

Virological and Serological Investigations of Bluetongue Virus (BTV) Infection in Sheep in Konya Region

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Abstract: This study was conducted for determination of recent situation of BTV infection and seroprevalence of the infection in sheep in Konya region. For this purpose, 320 blood samples were collected from randomly selected sheep, slaughtered in a local private slaughterhouse, from nonvaccinated sheep herds from different locations of the region. To determine the present situation of the BTV infection (new infection) in the animals, a commercial antigen capture ELISA was used. The infection rate was changed between 0-5.55% among the herds and overall the infection rate was found as 1.56%. To determine the specific antibodies to BTV in the blood serum samples of the sheep, a commercial competitive ELISA (c-ELISA) was used and the within herd seroprevalence was changed between 0-15% and individual animal seroprevalence was 4.68%. When, the results of this were compared with the results of seroprevalence and virological studies conducted previously in the same region, we concluded that the seroprevalence of the infection is decreasing in the region. But, new infections in the sheep population in the region are still present.

Key words: Sheep, bluetongue virus, antigen-capture ELISA, competitive ELISA, Konya, Turkey

INTRODUCTION

Bluetongue (BT) virus is an arthropod-borne pathogen, transmitted by species of the genus *Culicoides*. BTV can infect several species of domestic and wild ruminants, but sheep are most susceptible (Akhtar *et al.*, 1997). BTV is the type species of the Orbivirus genus, family *Reoviridae*, having double stranded RNA genome, which consists of ten segments. There are 25 serotypes of BTV currently recognized worldwide (Davies *et al.*, 1992). The diversity of viral serotypes and the wide host range of BTV complicate the development of diagnostic procedures. Two general types of tests primarily have been used in diagnostic veterinary medicine. Serologic assays, such as the complement fixation test, the Agar Gel Immunodiffusion (AGID) test and the more current c-ELISAs are useful in detecting animals that have had prior exposure to BTV (Afshar *et al.*, 1993; Reddington *et al.*, 1991); however, they do not answer questions regarding the current state of viremia in an animal (De la Concha-Bermejillo *et al.*, 1992). Currently, a number of other procedures are used to

detect BTV from the blood or tissues of infected animals: inoculation of susceptible mammalian or insect cells or intravenous inoculation into 10-12 days old Embryonated Chicken Eggs (ECE), Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), ELISA, immunofluorescence, and Dot Immunobinding Assay (DIA) and immunoelectron microscopy (OIE, 2009). However, some of diagnostic methods (virus isolation in cell culture, ECE and conventional RT-PCR) are laborious and time-consuming (Mecham, 1993; Niedbalski, 2007). Thus, there is need for the tests which are easy to perform, fast and cheap, such as Bluetongue Antigen Capture ELISA (BTACE). Although, BTACE generally gives positive results with blood and tissues taken from infected animals with bluetongue virus, it is less sensitive than virus isolation in ECE and RT-PCR. On the other hand, BTACE can be a useful herd test because, it gives positive result in the early acute phase of the infection (Stanislawek *et al.*, 1996).

This study was conducted for the determination of new BTV infections and seroprevalence of the infection in sheep in Konya region.

MATERIALS AND METHODS

Blood samples: For detection of specific antibodies to BTV in sheep blood serum samples by c-ELISA and BTV antigens in erythrocyte by BTACE, 320 blood samples (with and without EDTA) were collected by 10% random sampling method in unvaccinated sheep in 12 close system sheep managements in Konya region. Serum and erythrocyte were prepared by standard methods and kept at 22°C, until tested.

c-ELISA: Anti-BTV antibodies were detected in the serum samples by group specific, c-ELISA, using a commercial kit (VMRD Inc., Pullman, USA). The test is based on the competition between test sera and an anti-VP 7 MAb for a VP 7 antigen previously bound to the solid phase of an ELISA plate. This test was performed according to the producer's description and plates were read with an ELISA reader at 630 nm and results were calculated. Test sera were taken as positive according to produced optical density <50% to the negative control.

