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Serological Investigation of Bluetongue Virus and Rift Valley Fever Virus Infections in Sheep in Kars Province of Turkey

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SUMMARY In this study we surveyed Bluetongue Virus (BTV) and Rift Valley Fever Virus (RVFV) infections as serologically in sheep from private small scale production units of less than twenty sheep per unit, in Kars province of the Northeast Anatolia Region Turkey. For this purpose, blood samples randomly collected from 460 local sheep were analyzed for the presence of specific antibodies for BTV using a competative enzyme-linked immunosorbent assay (c-ELISA) and RVFV using blocking ELISA (b-ELISA), respectively. A total of 49 (10.65%) serum samples were found positive against BTV. None of the sheep were positive for antibodies to RVFV. On the basis of results it is suggested that infection was spreading in small private f1ocks. Furthermore, recommendation to the control of bluetongue infection were presented. In addition, the RVFV infection is not present in this region. This study is the first serological study on BTV and RVFV in sheep in Kars province of Turkey.

Key Words: *b-ELISA, BTV, c-ELISA, RVFV, Kars*

ÖZET

Kars ilindeki koyunlarda Bluetongue Virus ve Rift Valley Fever Virus infeksiyonlarının serolojik araştırılması

Bu çalışmada Kuzeydoğu Anadolu Bölgesinde yer alan Kars ilinde küçük aile işletmelerindeki koyunlarda Bluetongue Virus (BTV) ve Rift Valley Fever Virus (RVFV) enfeksiyonları serolojik olarak araştırıldı. Bu amaçla, yerel ırka ait 460 adet koyundan rastgele toplanan kan serumları BTV ve RVFV spesifik antikor varlığı yönünden sırasıyla yarışmalı enzyme linked immunosorbent assay (c-ELISA) ve bloklama ELISA (b-ELISA) testi ile analiz edildi. Koyunlarda BTV için %10.65 seropozitiflik saptanmasına karşın, RVFV yönünden antikor cevabı tespit edilemedi. Elde edilen veriler, kiiçük aile işletmelerinde enfeksiyonun yayılma gösterdiğini ortaya koydu. Bu bilgilerden hareketle bluetongue virus enfeksiyonunun kontrolüne yönelik önerilerde bulunuldu. Ayrıca bu bölgede RVFV varlığı tespit edilmedi. Bu çalışma Kars ilindeki koyunlarda BTV ve RVFV enfeksiyonlarının serolojik olarak araştırıldığı ilk çalışmadır.

Anahtar Kelimeler: *b-ELISA, BTV, c-ELISA, RVFV, Kars*

INTRODUCTION

Bluetongue virus (BTV) and Rift Valley Fever virus (RVFV) induce diseases (primarily in cattle, sheep, and goats) that have considerable economic impact on domestic livestock production. Due to the potential for very serious and rapid spread, the diseases caused by these two viruses are classified by OIE, the World Organization for Animal Health, as notifiable diseases (formerly List A) (OIE 2008).

BTV infection is characterized by fever, congestion, oedema, haemorrhages, hyperemia and ulceration of oral mucosa, coronitis and lameness (Mellor and Witmann 2002). BTV belongs to the genus Orbivirus of the family Reoviridae. There are 25 serotypes of BTV currently recognized worldwide (Davies et al. 1992). The serotypes present in the Mediterranean basin are BTV-1, 2, 3, 4, 6, 9, 10, and 16 (Mellor and Witmann 2002). Of these BTV-4, BTV-9 and BTV-16 have most recently been isolated in Turkey (Erturk et al. 2004). BTV is transmitted by species of the genus Culicoides, family Ceratopogonidae. The virus occurs almost globally between latitudes 35ºS and 50ºN, in which Culicoides midges are extremely effective. Climate, vectors and presence of reservoir animals play important roles in the epidemiology of BTV infection. 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 (Gibbs et al. 1994). The disease can cause up to 100 % morbidity in sheep with 0–50 % case fatality rates (Schwartz-Cornil et al. 2008). Goats, cattle and wild

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ruminants generally remain asymptomatic, although the northern European strain of BTV-8 has also caused a low but significant level of clinical signs and mortality (<1 %) in cattle (Koumbati et al. 1999; Szmaragd et al. 2007).

Rift Valley Fever (RVF) is a zoonotic disease caused by a Phlebovirus in the family Bunyaviridae that affects animals and humans. In cattle and sheep, the disease may cause sudden abortion, and mortality rates close to 100% are observed in newborns. Many infected humans become severely ill, and serious hemorrhagic fevers often lead to death (Elliot 1996). The virus transmitted by the bite of infected mosquitoes or exposure to tissues or blood of infected animals. Epizootics of the disease occur periodically after heavy rainfall and in flooded area, which allow the mosquito eggs to hatch. RVFV has been detected across Africa, from Senegal to Madagascar and from Egypt to South Africa. The first outbreaks among human and livestock populations outside Africa were reported in the Arabian Peninsula in 2000 (Davies 2006). Because of the increasing range of the virus, the high numbers of competent vector species present in currently non-endemic RVF areas, such as Europe, the intensification of international trade in live animals, and the unknown impact of climate change, several national and international agencies have issued warnings about the heightened risk of introduction of RVFV into non-endemic RVF areas (Moutailler et al. 2008; Albayrak and Ozan 2013).

