



## Serological Investigation of Bluetongue Virus Infection in Sheep in Elazığ and Malatya Provinces of Türkiye

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Bluetongue (BT) is a non-contagious animal disease spread by blood-feeding midges (*Culicoides* spp.) reported in domestic and wild ruminants in many parts of the world, including Türkiye. Bluetongue virus (BTV), the causative agent, belongs to the genus *Orbivirus* in the family *Reoviridae* in virus taxonomy. In this study, we aimed to investigate the seroprevalence of BT in healthy sheep in the provinces of Elazığ and Malatya in the Eastern Anatolian region of Türkiye, using a competitive enzyme-linked immunosorbent assay (cELISA; IDEXX, USA) designed for detection antibody against the virion protein 7 (VP7) of BTV. For this purpose, a total of 186 healthy sheep (Elazığ, n:100; Malatya, n:86) from five different locations in each province were included in the study, and serum samples were separated from their blood samples collected from their jugular vein were used in cELISA. According to the cELISA results in this study, the BT seropositivity of sheep in Elazığ and Malatya was 10% (10/100) and 0% (0/86), respectively, while the overall seroprevalence was 5.38% for both provinces. Information on the epidemiology of BTV infections in Elazığ and Malatya provinces is limited. Although this is a small-scale study, the current data obtained will contribute to the epidemiology of BTV in Türkiye, but more detailed studies are needed in this region.

**Key Words:** Bluetongue, ELISA, Elazığ, Malatya, seroprevalence, Türkiye

### Türkiye'nin Elazığ ve Malatya İllerinde Yetiştirilen Koyunlarda Mavidil Virüs Enfeksiyonunun Serolojik Araştırılması

Mavidil, evcil ve yabani ruminantların bulaşıcı olmayan viral bir hastalığıdır, *Culicoides* türündeki kan emici tatarcıklar ile nakledilmektedir. Aralarında Türkiye'nin de yer aldığı Dünya'nın birçok ülkesinde varlığı bildirilmiştir. Etiyolojik ajan, virus sınıflandırmasında *Reoviridae* ailesi, *Orbivirus* genusunda yer alan Mavidil virüsüdür. Bu çalışmada Türkiye'nin Doğu Anadolu bölgesinde yer alan Elazığ ve Malatya illerindeki sağlıklı koyunlarda Mavidil enfeksiyonunun seroprevalansını araştırdık ve bunu BTV'nin virion protein 7 (VP7)'e karşı antikorların tespit edilmesine yönelik tasarlanmış ticari cELISA (IDEXX, USA) kullanarak gerçekleştirdik. Bu amaçla, her bir ildeki beş farklı lokasyondan toplamda 186 sağlıklı koyun (Elazığ, n:100; Malatya, n:86) çalışmaya dahil edildi. Çalışmaya dahil edilen koyunların vena jugularisinden toplanan kanlardan ayrıştırılan serumlar cELISA'da kullanıldı. Bu testin sonucunda Elazığ ve Malatya illerindeki koyunlarda BTV seropozitifliği sırasıyla %10 (10/100) ve %0 (0/86) olarak hesaplandı. Her iki il için genel seroprevalans %5,38'di. Elazığ ve Malatya illerinde BTV enfeksiyonunun epidemiyolojisine yönelik sınırlı bilgiye sahibiz. Mevcut çalışma küçük ölçekli olsa da Türkiye'de BTV'nin epidemiyolojisine güncel verilerle katkıda bulunacaktır, ancak bu bölgede daha detaylı çalışmalara ihtiyaç vardır.

