The Seroprevalance of Bluetongue Virus Infectıon in Cattle in the Kars Dıstrıct of Turkey

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ABSTRACT

In this study we surveyed the seroprevalence of bluetongue virus infection in cattle of six months of age to nine years of age from private small scale production units of less than twenty cattle per unit, in the Kars district of Turkey. For this purpose, blood was collected from 401 cattle from private small scale production units in Kars and environs and was tested for antibodies against bluetongue virus using a competative enzyme-linked immunosorbent assay (c-ELISA). The results showed that 74 cattle (18.5%) of the 401 were found antibody positive for bluetongue infection. On the basis of the results it is suggested that infection may be spreading in private small scale production units. Furthermore, recommendations for the control of bluetongue infection are presented.

Key Words: Competative enzyme-linked immunosorbent assay, Bluetongue virus, Cattle, Seroprevalence, Kars District, Turkey

INTRODUCTION

Bluetongue (BT) is an arthropod-transmitted disease of wild and domestic ruminants, which is characterized by fever, congestion, oedema, haemorrhages, hyperemia and ulceration of oral mucosa, coronitis and lameness (1). The disease is enzootic in many tropical and temperate regions, coincident with the distribution of competent *Culicoides* vector insects. There are three major components recognised in the epidemiology of the infection: reservoir animals, vector and climatic conditions. Cattle are most important reservoir. The viraemia lasts about 100 days in cattle while only 30 days in sheep. Thus it is reported that cattle play an active role either in transmitting the virus or in passaging the virus in appropriate climatic condition over winter during which the vector is biologically inactive (2). Although certain species of deer and sheep frequently become clinically ill, goats and cattle usually remain asymptomatic. It is believed that this reflects host-species-specific variations in the response of microvascular endothelial cells to bluetongue virus (BTV) infection (3). It is known that the vector (*Culicoides* spp.) has approximately 1000 subspecies, but only 17 of them have been directly related to transmission of the BTV, most probably, because of their genetic predisposition. Transmission only occurs when the climatic conditions are favourable for adult vector activity (4). BTV infection of ruminants usually occurs during late summer or early autumn, in which midges are extremely effective (2).

BTV is the prototype virus of the genus Orbivirus in the Reoviridae family. There are at least 24 serotypes of BTV worldwide. The BTV double-stranded RNA genome consists of 10 unequal segments that are encapsulated by a double-layered icosahedral shell, and encodes 7 structural (VP1-VP7) and 4 non-structural (NS1, NS2, NS3/NS3A) proteins (5). Laboratory diagnosis includes the determina-

tion of antibodies using agar-gel immunodiffusion (AGID) or enzyme-linked immunosorbent assay (ELISA) (6, 7, 8, 9), RT-PCR (10, 11), virus isolation and virus neutralisation (VN) (12, 13).

The VP7 protein has been identified as the soluble group-specific antigen and supposedly reacts specifically with the MAbs used in the blocking and competative ELISAs (7, 14). These ELISAs are all serogroup-specific, identifying primarily the highly conserved BTV VP7 of all 24 known serotypes. In competative enzyme-linked immunosorbent assay (c-ELISA) there is competition between the samples to be tested and a monoclonal antibody, which is coupled to the peroxidase and directed to the N-terminal part of the VP7 protein. c-ELISA is more sensitive and specific than the AGID, modified complement fixation, plaque neutralization tests by reducing the serogroup-level cross-reactions. In addition, as the c-ELISA is rapid and reliable, it is ideally suited for confirmation of exposure to a single BTV serotype and thereafter for serological surveillance to help determine the transmission and spread of BTV, particularly in the absence of disease (6, 7, 15).

In this study, the seroprevalence of BTV, which has been shown to be active in western Turkey, in Kars province in North-eastern Anatolia was determined by c-ELISA in cattle. These seroepidemiological data demonstrate the occurrence of BTV in private small scale production units and emphasize the necessity for effective control measures (vector, slaughter and vaccination)

MATERIAL AND METHODS

Clinical Samples

Blood serum samples were obtained from 401 clinically healthy local cattle six months to nine years between November in 2010 to March in 2011 from private small scale production units (less than twenty cattle) in Kars district in Turkey (Figure 1). Cattle did not receive vaccinations against BTV at the time of this study. Blood samples were

