SEROLOGICAL INVESTIGATION OF BLUETONGUE VIRUS INFECTION BY SERUM NEUTRALIZATION TEST AND ELISA IN SHEEP AND GOATS

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Abstract

Altogether 562 sheep and goat blood serum samples were collected from livestock located in Konya, Burdur, and their environs in Turkey. The samples were tested for bluetongue virus antibodies by ELISA and serum neutralization test (SNT). Fifty-four (17.1%) out of 315 sheep serum samples from Konya and 1 (1.5%) out of 66 sheep serum samples from Burdur were detected as seropositive. Seventy-three (60%) out of 121 goat serum samples from Konya and 36 (60%) out of 60 goat serum sample from Burdur were found positive by ELISA. Fifty-three (16.8%) out of 315 sheep serum samples from Konya and none of 66 sheep serum samples from Burdur were detected to be seropositive. Sixty-five (53.7%) out of 121 goat serum samples from Konya and 32 (53.3%) out of 60 goat serum samples from Burdur were found as positive by SNT. The results demonstrate that ELISA is more sensitive than SNT.

Key words: sheep, goats, bluetongue virus, ELISA, serum neutralization test.

Bluetongue (BT) is a non-contagious arthropod-borne orbiviral infection of domestic and wild ruminants including sheep, cattle, goats, deer, antelopes and elk. Bluetongue virus (BTV) infection is widely distributed in the tropics and subtropics where competent Culicoides sp. exists. At lower latitudes and altitudes the infection may be endemic, but elsewhere infection is epidemic, sporadic or non-existent. BT can be introduced into distant areas by movement of infected Culicoides. The epidemiology of BT depends on interaction of host, vector, climate and virus types (2).

BT is characterized by torticollis, loss of condition, AH (arthrogryposis and hydranencephaly) syndrome in lamb, decrease in milk secretion and fertility, and death. In most countries including Turkey, BT is an economically important infection (5).

Material and Methods

Blood samples were collected from sheep and goats from farms located in Konya, Burdur and their environs. Out of ovine blood samples, 315 were from Konya area and 66 from Burdur area; out of caprine blood samples, 121 were from Konya area and 60 from Burdur area.

The blood samples were collected from the caudal vein into vacutainers with no additive. The samples were stored overnight at 4°C and, after being spun at 2500-3000 rpm for 30 min, the sera removed and put in a waterbath at 56°C for 30 min, left to cool and then stored at -20°C until required.

BTV-4 antibodies in serum samples were detected using a commercial ELISA kit (VMRD, Inc., Pullman, ABD), according to the procedure described by the manufacturer. The absorbance was read at 450 nm in the ELISA reader (Anthos II, Anthos Labtec Instruments GmbH, Austria). The ELISA results were expressed as positive and negative.

Serum neutralization test (SNT) was done by Frey and Liess (6) method. For each serum sample, two wells were used on a 96 well-microplate. Each of 0.05 ml heat inactivated test serum samples was mixed with equal volume of BTV-4, containing 100 TCID50. The mixtures were incubated for 1 h at 37°C in a CO2 incubator for the serum neutralization. At the end of this period, diluted African green monkey kidney (Vero) cells as 3x105 cell/ml (0.05ml) were added into all wells. The plates were put into an incubator with CO2 at 37°C.
On the 5th day, cytopathic effects (CPE) seen in the cells were studied in tissue culture microscope and the results were evaluated. Sensitivity and specificity of both tests were calculated according to the method described previously by Martin et al. (9).

Results

A total of 562 sheep and goat serum samples were examined by ELISA to detect BTV antibodies. It was found that 54 (17.1%) out of 315 sheep serum samples from Konya and 1 (1.5%) out of 66 sheep serum samples from Burdur were detected as seropositive. Seventy-three (60%) out of 121 goat serum samples from Konya and 36 (60%) out of 60 goat serum samples from Burdur were found positive (Table 1).

<table>
<thead>
<tr>
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<th>Konya Positive (%)</th>
<th>Burdur Positive (%)</th>
<th>Total Positive (%)</th>
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<tbody>
<tr>
<td>Sheep</td>
<td>54 (17.1)</td>
<td>1 (1.5)</td>
<td>55 (14.4)</td>
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<tr>
<td>Goats</td>
<td>73 (60.0)</td>
<td>36 (60.0)</td>
<td>109 (60.0)</td>
</tr>
<tr>
<td>Total</td>
<td>127 (29.1)</td>
<td>37 (29.3)</td>
<td>164 (29.1)</td>
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The same serum samples were also examined by SNT. Fifty-three (16.8%) out of 315 sheep serum samples from Konya and none of 66 sheep serum samples from Burdur were detected as seropositive. Sixty-five (53.7%) out of 121 goat serum samples from Konya and 32 (53.3%) out of 60 goat serum samples from Burdur were found positive (Table 2).