**Anahtar Kelimeler:** Mavidil, ELISA, Elazığ, Malatya, seroprevalans, Türkiye

### Introduction

The causative agent of Bluetongue (BT) disease, a non-contagious disease spread by blood-feeding midges of the genus *Culicoides* spp. (Diptera: Ceratopogonidae), is Bluetongue virus (BTV; family *Reoviridae*, genus *Orbivirus*) (1, 2). The virus has a wide host range, infects most domestic (sheep, cattle, and goat) and wild ruminants (antelope, deer, elk, elephant, llama, mithun), and causes variable clinical manifestations depending on the species and breed (3). Sheep are more sensitive to disease than others and natural infection of native sheep can lead to severe clinical manifestations including fever, vascular permeability, facial oedema, cyanosis of the tongue, bloody nasal/oral secretions, oral lesions, and ulcers, hyperaemia of the coronary band and limping (4). In an outbreak of BTV caused by highly virulent BTV strains, the mortality rate in some breeds of sheep (especially European) may be as high as 70% (3). Apart from deaths, direct economic losses caused by BTV are a decrease in milk production, early culling, weight loss, reproductive problems, abortion, stillbirths, a decrease in male fertility, and a decrease in birth weights (5). Diagnosis, treatment, control measures, and trade restrictions in outbreak areas are indirect economic losses. Bluetongue is a notifiable disease listed by the World Organisation for Animal Health (OIE) due to its important economic effects (6). The virion particle of BTV consisting of the inner core and outer capsid is icosahedral and non-enveloped. The BTV genome (19 kbp) has ten segments of linear dsRNA, encoding seven structural proteins (VP1, VP2, VP3, VP4, VP5, VP6, and VP7) for six non-structural proteins (NS1, NS2, NS3, NS3A, NS4, and NS5) (1, 7). The outer capsid layer consists of VP2, the main defining protein that stimulates serotype-specific immunity, and VP5, which helps it. The inner core consists of VP3 and VP7. VP7 is an immunodominant protein and

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contains serogroup-specific epitopes, making it often preferred in ELISA for the detection of antibodies to BTV. VP1 polymerase, VP4 capping enzyme, and VP6 helicase form an enzymatic complex (1, 7, 8, 9). Based on neutralization tests and Seg-2/VP2 sequence analysis, while 24 classical BTV serotypes were identified until 2008, this number has increased to 35 with the inclusion of atypical serotypes over the last decade (10). Bluetongue is endemic in tropical, subtropical, and temperate regions of the world including Africa and some regions of Asia, Australia, and America, between latitudes 40°S and 53°N, during times of the year that are optimal for vector activity (1, 2). In Europe, outbreaks of BTV were rarely observed in the Mediterranean Basin (40°N latitude). Since the 2000s, this global distribution of BTV has changed, with its continued emergence especially in Europe and in the Mediterranean Basin (11). So far, many BTV serotypes have been reported from America (BTV-1,-2,-3,-4,-6,-8,-10,-11,-12,-13,-14,-17,-18, and-24) (12) Europe (BTV-1,-2,-3,-4,-6,-8,-9,-11, and -16), China (BTV-1,-2,-3,-4,-12,-15, and-16) and Australia (BTV-1, -2, -3, -5, -7, -9, -12, -15, -16, -20, -21, and -23) (13, 14, 15).

In this study, we aimed to investigate the seroprevalence of BTV infection in sheep in private production units in the provinces of Elazığ and Malatya in the Eastern Anatolia Region of Türkiye by competitive ELISA.

**Material and Methods**

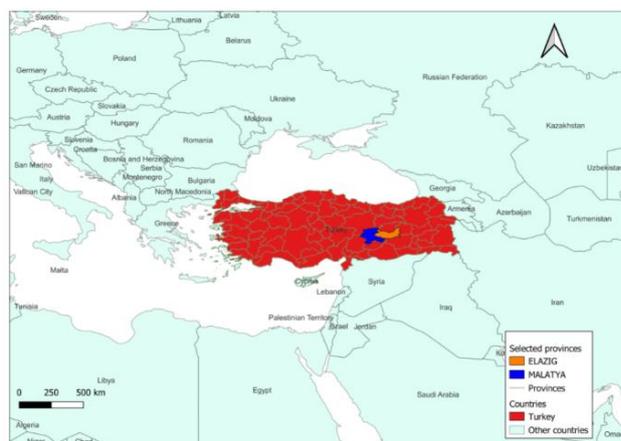
**Ethical statement:** Ethical approval for this study was obtained from Fırat University Local Ethics Committee for Animal Experiments (131929) and from Elazığ Provincial Directorate of Agriculture and Forestry (3842468).

