konya	30	3	24	36	-	8	55	1	7	82	3	14	40	11	17	25	5	21
Niğde	75	-	20	38	2	22	42	-	15	79	3	11	27	1	12	21	2	24
Antalya	20	-	25	17	1	8	54	1	9	134	4	5	11	10	10	1	4	13
Burdur	4	-	20	14	-	9	23	-	8	-	-	9	6	-	8	3	-	20
Isparta	12	-	30	18	1	14	77	-	4	17	-	5	2	3	11	2	-	1
Total	**162**	**3**	**135**	**159**	**4**	**80**	**298**	**4**	**63**	**346**	**31**	**55**	**117**	**27**	**67**	**62**	**13**	**92**

O: Ovine, C: Caprine, B: Bovine

Figure 1. Geographic location of the study area (pink colour) where the study was conducted

Among the studied provinces, Antalya Province has the lowest elevation (30 m), whereas Niğde Province has the highest elevation (1229 m). The climatic conditions in the Mediterranean, Aegean, and Central Anatolian regions are characterised by high temperatures and humidity, cold winters and hot summers, hot and dry summers, and cold and snowy winters, respectively. Average temperatures in the Mediterranean, Aegean, and Central Anatolian regions are 19°C, 15°C, and 16°C, respectively (Turkish state meteorological service, 2020). Domestic ruminant production plays a crucial role in the rural economic development of the studied regions.

Tissue collection

Necropsy was performed on each foetus under aseptic conditions to prevent contamination. During necropsy, tissue samples from the brain, spleen, lung, kidney, and liver were collected, placed into sterile tubes, and stored at -20°C until nucleic acid extraction. Furthermore, information related to abortions, the age of the pregnant animals, the clinical signs, and date of abortion was obtained from farmers using a semi-structured questionnaire.

Viral RNA extraction

The tissues of each foetus were pooled (30 mg) and homogenized in sterile phosphate-buffered saline by using a tissue homogenizer (Qiagen, Germany). The supernatant of tissue homogenates (200 µl) was collected and used for viral RNA extraction. The extraction was performed with a commercially available kit (QIAamp Cador Pathogen Mini Kit, Qiagen, Germany) according to the manufacturer's instructions, and nuclease-free water was used as a control sample to verify the absence of contamination. Viral RNA measurement was performed using a spectrophotometer (DeNovix DS-C, DeNovix Inc., USA), and viral RNA was kept at -85°C until analysis.

One-step real-time RT-PCR assay

Viral RNAs were denatured at 98°C for 5 min on a heating block (Stuart Scientific, UK), then placed at -20°C for 5 min. The PCR reaction mix for a one-step real-time RT-PCR assay was prepared with a OneStep RT-PCR kit (Qiagen, Germany) in a final volume of 25 µl, containing 5 µl 5 X RT-

PCR buffer, 0.8 µM of each primer, 0.1 µM probe, and 6 µl RNA of the sample. The primers and probe used in one-step real-time RT-PCR assay are listed in Table 2. One-step real-time RT-PCR assay was performed using a real-time PCR machine (Rotor-Gene Q, Qiagen, Germany) with the following amplification conditions: 55 °C for 30 min, 95 °C for 10 min, and 45 cycles of 95 °C for 15 sec, and 60 °C for 1 min. In the current study, samples with cycle threshold (Ct) values > 35 were considered negative, whereas samples with Ct values < 35 were considered positive (Shaw et al., 2007). BTV-4 RNA obtained from the Central Veterinary Control and Research Institute (Ankara, Türkiye) was used as a positive control, and nuclease-free water was used as a negative control in one-step real-time RT-PCR assay.

Table 2. Primers and probe sequences used for one-step real-time RT-PCR assay for the detection of BTV segment 1.