The Kars district's economy is primarily based on animal husbandry and about %80 of the population rely on it for their livelihoods. In addition, Kars is a mountainous province and almost 90% of the provice has an altitude of over 1500 meters. In Kars province where the study was performed, winter climate conditions are extremely harsh. Even though one might think that infections spread by vectors would occur less frequently due to the harsh climate conditions, the abundance of wetlands and the large numbers of cattle in the Kars district in Northeast Anatolia Region should be viewed as important factors that could keep the virus in circulation for a long time in this area. The objective of this study was to serologically determine the BTV and RVFV in sheep in the Kars district of Turkey. These seroepizootological datas demonstrate the occurrence of BTV and emphasize the necessity to effective control measures. This study is the first serological study to determine seroprevalence of BTV and RVFV infections in sheep in the Kars district of Turkey.

MATERIALS and METHODS

Clinical samples

Blood samples were collected from 460 local healthy unvaccinated sheep randomly, between November in 2013 to March in 2014 in private small scale production units (less than twenty sheep) in Kars district of Turkey (Fig.1). The age of the animals varied from 1 to 2 years. Blood samples were taken from the jugular veins of the animals. Blood tubes (without EDTA) were centrifuged at 3,000 × g for 10 min, and the serum samples were transferred to sterile tubes and stored in −20 °C until used.

Competitive enzyme linked immunosorbent assay (c-ELISA)

Anti-BTV antibodies were detected in serum samples by group specific, c-ELISA kit (Insitute Porquier, Cat.No: P00450/07, France) according to the instructions of the manufacturer. The test based on competitive between test sera and an anti-VP7 for a VP7 antigen previously bound to the solid phase of ELISA plate. 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 with an ELx800 absorbance microplate reader. Before interpretation of the results, all OD values in wells coated with BTV VP7 viral antigen were corrected by subtracting the ODs of negative control from the samples ODs ($OD_{corrected} = OD_{sample} - OD_{control}$). Percent positivity values (*PP* values) were evaluated. All corrected OD values for the test samples and the negative control were related to the corrected OD values of the positive control as follows:

PP = [Test sample or negative control ($OD_{corrected}$) ÷ Positive control ($OD_{corrected}$)] × 100

The *PP* value equivalent or lower than 70 were considered positive for BTV infection.

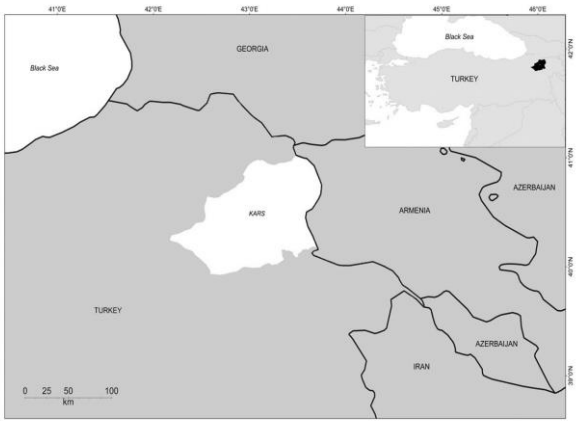

Figure 1. Geographical positioning of the Kars province in which the study was performed.

Blocking enzyme linked immunosorbent assay (b-ELISA)

A commercial b-ELISA (Ingezım, Cat.No: 13. FVR. K3, Spain) used for the detection of antibodies against the RVFV 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-N protein peroxidase conjugate were added to each well. Following incubation for 30 min at room temperature, unbound conjugate was removed by washing and 100 µl of enzyme substrate (hydrogen peroxide) and 2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS) were added to wells. After incubation at room temperature for 10 min, the enzymatic reaction was stopped by the addition of 100 µl of stop solution for ABTS substrate. The optical density (OD) was measured at 405 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 higher than 45% were considered as positive, as indicated in the kit procedure.

RESULTS and DISCUSSION

A total of 460 serum samples were tested for BTV and RVFV specific antibodies using c-ELISA and b-ELISA, respectively. Overall results revealed that 10.65% (49/460) of the sheep sampled were BTV seropositive, while 88.26% of the samples (406/460) had not antibody aganist BTV. Furthermore, the results of 1.09% of the samples (5/460) were of a dubious nature. All animals were negative for antibodies aganist RVFV.