**Climate conditions, study area, sampling:** Malatya and Elazığ have a continental climate and a Mediterranean climate in some regions, the temperature is between -4°C and +34°C depending on the season. Although Malatya and Elazığ are known to host many *Culicoides* spp., this number was found to be 42 in a study in Elazığ (16). Here, a total of 13 sheep herds from 5 different locations in the provinces of Elazığ and Malatya in the Eastern Anatolian region of Türkiye were included in the study, and the blood of 10-16 sheep (186

sheep in total) randomly selected from them were collected (Figure 1).

While the study material consisted of blood collected from healthy sheep of different ages (2-4 years) and breeds in January 2022, the study population consisted of unvaccinated and problematic sheep herds (herds with a history of abortion or abnormal offspring during the previous lambing periods). Detailed information about the study population is given in Table 1. Blood samples were taken into clot activator vacuum tubes from the jugular veins of the animals. Blood collection tubes were centrifuged at 2000 × g for 15 min at room temperature (21°C), and blood serum was transferred to new tubes and stored at -20°C until used.

**Competitive ELISA:** Detection of BTV group-specific antibodies (IgG against VP7-protein) in c-ELISA was performed using the Bluetongue Competition Ab Test Kit (IDEXX, USA) and following the manufacturer's instructions for use. The optical density (OD) values were measured at 450 nm by ELx800 (BioTek, USA) microplate absorbance reader. The tested sera were accepted positive when they produced an optical density less than or equal to 70% of the mean of the negative controls (S/N).



**Figure 1.** Locations on the physical map of Elazığ and Malatya provinces selected in this study

**Table 1.** Sampling places and seropositivity in this investigation

Elazığ			Malatya		
Sampling place	Sampling number	Seropositive case	Sampling place	Sampling Number	Seropositive case
Akcakiraz	15	0	Karagoz	15	0
Asagi demirtas	15	3	Orduzu	15	0
Orencay	25	0	Sahnahan	10	0
Yazikonak	30	0	Topsogut	15	0
Yukari demirtas	15	7	Yesilyurt	31	0
Total	100	10		86	0

## Results

A total of 186 samples were processed for detection of group-specific (VP7 protein) antibodies against BTV from 13 herds of sheep in Elazığ and Malatya provinces. According to cELISA results, two out of a total of 13 sheep herds (2/13, 15.38%) and 10 out of a total of 186 (5.38%) samples processed were seropositive for BTV. All of the sheep seropositive for BTV belonged to the province of Elazığ (10/100, 10%).

## Discussion

The Bluetongue disease was first described in South Africa in the early 19th century. The first well-documented epizootic of BT outside Africa appeared amongst sheep in Cyprus in 1943 (17). This disease was first clinically described in Hatay province in Türkiye's border region with Syria in 1944 (18, 19). The first officially defined outbreak of BT in Türkiye emerged in the province of Aydın in 1977 and spread to the neighboring provinces of the Mediterranean and Aegean between 1977-1979, creating an endemic. BTV-4 was the first serotype isolated from infected sheep in Türkiye and whose circulation was proven by serological tests (20). Then, this endemic was controlled by applying strict measures such as quarantine, animal movement control, disinfection, insecticide treatment, and vaccination (attenuated live vaccine, against BT-4) (21). Taylor and Melor (20) reported the presence of neutralizing antibodies against BTV-2, 4, 9, 13, and 15 in different animal species (ox, goat, sheep) from the Central Anatolian, Aegean, and Mediterranean regions of Türkiye from 1980 to 1981. In 1998, after 20 years of absence in Europe, BTV outbreaks occurred simultaneously in southern and western Anatolia. While BTV-9 was isolated from infected animals in Thrace during the epidemic that lasted for several years, BTV-16 was also isolated from infected animals in western Türkiye in 2000 (21). In Türkiye, the herd seroprevalence varies among geographic regions, but the percentage ranged from 1 to 91 (19; 22; 23). Ozgunluk (24) found 52.58% seropositivity against BTV-4 in cattle in the southeast Anatolia region. Yildirim and Burgu (25) reported that the rate of neutralizing antibodies to BTV-4 in cattle in 8 provinces (Iğdır, Agri, Kars, Erzurum, Bayburt, Gumushane, Artvin, Ardahan) in northeastern Türkiye was 48.02%. According to the results of the study conducted by Karaoglu et al., (26) in cattle in Thrace, the seropositivity rate for BTV-4, BTV-9, and BTV-16 was over 69%. Yildirim and Yılmaz (23) detected neutralizing antibodies against BTV-4, -9, and -16 serotypes in cattle in three provinces (Ardahan, Iğdır, and Kars) in northeastern Türkiye, and overall seroprevalence was quite high (91.7%) in the same study. Karaoglu et al., (27) determined the presence of BTV-9 circulation in the farm at the sampling time in the Southeast Anatolia region, according to the results of the SN50 test for BT-4, BT-9, and BT-16 in herds in the northeast and southeast Anatolia. Ozgunluk (28) detected 64.24% seropositivity with the serum neutralization test in cattle in Aydın province in the