Primer	Sequence (5'-3')	Reference
BTVrsa 291–311F	GCGTTCGAAGTTTACATCAAT	SHAW et al. (2007)
BTVuni 291–311F	GCTTTTGAGGTGTACGTGAAC	
BTVrsa 387–357R	CAGTCATCTCTCTAGACACTCTATAATTACG	
BTVuni 381–357R	TCTCCCTTGAAACTCTATAATTACG	
BTV-Probe	CYG GAT CAA GTT CAC TCC AYG GC	

RESULTS

Semi-structured questionnaire survey results

Flock owners reported that depression, anorexia, and nasal discharge were the common signs in ewes, whereas nanny goats showed anorexia before abortion. Abortions occurred in ewes and nanny goats at one to five months of gestation. No congenital defects were observed in caprine and ovine foetuses.

Herd owners reported that pregnant cattle did not show any clinical signs, and abortion occurred at 2 to 9 months of gestation. Congenital malformations were not observed in the examined bovine foetuses.

Detection of BTV-specific RNA by one-step real-time RT-PCR assay

BTV RNA was not detected in the examined foetuses. The Ct value of the positive control sample was 29.56 (Figure 2).

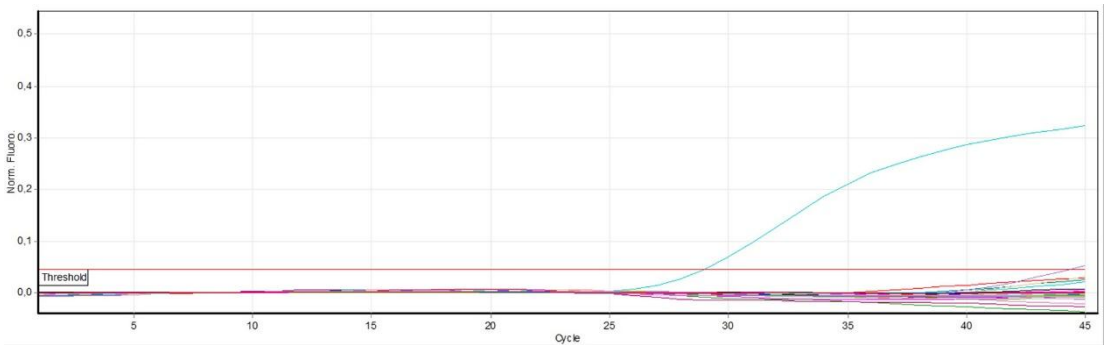

Figure 2. One step real time RT-PCR assay based on Segment 1 of BTV. Light-green line: positive control, red line: negative control.

DISCUSSION

Infectious agents have a significant impact on the livestock abortion (Alzuguren et al., 2023; Dorsch et al., 2022). BTV is one of the important viral abortion agents in cattle and small ruminants (Chauhan et al., 2014; Osburn, 1994). Although BTV infection has been reported in Türkiye, the role of BTV in cattle and small ruminant abortion cases in Türkiye remains uncertain. Therefore, this research aimed to fill this research gap by investigating the prevalence of BTV in cattle and small ruminant abortion cases. To the best of my knowledge, this is the longest study that has investigated the question

of whether BTV infection has a role in cattle and small ruminant abortion cases in Türkiye.

Real-time RT-PCR assays are more specific and sensitive when compared with conventional methods (Batten et al., 2008). Furthermore, WOAH (2021) recommended real-time RT-PCR assays for the detection of BTV in clinical cases. Therefore, in this study, a one-step real-time RT-PCR assay described by Shaw et al. (2007) was used to detect BTV genome segment 1 in ovine, caprine, and bovine foetuses.

BTV is one of the teratogenic infectious agents and can cause central nervous system defects that range from severe hydranencephaly to porencephaly (Maclachlan and Osburn, 2017). The disease is more severe in sheep than in cattle and goats, and central nervous system defects are mostly seen in lambs (Maclachlan, 1994; Maclachlan and Osburn, 2017). Furthermore, BTV can cause abortions without congenital abnormalities (Luedke, 1985). Therefore, in this study, all submitted foetuses without congenital abnormalities were analysed for the presence of BTV.