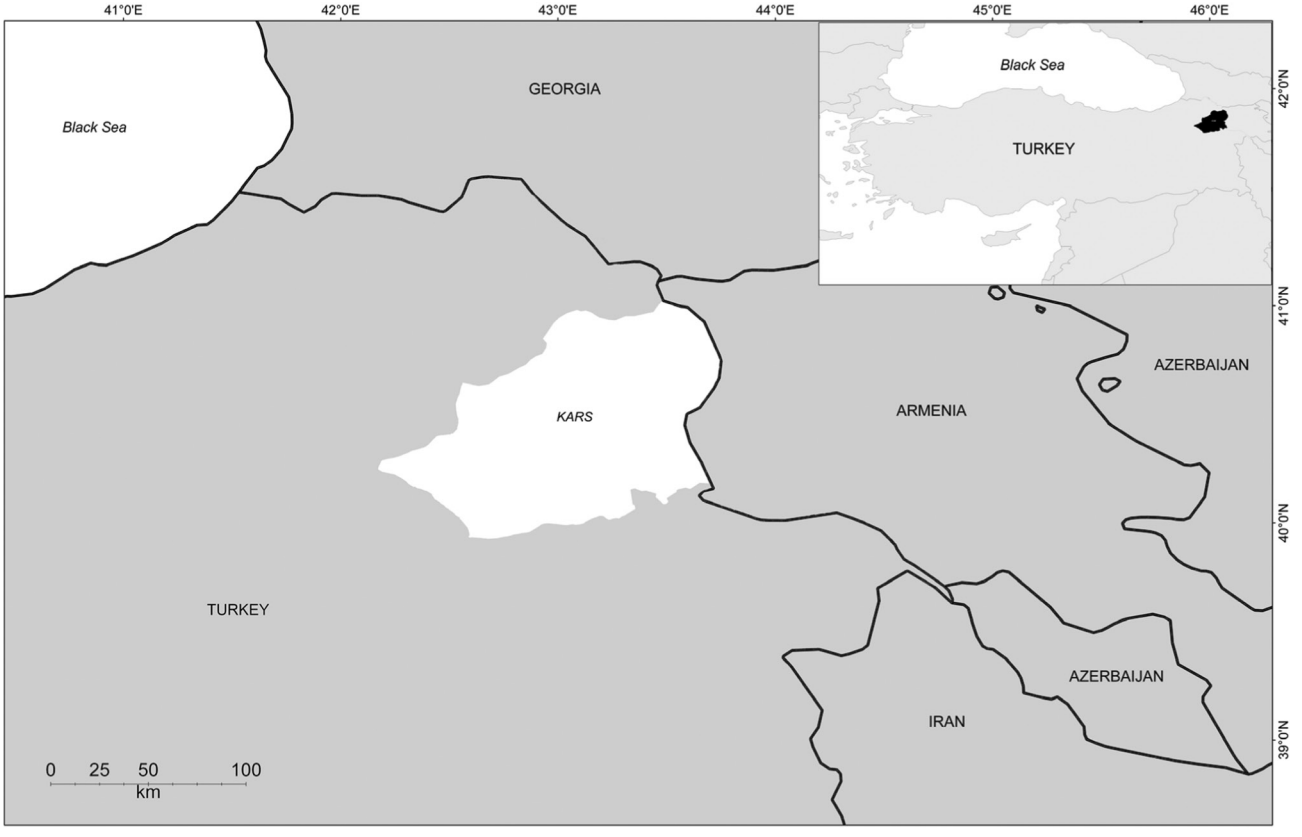

Figure 1: Geographical positioning of the Turkish province in which the study was performed.

collected directly into blood tubes with silicon and centrifuged at 1500 g for 10 minutes to separate the serum which was then stored at -20 ºC until use.

Competative Enzyme-Linked Immunosorbent Assay (C-ELISA)

A commercial c-ELISA (Insitute Porquier, Cat.No: P00450/07, France) used for the detection of antibodies against the BTV was carried out according to the instructions of the manufacturer. Briefly, 20 µl of test sera diluted at 1:5 in dilution buffer were added to wells and incubated for 45 min at room temperature. 100 µl of monoclonal anti-VP7 peroxidase conjugate diluted at 1:20 in wash solution were added to each well. Following incubation for 45 min at room temperature, unbound conjugate was removed by washing and 100 µl of enzyme substrate (hydrogen peroxide) and chromogen tetramethylbenzidine (TMB) were added to wells. After incubation at room temperature for 10 min, the enzymatic reaction was stopped by the addition of 100 µl of 0,5M H_2SO_4 solution. The optical density (OD) was measured at 450 nm. The ratio between the OD of the sample and the OD of the negative control (S/N percentage) was calculated for each sample. Samples with S/N percentage equal or lower than 70% were considered as positive, as indicated in the kit procedure.

RESULTS

A total of 401 serum samples were tested for BTV specific antibodies using c-ELISA. Overall results revealed that 18.5% (74/401) of the cattle sampled were BTV seropositive, while 76.6% of the samples (307/401) had no detectable antibody aganist BTV. Furthermore, the results of 5% of the samples (20/401) were of a dubious nature.

DISCUSSION

Cattle are a significant reservoir for BTV and the infection is generally subclinical in these animals. The prevalence of BT infection, which results in significant economic losses for reasons such as reduced fertility, abortions, congenital anomalies in calves, and restrictions on commercial semen imports, has been reported in research conducted in countries of the Caucasus, which border the region of Northeast Anatolia to vary between 25% and 93.5%. (16, 17, 18).

