

# Seroprevalence of culicoides-borne disease in cattle in European Turkey\*

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**Summary:** In this research, seroprevalence of BTV serotypes (BTV-4, BTV-9, BTV-16), and occurrence of Akabane and Ephemeral fever viruses were investigated in 557 cattle sera collected from 21 locations in European Turkey (Thrace). Virus neutralization test was used to discriminate antibody response against BTV serotypes while commercial blocking-ELISA systems were used to detect antibodies for Akabane and ephemeral fever viruses. The overall seroprevalence of BTV was recorded as 73.54%, comprising individual percentages for BTV-4, BTV-9 and BTV-16 were 69.04%, 71.38% and 80.2%, respectively. The seroprevalence of Ephemeral fever virus infection in Thrace varied between 2.5 and 15.3% on the province basis, whilst antibody percentage for Akabane virus was found as low as 0.14% in the study.

Key words: Akabane, bluetongue, ephemeral fever, seroprevalence.

## Trakya bölgesinde sığırlarda sivrisineklerle nakledilen enfeksiyonların seroprevalansı

**Özet:** Trakya bölgesinde, 2003 yılında 5 ile bağlı 21 ilçe/köyden toplam 557 sığır, Mavidil, Akabane ve Ephemeral Fever enfeksiyonlarının seroprevalanslarının tespiti amacıyla random sampling metoduyla örneklendi. Serum örnekleri Mavidil virusunun 3 farklı serotipine (BTV-serotip 4, BTV-serotip 9 ve BTV-serotip 16) karşı virus nötralizasyon testine tabi tutuldu. Aynı serum örneklerine Akabane ve Ephemeral Fever virus antikorlarının tespiti için blocking ELISA testi uygulandı. Trakya bölgesinde mavi dil enfeksiyonunun seroprevalansı %73.54 olarak tespit edilirken, BTV serotip-4 %69.04, BTV serotip-9 %71.38, BTV serotip-16 ise %80.2 olarak saptandı. Akabane enfeksiyonunun seroprevalansı Trakya bölgesinde %0.14 olarak belirlenirken, Ephemeral fever enfeksiyonunun seroprevalansı örneklenen iller bazında %2.5-15.3 olarak tespit edildi.

Anahtar sözcükler: Akabane, ephemeral fever, mavidil, seroprevalans.

## Introduction

Bluetongue is an economically devastating and Culicoides-born disease, which is characterized by congestion, oedema and haemorrhages in wild and domestic ruminants. In addition, this virus is responsible for fetal death, congenital defects, and reproductive failure in cattle (26).

The causative agent is classified in *Reoviridae* family genus *Orbivirus* as Bluetongue virus (BTV) has 24 serotypes (10,12,29). There are three major components recognised in the epidemiology of the infection such as reservoir animal, vector and climatic conditions. The most important reservoir is cattle and they can be infected rarely (10,12). The viraemia lasts about 100 days in cattle (22) while only 30 days in sheep (11). Thus it is reported that cattle play an active role either in transmitting the virus or in passaging the virus to appropriate climatic condition over winter in which the

vector midges are biologically in-effective (11,22). It is known that the vector midges (*Culicoides spp.*) has approximately 1000 subspecies in tropical and subtropical regions in which proper ecologic situations have been provided for their life cycle. However, only 17 of them have directly been related to transmission of the BTV, most probably, because of their genetic predisposition (25). BTV infection of ruminants are commonly seen in most of the tropical and subtropical regions between 35° south and 40° north latitudes, in which Culicoides midges are extremely effective (10,13). These areas are called as incursional zones and infection of ruminants usually occurs during midday of late summer or early autumn (10,27).

Akabane virus (AKAV) is a Simbuvirus classified in *Bunyaviridae* family, genus *Bunyavirus* (17,19). The virus is transmitted by blood sucking midges and accepted as an important cause of abortions, fetal

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mummification, still births, and various congenital malformations in ruminant species (17,19). Viraemia occurs in every case and the virus can reach to fetus by transplacental spread. The most important fetal pathology is arthrogryposis and hydranencephalie complex in affected animals (20,21,32). Akabane disease is subclinical in adult cattle although causes economical losses resulted from severe decrease in reproductive performance (32).

Bovine ephemeral fever (BEF) also causes a subclinical infection in ruminant species and is characterized by fever, acute febrile reaction, stiffness, lameness and total recovery within three days. The virus that classified in rhabdoviridae family may cause abortions (6,30) in female and transient sterility in males (28). The infection is mostly seen in tropical regions of Africa, subtropical and warm territories of Australia and Asia. All three infections are called as non-infectious arthropod-borne disease and blood sucking midges play role in transmission between infected and susceptible animals. Main objective of this study is to detect the seroprevalance the local serotypes (serotype 4, 9 and 16) of Bluetongue virus, and the occurrence of Akabane and Ephemeral Fever viruses in European Turkey.