Although isolation of the BTV and serological evidence of BTV infection in Türkiye has been reported (Ertürk et al., 2004; Yavru et al., 2015), there are currently no reports of detection of BTV in ovine, caprine, or bovine foetuses in Türkiye. In the present study, BTV was not also detected in any of the foetuses examined. A possible explanation for the lack of detection of BTV in foetuses could be related to vaccine-mediated immunity, since there is an official vaccine programme against BTV in Türkiye.

Another possible explanation could be that the insect vectors that play a role in the transmission of the BTV are not available in the research area. *C. obsoletus*, *C. imicola*, *C. dewulfi*, *C. bolitinos*, *C. scoticus*, *C. pulicaris*, *C. insignis*, *C. sonorensis*, and *C. brevitarsis* have a major role in the biological transmission of BTV (Duan et al., 2019; Hudson et al., 2023; Purse et al., 2015). *C. imicola*, *C. pulicaris*, and *C. circumscriptus* were detected in a field study in the Antalya Province (Dik et al., 2006). In the present study, Antalya is the only city where climatic conditions are suitable for the introduction and distribution of *Culicoides* biting midges throughout the entire season, whereas climatic conditions in other studied provinces are suitable for the abundance and distribution of *Culicoides* spp. only between spring and autumn. A possible explanation for the lack of detection of BTV in foetuses could be related to the sampling period, since the majority of aborted foetuses were collected in the winter period, when vector activity is not observed in the Aegean and Central Anatolia Regions.

BTV infection is one of the endemic viral diseases in Türkiye (Ertürk et al., 2004; Ozkul et al., 2009; Yavru et al., 2015), and it has been reported that immunity in animals infected with BTV is lifelong. Lifelong immunity has been linked to protection from severe disease (WOAH, 2021). This could explain why BTV was not detected in the foetuses examined.

It has been reported that BTV infection during the early stages of pregnancy can lead to abortion in infected pregnant animals (Saegerman et al., 2011). In the present study, foetuses were in different stages of pregnancy; the majority of the caprine and ovine foetuses were between 3- and 5-month gestations, whereas bovine foetuses were between 4- and 7-month gestations. The foetal immune response in a bovine foetus develops between days 125 and 150 of gestation (Baker, 1995), whereas in caprine and ovine foetuses it develops after day 70 of gestation (Lopez et al., 2012). Therefore, this study could not detect BTV in foetuses, which may be due to the presence of immune responses in foetuses to BTV, which could contribute to virus clearance in aborted foetuses (Maclachlan et al., 1984).

Contrary to the results of this study, previous studies carried out by Anderson et al. (1989) in the United States and Chauhan et al. (2014) in India detected BTV in bovine and caprine foetuses, respectively. Furthermore, an experimental study carried out by Van der sluijs et al. (2011) also detected BTV in ovine foetuses. This difference in the results of studies may be related to the sample type (malformed or aborted foetuses), the number of animals sampled, the sampling period, the virulence of the virus strain, the immune status of animals, the presence of biological vectors in investigated areas, and the differences in farm management.

CONCLUSION

In this study, results were obtained only from the studied regions. Climate change can affect the global distribution of *Culicoides* biting midges and aid their introduction into new geographic locations. Therefore, further epidemiological studies are needed to confirm the findings of this study.

ACKNOWLEDGMENT

The author wants to thank the Molecular Microbiology Laboratory, Veterinary Control Institute (Konya, Türkiye) for their support.

Financial support: The author did not receive any financial support for the research.

Conflict of interest: The author declares that there is no conflict of interest.

Ethical statement or informed consent: This study was authorized by the General Directorate of Food and Control (number E.333546, issued on December 27, 2017).

Author contributions: The study design, analysis, evaluation of the results, writing of the original draft, review and editing were carried out by MŞ.

Availability of data and materials: The author confirms that the data supporting the findings of this study are available within the article.

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