



## Investigation of Seroprevalence of Bluetongue Diseases in Sheep in the Province of Siirt

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### Abstract

Bluetongue is a viral infection that is commonly encountered in domestic and wild ruminants and is transmitted by biting midges. The cause of the disease is within Orbivirus genus of Reoviridae family and has 24 known serotypes. The infection is spread by the flies of Culicoides species. The aim of this study was to determine the serological presence of the bluetongue infection in sheep raised in various locations of Siirt province and to obtain information about the spread of the infection in the region as a whole. The study was conducted between August 2017 and February 2018, and the study consisted of 465 sheep (1-3 years old). 5 mL blood samples were taken from the jugular veins of the sheep to non-anticoagulant tubes for laboratory analyses. The blood samples were centrifuged at 3000 rpm/10 minutes and the serum were transferred to Eppendorf tubes, which were stored at -20 °C until analysis was carried out. Determination of the Bluetongue-specific antibodies in the serum samples was performed using a commercial test kit by ELISA device. The results of the specific-antibody analysis revealed that 340 (73.12%) of 465 sheep were seropositive, while 125 (26.88%) were seronegative. As a result, the seroprevalence of Bluetongue disease was found to be considerably high in various locations of Siirt province. Considering the findings of the study, in addition to the measures like the establishment of quarantine protocols and vector controls, utilization of different diagnostic methods and use of vaccines specific to the virus serotypes are suggested to fight the infection.

**Key Words:** Bluetongue, ELISA, seroprevalence, sheep, Siirt

### Siirt İli Koyunlarında Mavidil Hastalığı Seroprevalansının Araştırılması

#### Öz

Mavidil, evcil ve yabani ruminantlarda görülen ve sokucu sineklerle bulaştırılan viral bir hastalıktır. Hastalığın etkeni, Reoviridae ailesinin Orbivirus cinsi içinde yer alır ve bilinen 24 serotipi bulunmaktadır. Hastalık Culicoides türü sinekler ile yayılmaktadır. Bu araştırma, Siirt ilinin farklı lokalitelerinde yetiştirilen koyunlarda mavidil enfeksiyonunun varlığının serolojik olarak belirlenmesi ve yöredeki yaygınlığı hakkında bilgi edinilmesi amacıyla yapılmıştır. Bu çalışma Ağustos 2017- Şubat 2018 tarihleri arasında Siirt ilinin farklı lokalitelerinde yetiştirilen 1-3 yaş aralığında toplam 465 koyun üzerinde gerçekleştirildi. Laboratuvar analizleri için koyunların vena jugularisinden antikoagülan tüplere 5 mL kan örneği alındı. Alınan örnekler 3000 devirde 10 dakika santrifüje edilip serumları ependorf tüplerine aktarıldı ve analiz yapılıncaya kadar -20 °C de saklandı. Serum örneklerinde mavidil virüsü spesifik antikor tespiti, ticari test kiti kullanılarak ELISA reader ile gerçekleştirildi. Mavidil virüs antikor tespiti için yapılan analiz sonucunda çalışmanın materyalini oluşturan 465 koyunun 340 (%73.12)'i seropozitif bulunurken, 125 (% 26.88)'i seronegatif tespit edildi. Sonuç olarak; Siirt ilinin farklı lokalitelerinde mavidil hastalığının seroprevalansı oldukça yüksek tespit edilmiştir. Çalışma verileri dikkate alındığında teşhiste farklı tanı yöntemlerinin kullanılması, hastalıkla mücadelede karantina ve vektör kontrolüne yönelik önlemler yanında, virüs serotiplerine spesifik aşılardan yararlanılması gerektiği düşünülmektedir.

**Anahtar Kelimeler:** Koyun, ELISA, Mavidil, Seroprevalans, Siirt

### INTRODUCTION

Bluetongue (BT) is a viral infection encountered in domestic (cattle, sheep, goats, camels) and wild (antelope, gazelle, deer) ruminants and transmitted by biting midges (Culicoides spp) (1, 2). BT disease was first detected in sheep in South Africa in 1800 (3). At the end of the twentieth century, the disease spread to a very wide area and its presence was reported in many parts of the world (4). The cause of the disease is within Orbivirus genus of Reoviridae family and has 24 known serotypes (1, 5, 6). The infection is spread by the flies of Culicoides species (7). Of the hund

reds of species of Culicoides flies, 17 are believed to take part as vectors for the BT disease, and climatic changes are reported to play a significant role in the spreading of the infection (1). The geographical conditions these biting midges are most active in are within the tropical and subtropical regions between 40 degrees north and 35 degrees south latitudes (8). As a result of the increased survival rate of vectors in winter conditions due to global warming, the disease has spread from the European continent towards the north, and since 2007 it has emerged as a major problem in susceptible animal populations in Switzerland and Scandinavia (9, 10). As of the end of the twentieth century,

the disease is quite widespread and is reported in numerous regions of the world (4).