Aegean region of Türkiye and found neutralizing antibodies against BTV-4, -9, and -16 serotypes. Erturk (19) detected 34.4%, 15%, and 1% seropositivity in sheep, goats, and cattle in 21 provinces of Türkiye by agar-gel precipitation test, respectively. Ozturk et al. (29) found 36.04% positivity with the microneutralization test in sheep in Konya province in the Central Anatolian region. According to ELISA results, which Bulut et al. (22) found more sensitive than SNT, the seroprevalence of BTV in sheep and goats in Konya province was 17% and 60%, respectively. In the same study, this rate was 1.5% and 60% for sheep and goats in Burdur province. Yigit (30) found 10% seropositivity against BTV by c-ELISA in sheep in Konya province. BTV seropositivity in Kars province in eastern Türkiye was 10.65% and 15.5% in sheep and cattle, respectively, according to the results of two studies conducted at different times (31, 32). Celik and Sahin (33) detected 73.12% BTV seropositivity in sheep in Siirt province in eastern Türkiye. Gur (34) found BTV seropositivity of 24.8% in sheep in Afyonkarahisar province. Azkur et al. (35) reported that BTV seroprevalence was 49.8% in sheep in Kirikkale province in the Central Anatolian region. Albayrak and Ozan (36) reported that BTV seropositivity of cattle and sheep in five provinces (Samsun, Sinop, Ordu, Amasya, and Tokat) in the Black Sea region was 11% and 3%, respectively. Kulac et al. (37) detected 25% seropositivity in cattle in Rize province in northeastern Türkiye. Ozgunluk and Cabalar (38) reported that BTV seroprevalence in sheep and goat herds in Sanliurfa province in southeastern Türkiye was 87.50% and 81.17%, respectively, using the agar-gel immunodiffusion method (AGID). Simsek et al., (39) reported that the seroprevalence of Bluetongue disease was 17.43% in animals with digestive and respiratory system disorders in Diyarbakir province. Pestil (40) determined 38.7% BTV seropositivity in abortion and postnatal cases in sheep in the Marmara region. Pestil et al., (41) determined 53.5% BTV seropositivity in camels in 7 provinces of Türkiye (Antalya, Aydın, Canakkale, Denizli, Izmir, Muğla and Sanliurfa). Since Türkiye is an important gateway between the continents of Europe and Asia, epidemiological studies are of great importance and outbreak reports are followed closely by Europe and the OIE. Studies on the prevalence of BTV in Türkiye's Elazığ and Malatya provinces are limited. Bolat (42) reported with the AGID test that the seropositivity of sheep in Elazığ province was 33.25% and the overall seroprevalence was 21.32% in 8 provinces, including Elazığ in central, eastern, and southern Anatolia. In this study, we found the BTV seropositivity to be 10% and 0% for Elazığ and Malatya, respectively, while the overall seropositivity was 5.38%. Serotype determination in seropositive animals may reveal that the current BTV strain is typical or atypical form, but this was not performed in this study. The most likely vectors of BTV in western Türkiye have been identified as *Culicoides imicola*, *C. obsoletus*, and *C. schultzei* (21). It is known that there is a culicoides population in the region where the study was conducted (16), but there is no study that has been identified.

Although this is a small-scale study, the current data obtained will contribute to the epidemiology of BTV in

Türkiye, but more detailed studies are needed in this region.

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