## Materials and Methods

### Serum samples

Blood serum samples were collected randomly from 557 cattle of 21 counties and/or villages between September-October 2003. Geographical localization and number of sampled animals are given in Figure and Table 1, respectively. The serum samples were stored at  $-20^{\circ}\text{C}$  freezers following inactivation at  $56^{\circ}\text{C}$  for 30 minutes.



Figure 1. Geographical position of villages that have been sampled in the research. The points indicate exact location of villages.

Şekil 1. Örnekleme yapılan merkezler ve örneklenen hayvan sayıları.

Table 1. The numerical distribution of samples based on province and town.

Tablo 1. Örnekleme yapılan merkezler ve örneklenen hayvan sayıları.

Code	Province	Town/Village	Animal number
01	Canakkale	Gelibolu, Eceabat, Gokceada	164
02	Edirne	Lalapasa, Kesan, Uzunkopru, Ipsala, Enez, Meric	163
03	Istanbul	Gaziosmanpasa, Silivri, Catalca, B.Cekmece	70
04	Kirklareli	Merkez, Demirkoy, Kofcaz, Luleburgaz, Vize, Babaeski	130
05	Tekirdag	Malkara, Saray	30
Total			557

### Antibody detection

*Virus Neutralization Test (VNT)*: Presence of antibodies against Bluetongue virus serotypes (BTV-4, BTV-9, BTV-16) were screened using virus neutralization assay (9). Two subsets of sera were tested for their neutralizing activity against BTV serotypes. The fifty  $\mu\text{l}$  serum samples in duplicate were mixed with equal volume of 100 TCID<sub>50</sub> diluted BTV suspensions in 96-well plates. After neutralization at  $37^{\circ}\text{C}$  for 1 h, 50  $\mu\text{l}$  of Vero cell suspension was added all wells used of the 96-well plate. The plates were then incubated under 5 % CO<sub>2</sub> atmosphere at  $37^{\circ}\text{C}$  for 4 days. Then the results were assessed by microscopically monitoring of cytopathic effect.

*Blocking ELISA System*: For detection of AKA and BEF virus antibodies a commercial blocking ELISA systems (Elisabeth Mc Artur Agriculture Institute, Australia) were used. Tests were performed according to the manufacturer's directions. Briefly, serum samples were used in 1:10 and 1:8 dilutions in order to detect antibodies against AKA and BEF viruses, respectively. Test and control serums were placed into antigen-coated wells in duplicate and incubated for 1 h. Additional 1 h. incubation of monoclonal antibodies mixed with serum dilutions were followed by conjugate and chromogenic substrate incubations, sequentially. The results were evaluated by reading of plates in 450 nm spectrophotometer at the final step.

## Results

Prevalence of BTV antibodies in European Turkey varied between 69.9-77.9% in province basis. The highest BTV prevalence detected was 77.9% in Edirne province, which is followed by Kırklareli (76.9%), Canakkale (71.5%), İstanbul (71.4%) and Tekirdag

(69.9%) provinces. The seroprevalence of the BTV serotypes in Thrace were 80.2%, 71.38%, and 69.04% for BTV-16, for BTV-9 and for BTV-4, respectively (Table 2).

Table 2. Seroprevalence of Bluetongue, Akabane and Ephemeral fever infections in Thrace.

Tablo 2. Trakya bölgesinde Mavidil, Akabane ve Ephemeral fever enfeksiyonlarının seroprevalansı.

Code	Province	Bluetongue			Akabane	Ephemeral Fever
		BTV-4	BTV-9	BTV-16		
01	Canakkale	71,5	63,4	79,6	-	2,5
02	Edirne	65,9	84,3	83,5	-	15,3
03	Istanbul	64,4	73,4	76,5	-	2,8
04	Kirklareli	73,4	72,5	84,8	0,7	13
05	Tekirdag	70,0	63,3	76,6	-	6,6
Total		69,04	71,38	80,20	0,14	8,04

The blocking ELISA revealed single antibody positive serum specimen for AKAV from Kirklareli, indicating low prevalences based on province (0.7%) and Thrace (0.14%) region.

The seroprevalence of Ephemeral fever virus was found between 2.5-15.3% in the study. The highest seroprevalence of the infection was in Edirne (15.3%), which was followed by Kirklareli, Tekirdag, Istanbul and Canakkale, with 13%, 6.6%, 2.8%, and 2.5%, respectively.

### Discussion and Conclusion

In this research serologic survey of some *Culicoides*-borne virus diseases was carried out in randomly selected cattle of European Turkey. Results revealed that prevalence of infections caused by BTV serotypes somewhat higher (73.54%) than that of Akabane (0.14%) and Ephemeral fever (8.04%) virus infections in the region.