The first data related to BT infection in Turkey date back to 1944. For many years subsequently the existence of BT infection in Turkey was not reported, but in October of 1977, it was observed in sheep in 7 provinces surrounding Aydın. After this incident, the infection was encountered in other provinces (19). Gürtürk *et al*. (20) collected blood serum from 21 cattle in the Aydın region, and found for the first time in Turkey neutralizing antibody against BTV-4 in 19 of the 21 samples. A number of studies (19, 21, 22, 23, 24) conducted regarding BT infection in Turkey have demonstrated that the infection is widespread in populations of cattle, sheep and goats. These studies have reported that in various regions of Turkey BTV-4 seroprevalence varies between 0-100% in sheep, 2.8-50% in goats and 0-75.2% in cattle. Bulut *et al*. (25) checked blood serum samples from 562 sheep and 562 goats in the region of Konya for the existence of BTV-4 antibodies using the ELISA and serum neutralization techniques. The ELISA test revealed that the seropositivity for BTV-4 in sheep and goats was 14.4% and 60% respectively, while the serum neutralization test found it to be 13.9% and 53.5%, respectively. Using the virus neutralization technique, the blood serum of 557 cattle collected from 21 towns/villages in 5 provinces in the Trakya region was checked by Karaoglu *et al*. (26) for the presence of BTV-4, BTV-9 and BTV-16 antibodies. Serotype specific BTV seroprevalence was found to be 69.0%, 71.4% and 80.2% respectively. Ozgunluk (27) reported that BTV-9 and BTV-16 seroprevalence as being 20.0% and 22.0%, respectively by the virus neutralization tests conducted on serum from 740 cattle collected from 9 provinces in the Southeast Anatolia Region. Albayrak and Ozan (28) reported that seroprevalence for bluetongue infection was 3% in sheep and 11% in cattle using a competitive ELISA to identify bluetongue antibodies in blood serum samples collected from 200 cattle and 200 sheep in 5 provinces in the Black Sea Region. Using the virus neutralization technique, the blood serum of 352 cattle collected from the provinces of Iğdır, Kars and Ardahan in the Northeast Anatolian Region was checked by Yildirim and Yilmaz (29) for the presence of BTV-4, BTV-9 and BTV-16 antibodies and serotype specific BTV seroprevalence was found to be 72.2%, 42.1% and 36.9% respectively.

In this study, however, seropositivity was found to be only 18.5% in blood samples from 401 local cattle six months–nine years of age in private small scale production units in the province of Kars. This percentage is lower than the percentages found in studies (23, 24, 26, 27, 29) conducted in past

years and is similar to the percentages reported by Albayrak and Ozan (28).

In light of the data from this study which demonstrated the presence of blue tongue infection, it is clear that the infection is significant for the Northeast Anatolian Region and serious measures should be taken to prevent it. Even though this study found a low rate of seropositivity in cattle sampled from private small scale production units, this situation should be evaluated bearing in mind the limited animal population and the fact that the sampling was conducted in the winter months when the vectors that carry the infection are inactive.

The most effective means of prevention and control is individual monovalent or polyvalent immunization. Live attenuated BTV vaccines that provide long-lasting immunity cause abortions and malformations in the brain of foetuses in pregnant animals (30). It has been reported that vaccinations should be given when insect vectors are not active because the live attenuated vaccine virus may acquire virulence due to possible reassortments during transfer and replication with insects. In addition, new BTV serotypes may be created as a result of genetic reassortment occurring between the vaccine virus and wild BTV strains (31). Inactivated vaccines can be safely used during seasons when vectors are present and are safer than the live attenuated vaccines but they only provide short-term immunity. It has been observed that after using these vaccines the viremi phase is shortened in cattle and goats. Because there is no close antigenic relationship between BTV serotypes, using polyvalent vaccines in epidemics caused by simultaneous infection with multiple BTV types is the most effecient method. In the preparation of polyvalent vaccines in South Africa, where 21 of the 24 known serotypes are found, difficulty has been encountered with combinations because they do not provide sufficient immunity against some strains. Therefore, care must be exercised in the choice of strains in polyvalent vaccines (32).

In the near future dams will be built in the province of Kars with the resultant formation of large lakes. The milder weather in the winter months due to irrigation combined with a drop in temperatures during the summer and an increase in humidity will cause both an increase in the population of insects and heightened flight activity. These changes are extremely important in terms of the epidemiology of the BT infection. As a general observation, it is possible to reduce, in a limited fashion, the prevalence of the infection by bringing animals indoors during the night when insect activity is particularly high and with the appropriate use of insect repellents.

In conclusion, we are of the opinion that those involved in animal husbandry should be encouraged to implement programs aimed at controlling the infection in light of the data obtained from this study and previous reports related to the issue. Furthermore, animal movement (entrance of contraband animals or transfers between operations or from other countries via importation) should be monitored diligently to prevent the spread of the disease to a larger population. It is also thought that conducting blue tongue virus antibody screening on cattle will be very beneficial in terms of identifying the presence of this disease in the region and the extent of the economic losses that could result and for determining programs to be implemented for the control of the disease.

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