SEROPREVALENCE OF BLUETONGUE VIRUS INFECTION IN SHEEP IN TEKAB AREA IN IRAN

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ABSTRACT
This study was conducted on 198 sheep blood samples from 20 sheep flocks in Tekab city in the West–Azarbaijan province in the West-North of Iran. 172 sheep are ewe and 26 are male. The objective was describing the prevalence and distribution of serum antibodies to Bluetongue virus (BTV) in a sample. Competitive ELISA was applied to detect antibodies. 35.9% were positive and 64.1% were negative. 26.9% of males and 37.2% females were positive. The difference prevalence of antibodies in serum between male and females was significant (p<0.05). The difference of percent positive (PP) mean was significant between positive and negative dams in the male and females (p= 0.000 and p= 0.007, respectively). 38 ewes (47.5%) with history of abortion were seropositive but 26 ewes (28.6%) without history of abortion were seropositive. The relationship between prevalence antibodies in serum and ageing was significant (p = 0.010). From this study it is concluded that the bluetongue antibodies presence in the sheep sera from Tekab area can create a disease.

Keywords: Seroprevalence, Bluetongue Virus, Sheep, Competitive ELISA, Tekab

INTRODUCTION
Bluetongue is a seasonal disease generally observed in the late summer and early falls in the Iran. Virus transmission begins in the early spring with the onset of insect flight activity and continues until the first hard frosts. Bluetongue viruses are spread from animal to animal by biting gnats. Animals cannot directly contract the disease from other animals. There have been reports of BTV infection in the Iran and other countries such as Austria, India, Turkey, Pakistan and others (Radostitis et al., 2007).

Bluetongue virus (BTV) is a vector-borne disease of ruminants disseminated in the tropic and sub-tropic zone of the world. Bluetongue (BT) is an insect–transmitted, viral disease of sheep, cattle, goats, and other ruminants, such as white–tailed deer and pronghorn. Bluetongue is an orbivirus which cross-reacts with many antigenically related viruses including Palyam virus and the viruses that cause epizootic hemorrhagic disease of deer and African Horse sickness. Bluetongue virus replicates in both arthropod and mammalian host cells. The virulence of BTV varies quite markedly; even strains with matching serotypes have variable virulence. It is particularly damaging in sheep; half the sheep in an infected flock may die (Darapel et al., 2007; Veronesi et al., 2005). In cattle and goats, however, bluetongue viruses cause very mild, self–limiting infections with only minor clinical consequences. Bluetongue is clinically manifested as two syndromes: 1) vascular insult of several organ systems and 2) a reproductive syndrome. Sheep are commonly seen with clinical disease, but other domestic ruminants such as cattle and goats only rarely show clinical signs. Differential diagnoses of Bluetongue in sheep include Orf (contagious ecthyma), foot and mouth disease, any vesicular disease, and sheep pox. A bluetongue virus infection causes inflammation, swelling, and hemorrhage of the mucous membranes of the mouth, nose, and tongue. Inflammation and soreness of the feet also are associated with bluetongue. In sheep, the tongue and mucous membranes of the mouth become swollen, hemorrhagic, and may look red or dirty blue in color, thus giving the disease its name bluetongue. The reproductive portion of the disease varies
greatly. Signs include abortions, stillbirths, and weak “dummy lamb” live births. BTV can be both abortigenic and teratogenic in cattle experimentally, but neither is commonly seen in field conditions (Housawi et al., 2004). Due to the complexity of the serotypes of BTV, current procedures for monitoring the prevalence of BT infection are generally based on the determination of the serotype specific antibodies in animal serum samples. Although highly serotype specific, these procedures are cumbersome, because they require determination of the capacity of test sera to inhibit the infectivity of panels of known virus serotypes in time-consuming neutralization tests. Therefore it is imperative to use simplified tests for the purpose of sero-monitoring of BTV in a particular animal population in order to demonstrate that the population has been exposed to BTV infection. Until recently, tests such as agar gel immunodiffusion and indirect enzyme-linked immunosorbent assay (ELISA) were used to detect BTV serogroup-specific antibody. However, apart from being less sensitive, these tests have the major drawback of being unable to consistently distinguish between antibodies against BTV and the closely related epizootic haemorrhagic disease virus serogroups (Afshar et al., 1989). Recently, monoclonal-antibody-based competitive ELISA (cELISA) has been used as highly specific and sensitive test for detection of BTV group specific antibodies. Apart from AGID, cELISA and PCR is now recommended as an official test by OIE for serological monitoring of BTV antibodies in small ruminants like sheep and goats (Shaw et al., 2007). The objectives of this study were to describe the prevalence and distribution of serum antibodies to Bluetongue virus (BTV) in a sample of sheep flocks in Tekab area in West – Azarbajian province in the West-North of Iran which has a tropical climate.