Small ruminant production in the Kars district of Turkey is gaining more importance with the reasons of decreasing number of animals, increase in meat prices and more demand on small animal meat. Some viral agents are big threats to this sector. ELISA is a simple, sensitive, rapid test; modifications of ELISA are most widely used for antibody and antigen detection. The c-ELISA is more sensitive and specific than the agar gel immunodiffusion test (AGID), modified complement fixation, plaque neutralization tests by reducing the serogroup-level cross-reactions. In addition, c-ELISA 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 (Afshar et al. 1989; Reddington et al. 1991).

The prevalence of BTV infection, which results in significant economic losses for reasons such as reduced fertility, abortions, congenital anomalies in sheep, and restrictions on commercial semen imports, has been reported in research conducted in countries of the Caucasus, which border the region of Northeast Anatolia Region to vary between 25% and 93.5%. (Lundervold et al. 2004; Shoorijeh et al. 2010; Hasanpour et al. 2008). The location of the study was chosen because of the fact that Kars is located in the middle of animal transit route between Northeast Anatolia Region and Caucasus which functions as a bridge between Asia and Europe. In the studies conducted in later years, the seropositivity rates among sheep were detected between 0-50% (Burgu et al. 1992; Bulut et al. 2006; Okur Gumussova et al. 2006; Duman et al. 2009; Albayrak and Ozan 2010; Azkur et al. 2011; Ozan et al. 2012). Burgu et al. (1992) determined BTV seropositivity rates in sheep as 25.5% in South, South-East and Aegean regions of Turkey. Bulut et al. (2006) examined total 562 sheep and 562 goat serum samples collected from Konya, Burdur and surrounding farms by ELISA and serum neutralization technique (SNT). The ELISA test revealed that the seropositivity for BTV in sheep and goats was 14.4% and 60% respectively, while the SNT found it to be 13.9% and 53.5%, respectively. Okur-Gumusova et al. (2006) detected 2.44 % seropositivity rate in sheep in the middle Black Sea Region. Duman et al. (2009) tested 320 sheep blood serum samples for the presence of BTV specific antibodies by c-ELISA and found seropositivity rate as 4.68% in Konya district of Turkey. Albayrak and Ozan (2010) reported that seroprevalence for BTV was 3% in sheep and 11% in cattle using a c-ELISA to identify BTV specific antibodies in blood serum samples collected from 200 cattle and 200 sheep in 5 provinces in the Black Sea Region. It was announced that BTV seroprevalence was 49.8 % in sheep in Kirikkale district of Turkey (Azkur et al. 2011). Using the ELISA, the blood serum of 144 sheep and 50 goats collected from the Samsun province of Turkey was checked by Ozan et al. (2012) for the existence of BTV antibodies and BTV seroprevalence was found to be zero% in sheep and 4% in goats.

In this study, the seroprevalence has been found quite low for BTV in Kars province. It is thought that is because of low Culicoides population in the province having a cold and long dry season. This percentage (10.65%) is higher than the percentages found in studies (Okur Gumusova et al. 2006; Duman et al. 2009; Ozan et al. 2012) conducted in past years and lower than the percentages of some studies (Burgu et al. 1992; Bulut et al. 2006; Azkur et al. 2011).

In light of the data from this study which demonstrated the presence of BTV 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 sheep 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. It is common place knowledge that the result of the seroprevalance studies are influenced by many factors such as the number of sampled animals, the age of the animals, the time of sampling, the conditions of care and feeding, individual differences and so on.

The most effective means of prevention and control is individual monovalent or polyvalent immunization. One of the important features of the BTV infection is plurality of the virus and outbreaks caused by several serotypes of the virus could be detected in the same region. However, presence of several serotypes in country should be taken into consideration in both preparation and administration of the vaccine as there is no close antigenic relation between BTV serotypes. Therefore, successful protection against BTV infection can be achieved by using polyvalent vaccines included of local BTV serotypes.

In the near future dams will be built in the Kars district 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 BTV 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.

Albayrak and Ozan (2013) performed the first serological study about presence of RVFV antibodies in different mammalian species (cattle, horse, sheep, goat and water buffalo) in the northern Turkey, but they did not detect any antibody to RVFV. Similarly, no antibody response was detected aganist RVFV in this study. Although mosquito species known to transmit RVFV have been observed, there has been no report of acutely infected humans and animals in Turkey (Dik et al. 2006; Albayrak and Ozan 2013). This may suggest that the infection is not present in the Kars district in Northeast Anatolia Region. In addition, the vectors in this area may not carry RVFV.

The results obtained in the present study showed that BTV infection may be spreading in Northeast Anatolia Region, which is the most important cattle and sheep production area in Turkey. 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 BTV antibody screening on cattle and sheep 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. In addition, the vectors in this area may not carry RVFV. This study showed that RVFV might not become a risk potential for animals in Kars. In order to know the RVFV status in Northeast Anatolia Region of Turkey, further studies focusing vector and risk factors of exposure are needed.

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