<table>
<thead>
<tr>
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<th>Konya Positive (%)</th>
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<th>Total Positive (%)</th>
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</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>53 (16.8)</td>
<td>-</td>
<td>53 (13.9)</td>
</tr>
<tr>
<td>Goats</td>
<td>65 (53.7)</td>
<td>32 (53.3)</td>
<td>97 (53.5)</td>
</tr>
<tr>
<td>Total</td>
<td>118 (27.0)</td>
<td>32 (25.3)</td>
<td>150 (26.6)</td>
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Table 2

Compared with SNT as the reference test, the sensitivity and specificity of the ELISA were 100 and 96%, respectively.

Discussion

The SNT and various modifications of ELISA are most widely used for the antibody detection. The simplicity, sensitivity and rapidity of the ELISA make it useful for indirect diagnosis (10). The sensitivity and specificity of SNT and ELISA were compared by several researchers (4, 14).

Recently, the competitive ELISA (c-ELISA) has been recommended as the test of choice for regulatory purposes for BT diagnosis (1). The test is sensitive and specific for BT, with no cross reactivity with epizootic haemorrhagic disease virus (EHDV), as has been the case with the agar gel immunodiffusion (AGID) test (11).

An indirect ELISA and a competitive ELISA, using a group-specific monoclonal BTV antibodies were described for the detection of antibodies to BTV in cattle and sheep sera by Afshar et al. (1). They reported that while both ELISAs proved reliable, under the present test conditions involving detection of early postinfection reactions in experimentally infected animals, the competitive-ELISA was always more sensitive than the standard agar gel immunodiffusion test, the modified complement fixation test, and the plaque neutralization tests in the detection of BTV antibodies.

Lundervold et al. (8) reported the results of the first serological survey for BTV infection in Kazakhstan. They analysed blood samples which were collected from 279 cattle, 542 sheep, 137 goats and 513 wild saiga antelopes over large area of the country by ELISA, and found 25.4% seroprevalence in cattle, 21.4% in sheep, 25.5% in goats and 0% in saigas.

In this study, our seroprevalence rates (14.4% by ELISA and 13.9% by SNT in sheep) were found much less than the seropositivity rates (21% in sheep) as detected by Lundervold et al. (8). They reported that vector distribution, abundance, infection rates, efficiency and host preferences might all be important.

According to the results of this study, seroprevalence rates of BTV in goats have been found higher than in sheep both in SNT and ELISA. Probably it depends on goat breeding system. Goats are not usually in their folds in summer season. Thus, they can be exposed to midges easily in day time and especially night time.

In this country, BTV infection was usually diagnosed serologically and was tested by SNT. Bolat et al. (3) examined by SNT and AGID tests 1290 blood serum samples collected from sheep in Eastern and Southeastern Anatolia regions (Diyarbakır, Elazığ, Sanliurfa, Gaziantep, Kayseri, Muş, Van and Tunceli). They found 275 (21.32%) positive serum samples in these regions. Oztürk et al. (13) studied 86 sheep serum samples which were collected from Konya Livestock Research Centre. The researchers examined them to determine the presence of neutralizing antibodies to BTV type SA4 by SNT and detected 36.04% seropositivity. They sampled blood serum from one breeding farm and found higher seropositivity rate (36.04%) than we did (14.4% by ELISA and 13.9% by SNT in sheep) in the same region. But our samples were taken from livestock located in Konya and its environs. Girgin and Yonguç (7) detected by SNT the presence of neutralizing antibodies to BTV in 46% of sheep and in 44% of goats in the Western Anatolia regions.

Ozgünlük and Alkan (12) determining seroprevalence rates of BTV infections in cattle in Southeastern Anatolia project area detected 52.58%
seropositivity by SNT. They thought that ecologic alternation caused by the project would cause an increase in the population of bitting midges and their increased flying activity. Under these circumstances, if necessary control measures would not be taken, this infection transmitted by bitting midges would raise and cause economical losses.

We observed low rates of seropositivity when compared to others (7, 12) in Turkey, because the results of studies could be affected by season period. BTV infections are seen often in the regions that have a long-term summer season, humidity and raining. The regions examined in our study (Konya and Burdur) have a short summer season resulting in a short flying period of Culicoides.

In this study, 150 (26.6%) out of 562 sheep and goat blood serum samples were found positive by SNT, and 164 (29.1%) serum samples were detected as positive by ELISA. This indicated that ELISA was more sensitive than SNT. Besides, Chander et al. (4) reported that BTV antibodies in animals were detectable by ELISA at the 7th d post infection, while neutralizing antibodies were detected 14 d post BTV infection. Our results were in agreement with previous studies by Chander et al. (4) and Patton et al. (14) who reported that ELISA was more sensitive than SNT.

In conclusion, it was detected that there are various rates of seropositivity for BTV in Turkey as documented by several studies including the present study. For understanding epidemiology of BT more detailed studies should be planned in the future.

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References