MATERIALS AND METHODS

1) Sample Population
This study was achieved on 198 sheep blood samples from 20 sheep flocks in Tekab city in the West – Azarbajian province in the West-North of Iran. 172 sheep are ewe and 26 are male. The sampling was stratified random sampling. Blood samples were taken from jugular vein and serum separation was achieved by centrifuging in the laboratory of Veterinary Medicine Organization, East-Azarbajian province office.

2) Testing
Competitive ELISA was applied to detect antibodies against bluetongue virus in the Mabna Veterinary laboratory. For this study ID.VET kit was applied. This diagnostic kit is designed to detect antibodies secreted against the bluetongue virus vp7 protein. The samples to be tested and the controls are added to the microwells. The anti-vp7 antibodies, if present, from an antibody-antigen complex which makes the vp7 epitopes. An anti-vp7 peroxidase (po) conjugate is added to the microwells. It fixes to the remaining free vp7 epitopes, forming an antigen-conjugate-peroxidase complex. After washing in order to eliminate the excess conjugate, the substrate solution (TMB) is added. The resulting coloration depends on the quality of specific antibodies present in the sample to be tested: In the absence of antibodies, a blue solution appears which becomes yellow after addition of stop solution. In the presence of antibodies, no coloration appears. The micro plate is read spectrophotometrically at 450nm. The kit components: microplate coated with vp7 (8strips of 12 microwells), anti-vp7-conjugate (10x), positive control, negative control, dilution buffer 2, wash concentrate (20x), substrate solution, stop solution (H2SO4, 0.5 M).

3) Testing Procedure
Allow all the reagents to come to room temperature (21 ± 5 ºc) before use. Homogenize all reagents by inversion or vortex.
1. Add:
-50 µl of dilution buffer 2 to each well.
-50 µl of the positive control to wells A1 and B1.
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- 50 µl of the negative control to wells C1 and D1.
- 50 µl of each sample to be tested to the remaining wells.
2. Incubate 45± 4 minutes at 21 ± 5 ºc.
3. Prepare anti-vp7 conjugate 1x by diluting the anti-vp7-po conjugate (10x) to 1/10 in dilution buffer 2.
4. Add 100 µl of the anti-vp7-po conjugate to each well.
5. Incubate 30±3 minutes at 21±5 ºc.
6. Wash each well 3 times approximately 300 µl of the wash solution. Avoid drying of the wells between washing.
7. Add 100 µl of the substrate solution to each well.
8. Incubate 15±2 minutes at 21±5 ºc
9. Add 100 µl of the stop solution to each well in order to stop reaction
10. Read and record to O.D. at 450 nm.

The test is validated if:
- The mean value of the negative control O.D. (ODnc) is greater than 0.7 (ODnc>0.7)
- The mean value of the positive control O.D. (ODpc) is less than 30% of the ODnc (ODpc/ODnc<0.3)

For each sample, calculate the competition percentage

\[
\text{Competition \%} = \frac{\text{ODsample}}{\text{ODnc}} \times 100
\]

Samples presenting a competition percentage (PP):
- Greater than or equal 40% are considered negative
- Less than 40% are considered positive.