Bluetongue virus infection predominantly detected in Edirne (77.9%), while seroprevalence of BTV in remaining provinces was between 69.9-76.9%. This variation between provinces was accepted as traceable. On the other hand, serotypic occurrence of the BT viruses showed the superiority of BTV-16 (80.2%) and BTV-9 (71.38%) over BTV-4 (69%).

Two provinces (i.e. Edirne and Kirklareli) were perceived (distinguished) because of their high seroprevalence status for BTV-9 and BTV-16. On the basis of geographic features or BT occurrence data from neighboring countries, one might be postulated that

BTV-9 is predominately spread in Bulgaria, Greece and Turkey while BTV-16 is the most threaten serotype for Bulgaria and Turkey (1,2,3,4,24). This speculation need to be further investigated by species distribution along the region.

Turkey has a very critical geographic location between Asia and Europe. Because of its bridge like position, European Turkey, particularly, is accepted as buffer zone in which transmission of some viral disease has been prevented by application of various central measurements in epidemiologic manner.

Common feature of the project diseases is to be transmissible under certain climate and vector conditions. Some regions of Turkey seem to have proper conditions for these infections. Wind streams coming from neighboring countries in Mediterranean basin and Middle East may cause passively transport of the vector *Culicoides spp.* so that new serotypes of the BTV may be acquired by the mentioned countries.

Bluetongue is an economically important infection of ruminants that causes serious decreases in reproductive ability, abortions and congenital malformations (26). *Culicoides spp.* responsible of transmitting project disease have been investigated in Turkey and 57 specieses of them have previously been identified by Dik (7) Mellor et al. (23) have identified 13 *Culicoides spp.* in Southern, Southeastern and Western Turkey in which congenitally deformed calf births have been reported during the same period. They also reported seroprevalence of bluetongue, AKA and BEF viruses in cattle of the region as 2.3%, 12.3% and 9.2%, respectively, using the competitive ELISA.

After the first declaration in 1944 in Turkey, Bluetongue infection incursions have been reported by numerous investigators (5,8,14,23,31,33). Seroprevalence of BTV infection in cattle of South, southeast and Aegean regions of Turkey was detected as between 0-52.1 % in convalescence serum samples by Burgu et al (5).

The most recent isolation studies revealed presence of the different serotypes in Turkey. The virus isolated in 1999 in European Turkey has been identified as BTV-9. Mellor and Witthmann (24) reported that BT infection has been circulating within Southern and Western Anatolia and BTV-16 has been isolated in Aegean region during the epidemic of 2000. Recently, BTV outbreaks were officially declared in Bulgaria, Greece (mainland and Aegean islands), Italy, and Portugal (24). So far, presence of four BTV serotypes (BTV-2, BTV-4, BTV-9, BTV-16) has been reported in Turkey (8,24).

Akabane disease which causes abortion, fetal mummification, stillbirths and arthrogriposis/

hydranencephalie complex in ruminant species (17,19) was serologically detected in one (0.7 %) animal in Kirklareli.

The occurrence of ephemeral fever in basis European Turkey provinces (i.e. Edirne, Kirklareli, Tekirdag, Istanbul, Canakkale) were detected as 15.3 %, 13 %, 6.6 %, 2.8 % and 2.5 %, respectively. The overall seroprevalence of the disease was 8.04 % in the region. This results showed that BEF has higher occurrence than AKA in the region.

By means of this study, valuable data were obtained on some Culicoides-born virus disease of ruminants. These data led to figure out the occurrence of BT, AKA and BEF in province basis of Thrace. Results indicated that high prevalence of BTV infection may reflect the presence of novel serotypes of BTV in the region. The well-known features of the disease, such as plurality and persistency in cattle may complicate local attempts to eradicate and/or control the disease (18).

Preventive approaches based on the fight with vector midges are accepted as to be non-practical and unsuccessful (15,16). Because Turkey has an adequate geographic position and climatic feature, various vector-borne exotic diseases able to invade the country from its neighboring countries. Thus, animal health records showed that Culicoides-borne infections, (e.g. Bluetongue, Akabane, Ephemeral Fever) have often occurred due to satisfactory environmental conditions in West, South and Southeastern Turkey.

One of the important features of the Bluetongue infection is plurality of the virus and outbreaks caused by several serotypes of the virus could be detected in the same region. The most valuable protective and control measurement against BT is effective vaccination. However, presence of several serotypes in country should take into consider in both preparation and administration of the vaccine as there is no close antigenic relation between BTV serotypes. Therefore, successful protection against BT can be achieved by using polyvalent vaccines included of local BTV serotypes.

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