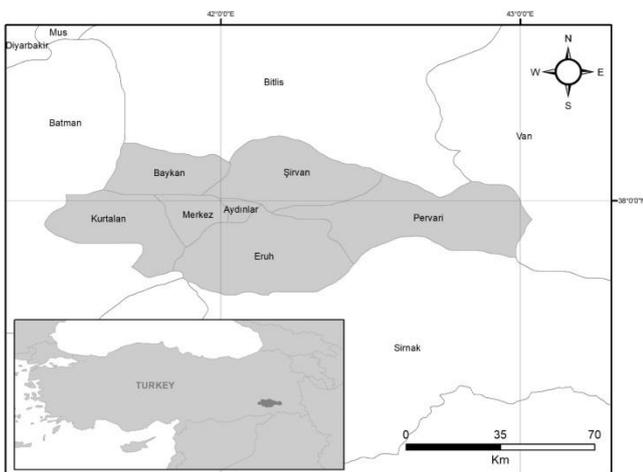
It is reported that sheep are the most susceptible species to the infection (11). The disease might have an acute, chronic, or sub-clinical course in sheep (12). Infected sheep may display symptoms like fever, depression, excessive salivation, dyspnea, mucopurulent nasal discharge, edema in face, hyperemia and ulcer in the mouth mucosa, coronitis, and muscle weakness (5, 7, 13), or may not display any symptoms at all (14, 15).

The aim of this study was to determine the serological presence of the BT infection in sheep raised in various locations of Siirt province and to obtain information about the spread of the infection in whole region.

## MATERIAL AND METHOD

### Study area

The Siirt province (Figure 1) is located in a sub-humid climate region according to the Thornthwaite Climate Classification (C2, B'3, S2, B'2). The annual precipitation in the province of Siirt in Turkey is 715.4 mm. The average highest and lowest temperatures are between 36.9 °C and 18.9 °C in summer, and 8.7 °C and -0.5 °C in winter (16).



**Figure 1.** Geographical positioning of the Siirt province in which the study was performed

### Ethical approval:

Ethical approval for this study was obtained from Siirt University Local Ethics Committee for Animal Experiments (Approval Numer: 2018/13).

### Animal Material

The study was conducted between August 2017 and February 2018, and the live material of the study consisted of 465 sheep of ages 1 to 3 raised in Siirt province. 5 mL blood samples were taken from the jugular veins of the sheep to non-anticoagulant tubes for laboratory analyses. The blood samples were centrifuged for 10 minutes at 3000 rpm and the serum were transferred to eppendorf tubes, which were stored at -20 °C until analysis was carried out. It was also determined that the herds of the sheep from which the

samples were collected were not vaccinated against the BT disease and they did not display any of the clinical findings specific to the infection.

### ELISA Test Procedure:

Determination of the BT -specific antibodies in the serum samples was performed using a commercial test kit (Bluetongue Virus VP7 Ab Test Kit, IDEXX, USA) by ELISA device and ELISA reader (Multiskan GO, Thermo Scientific, Finland). It is based on competition between the serum to be tested and a monoclonal antibody, which is coupled to the peroxidase and directed to the N-terminal part of the VP7 protein, a major core protein of the Bluetongue Virus (BTV).

## RESULTS

In this study, blood samples of 465 sheep were examined for bluetongue disease. The results of the specific-antibody analysis revealed that 340 (73.12%) of 465 sheep were seropositive, while 125 (26.88%) were seronegative.

## DISCUSSION AND CONCLUSION

In various studies around the world, the prevalence of BT disease in sheep was determined as follows: Kyrgyzstan 36.94% (17), Georgia 34% (18), Kosovo 9.01% (15), India 28.6% (19), Albania 4.4% (20), Egypt 14.7% (21), Iran 74.4% (22), Eastern Azerbaijan in Iran 76.44% (23), Ethiopia 69.01% (24), Saudi Arabia 54.1% (25), and China 20.3% (26).