BTACE: The red blood cells mixed with an equal volume of sample diluent were tested for BTV antigen using the commercial BTACE (New South Wales, Australia) according to the manufacturer's instructions. The Optical Densities (ODs) on an ELISA reader at a wavelength of 450 nm were read. The mean of the duplicates for each sample was calculated and the final result (the Positive to Negative (P/N) ratio) was evaluated as the ratio of the mean OD of the absorbance obtained for the test sample compared to the mean OD of the absorbance obtained for the Negative Control (NC) antigen. The interpretation for the test samples was determined by both the OD and the P/N ratio. The ratio was taken priority, provided target OD values are achieved as follows:

- A P/N ratio >2.0 is positive, if OD of the Positive antigen Control (PC) is at least 0.900
- A P/N ratio <2.0 is negative, if and that of the Negative Control antigen (NC) is 0.100 or less
- Samples were retested, if the OD of the PC was <0.900 and the P/N ratio of the sample was <2

RESULTS AND DISCUSSION

The presence of the specific antibodies to BTV sheep blood samples ranged between 0-15% among the managements and 4.68% of the animals (15/320 animals) were seropositive for bluetongue infection. To detect infection status in 320 animals at present, positivity ranged between 0-5.55% among the managements and 1.56% of the animals were positive, when antigen capture ELISA was performed in erythrocyte samples (Table 1).

In Turkey, BTV infection was diagnosed with Serum Neutralization Test (SNT) and the studies used the SNT in investigation of BTV infection. The first data on BTV infection in Turkey was from 1944 (Anonymous, 1980). Then, for a long time, BTV infection was not reported in Turkey and in 1977, in Aydin and seven states around Aydin province, BTV infection was detected in sheep (Yonguc *et al.*, 1982). In the studies conducted in later years, the seropositivity rates among sheep were detected between 0-46% (Burgu *et al.*, 1984; Bolat, 1986; Ozturk *et al.*, 1987; Girgin and Yonguc, 1988; Burgu *et al.*, 1992; Yavru *et al.*, 1999; Bulut *et al.*, 2006; Gur, 2008). Burgu *et al.* (1984), conducted a serological study in 52 sheep in Tahirova State Farm and micro neutralization test (mNT), all serum samples were found negative for antibodies to BTV. Bolat (1986), tested 1290 sheep blood samples from East and Southeast of Turkey by SNT and AGID and found 273 sheep (21%) seropositive for BTV. Ozturk *et al.* (1987), tested 86 sheep blood serum samples

Table 1: Results of BTACE in sheep erythrocyte samples and c-ELISA in sheep serum samples

Managements	Total number of animals	Sampling of animals*	ELISA antibody		Positive (%)	ELISA antigen		Positive (%)
			-	+		-	+	
1	300	30	28	2	6.66	30	0	0
2	200	20	17	3	15.00	20	0	0
3	250	25	22	3	12.00	25	0	0
4	230	23	23	0	0	22	1	4.34
5	250	25	25	0	0	25	0	0
6	300	30	30	0	0	30	0	0
7	280	28	25	3	10.71	28	0	0
8	500	50	46	4	8.00	50	0	4.00
9	280	28	28	0	0	27	1	3.57
10	250	25	25	0	0	24	1	4.00
11	360	36	36	0	0	34	2	5.55
Toplam	3200	320	305	15	4.68	315	5	1.56

*10% random sampling method

from Konya Livestock Research Center for neutralizing antibodies to BTV SA₄ type and found 36.04% seropositivity. Girgin and Yonguc (1988) found 46% of the sheep and 44% of the goats in East Anatolia positive for neutralizing antibodies to BTV.

Burgu *et al.* (1992) determined BTV seropositivity rates in sheep as 25.5% and in cattle as 15.5% South, South-East and Aegean regions of Turkey. Yavru *et al.* (1999) examined 115 blood serum samples collected from healthy sheep in Bala agriculture management by mNT and they detected 29 (25.21%) seropositive animals. Bulut *et al.* (2006) examined total 562 sheep and goat serum samples collected from Konya, Burdur and surrounding farms by ELISA and SNT. They found 54 out of 315 sheep blood samples positive in Konya and 1 out of 66 sheep blood serum samples positive in Burdur. Gur (2008) tested 82 gazelle, 684 sheep and 100 cattle blood serum samples for the presence of BTV antibodies and found seropositivity rates as 40.2, 29.5 and 88%, respectively.

In this study, 4.68% seropositivity for BTV was detected by c-ELISA in healthy nonvaccinated sheep from private managements in Konya region. Although, c-ELISA can not differentiate the antibodies whether, due to infection or vaccination with live attenuated vaccine (Hamblin, 2004), this result shows the true prevalence of BTV in this region since unvaccinated sheep were sampled.

This rate (4.68%) is higher than the rates of some studies (Burgu *et al.*, 1984; Bulut *et al.*, 2006) conducted in Turkey and lower than the rates of some studies (Bolat, 1986; Ozturk *et al.*, 1987; Girgin and Yonguc, 1988; Burgu *et al.*, 1992; Yavru *et al.*, 1999; Gur, 2008). Lundervold *et al.* (2003) stated that distribution and amount of vectors and host of the vectors can be important factors for different prevalence rates of the infection. On the other hand, the prevalence of BTV in this study (4.68%) was lower than the prevalence in previous study (Ozturk *et al.*, 1987; Yavru *et al.*, 1999; Bulut *et al.*, 2006) conducted in Konya. Ozturk *et al.* (1987) sampled the blood serum samples collected from a farm in the same region and they found higher (10%) seropositivity rate than our study.

But in the study, we collected the serum samples from private managements in Konya with close management model. On the other hand, the reason for lower seropositivity in this study than the seropositivity of the studies conducted by Yavru *et al.* (1999) and Bulut *et al.* (2006) can be due to especially, the strict measurements (control of animal transports and addition of new animals

to the herds, establishment of sheep farms far from cattle farms and at high altitude, strict hygiene rules, separation of the animals having clinical signs from healthy animals, etc.) taken by Department of Agriculture in Konya and private sheep managements applied these measurements in their farms.

In this study, to detect the present situation of the infection (new infections), we used commercial BTACE (Stanislawek *et al.*, 1996), which allows detecting the BTV antigen in blood samples in acute phase of the infection. Stanislawek *et al.* (1996) developed antigen capture ELISA to investigate the antigen in 40 sheep, which were infected with one of 20 South African BTV serotype and used 4 blood preparates (whole blood, leucocyte layer, washed leucocyte and plasma) taken preinoculation and 6-9th days postinoculation. At the end of the study, they detected 17 of the 20 serotype by BTACE and 27 of 40 sheep were detected positive by Antigen-ELISA. During sampling period most of the positive results were found in whole blood samples taken at postinoculation 6th and 7th days, but in same samples amount and time of the antigen were higher in leucocyte layers and washed erythrocyte preparates.

Portanti *et al.* (2005) developed BTV antigen capture ELISA in which tissue culture supernatant used to detect BTV serotypes (1, 2, 4, 9 and 16) responsible from a recent epidemic in the Mediterranean region and determined the sensitivity and specificity of it as 100%. Shad *et al.* (1997) developed a new RT-PCR which allows the diagnosis of BTV fast and reliable and they compare it with BTACE and virus isolation. They inoculated 8 Warhill crossbred sheep by subcutaneously with BTV serotype 10 and they collected blood samples during 28 days period. RT-PCR can detect viremia peak from the 5th-14th day of the immunity, as virus isolation and BTACE. In this study to reveal the presence of circulating new BTV infections in sheep in Konya region, commercial BTACE (New South Wales, Australia) and erythrocyte suspension as material were used.

CONCLUSION

Five of 320 sheep (1.56%) were found positive for BTV antigen. When, we evaluate the results of this study with the results of the previous serological and virological studies, we concluded that seroprevalence of BTV infection in sheep in Konya region is decreasing, but the presence of active BTV infection in sheep in the region continue.

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