In Turkey, BT was first reported in 1944 in Hatay province, and later in 1977 in the Aegean region, and in later years, it was also reported in the Mediterranean and Marmara regions. When the disease first appeared in Turkey, it was reported to have high mortality rates (27, 28). Savini et al. (29) reported that losses due to epidemics of BT infections decreased in previous years.

According to a study performed by Bulut et al. (30) in Konya region in order to determine BTV antibodies in sheep and goats the seropositivity was determined as 17% and 60% respectively. In another study conducted by the same researcher in the province of Burdur, a 1.5% seropositivity for sheep and a 60% seropositivity for goats were reported. In a study conducted by Celik and Sahin (31) on goats raised in the province of Siirt where the presence of BTV antibodies was inspected using the ELISA method, the seroprevalence of the disease was determined as 67.74%.

Öztürk et al. (32) inspected the blood serums of 86 sheep located in the Konya Livestock Central Research Institute with micro-neutralization method to determine the neutralizing antibody presence against the SA4 strain of the BTV. A positivity rate of 36.04% was detected as a result of this study. In a study carried out by Gür (27) on the sheep in the province of Afyonkarahisar with the competitive ELISA method to determine the BTV antibodies, the seroprevalence of the disease was found to be 24.8%.

In another study carried out by Yiğit (33) using the competitive ELISA method to determine the BTV antibodies in Konya, the seroprevalence of the disease in sheep was reported as 10%. Albayrak and Özan (5) conducted a study in 5 cities (Samsun, Sinop, Ordu, Amasya, and Tokat) loca-

ted in the Black Sea region using the competitive ELISA method and reported a seroprevalence of 5% for Sinop and 10% for Amasya, while no seropositivity was encountered in the other cities. In a study conducted by Yılmaz et al. (34) on the sheep of Kars province to determine the BTV antibodies, the seroprevalence of the disease was reported as 10.65%. Girgin and Yonguç (35) reported the average seropositivity for BT infection in 14 provinces of Turkey as 46%, a stated that the rate varied between 0 and 93% according to provinces. The findings of our study in this regard are in line with the findings of these researchers (35).

The serum samples collected from sheep and goat herds of the Şanlıurfa region by (36) were inspected for BTV antibodies with agar gel immunodiffusion (AGID) method, and the average seropositivity for sheep herds was determined as 87.50%, and as 81.17% for the goatherds. The high seroprevalence of the infection was attributed to the geographical location of the region. The province of Siirt in which our study was conducted in is in the same region with Şanlıurfa, and in that regard, the relatively high seropositivity determined in our study might be related to the geographical location of the province as well.

Culicoides spp. are reported to be active between April-October, and their activity peaks during July-September. The optimum temperature for Culicoides activity was reported as 13 - 24 °C, and reportedly could be as high as 35 °C for the members of *C. nubeculosus* complex, while it was reported that Culicoides did not take flight under temperatures 9-10 °C (37). The province of Siirt provide a suitable environment for the thriving of Culicoides species, and the animal populations in the region move a lot during the spring and fall months.

BTV seroprevalence in sheep was determined as 74.4% in Iran (22), and as 76.44% in Eastern Azerbaijan in Iran (23). The same researchers have indicated factors like the density of cattle in the animal population, lack of limitation on the animal movement, lack of vaccination and eradication programs, and presence of a climate suitable for the thriving of Culicoides species as the reasons for the high seroprevalence of the infection. It is possible that the reasons reported by these researchers (22, 23) are mirrored in our own study regarding the relatively high seropositivity determined for the infection.

Researchers (14, 38) have also reported that it's possible that the disease would not display any clinical findings, and most epidemics in the world have a subclinical course. Similarly, the absence of disease-specific clinical symptoms in the herds from which the samples were taken is consistent with the findings of the researchers (14,38).

As a result, the seroprevalence of BTV was found to be high in various locations of Siirt province. Considering the findings of the study, in addition to the measures like the establishment of quarantine protocols and vector controls, utilization of different diagnostic methods and use of vaccines specific to the virus serotypes are suggested to better fight the infection. Researching the genetic and phenotypic diversity of BTV is also recommended for future research.

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