Statistical Analysis

Percent positivity (PP) was analyzed as percent. The difference of means of PP between cities and the relationship between cities and prevalence of the bluetongue were analyzed by using x² test. The difference of numbers of positive and negative between cities was analyzed by ANOVA. The difference of means of PP between two genders was analyzed by T test. A P value of 0.05 and 0.01 was considered significant.

RESULTS AND DISCUSSION

Results

35.9% were positive and 64.1% were negative. 26.9% of males and 37.2% females were positive. The difference prevalence of antibodies in serum between male and females was significant (p<0.05). The difference of percent positive (PP) mean was significant between positive and negative dams in the male and females (p= 0.000 and p= 0.007, respectively).

In affected Male Animals the average PP rate was significantly greater than of affected female (P = 0.034). Table 3 shows the Comparison of healthy and affected animals. In male healthy animals the mean PP was greater than its rate in affected animals (P = 0.042).

Table 1: Frequency of blue tang virus and comparison of positive PP percentage in understudied affected and healthy animals

<table>
<thead>
<tr>
<th>Total number</th>
<th>result</th>
<th>Number</th>
<th>Percent</th>
<th>PP mean</th>
<th>SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>198</td>
<td>Positive</td>
<td>71</td>
<td>35.9</td>
<td>50.99</td>
<td>1.13</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>127</td>
<td>64.1</td>
<td>28.23</td>
<td>0.56</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Frequency of blue tang infection and comparison of positive PP percentage in understudied affected and healthy ewes

<table>
<thead>
<tr>
<th>Number</th>
<th>Result</th>
<th>Number</th>
<th>Percent</th>
<th>PP mean</th>
<th>SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>172</td>
<td>Positive</td>
<td>64</td>
<td>37.2</td>
<td>50.56</td>
<td>1.12</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>108</td>
<td>62.8</td>
<td>27.79</td>
<td>0.62</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Frequency of blue tang infection and comparison of positive PP percentage in understudied affected and healthy rams

<table>
<thead>
<tr>
<th>Number</th>
<th>Result</th>
<th>Number</th>
<th>Percent</th>
<th>PP mean</th>
<th>SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>Positive</td>
<td>7</td>
<td>26.9</td>
<td>54.86</td>
<td>4.82</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>19</td>
<td>73.1</td>
<td>30.74</td>
<td>1.10</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Frequency of blue tang infection and comparison of positive PP percentage in affected cases by sex

<table>
<thead>
<tr>
<th>Number of positive cases</th>
<th>Sex</th>
<th>Number</th>
<th>PP mean</th>
<th>SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>71</td>
<td>Female</td>
<td>64</td>
<td>50.56</td>
<td>1.12</td>
<td>0.034</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>7</td>
<td>54.86</td>
<td>4.82</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Frequency of blue tang infection and comparison of positive PP percentage in healthy cases by sex

<table>
<thead>
<tr>
<th>Number of negative cases</th>
<th>Sex</th>
<th>Number</th>
<th>PP mean</th>
<th>SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>127</td>
<td>Female</td>
<td>108</td>
<td>27.79</td>
<td>0.62</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>19</td>
<td>30.74</td>
<td>1.10</td>
<td></td>
</tr>
</tbody>
</table>

There was abortion in the history of 80 understudied cases in the herd of cattle and 92 ewes had no abortion. Animals with a history of abortion had a higher PP average (P = 0.010) compared with other ewes. The rate of positive cases was greater among ewes with Abortion compared with ewes with no abortion history; so, blue tang infection was positive in 38 (47.5%) and 26 (28.26%) animals with abortion and non-abortion history, respectively.

Table 6: Comparison of the mean percentage of positive understudied cases based on abortion or non-abortion history

<table>
<thead>
<tr>
<th>Total number of ewes</th>
<th>Abortion history</th>
<th>Number</th>
<th>PP mean</th>
<th>SD</th>
<th>Infection result</th>
<th>number</th>
<th>Percent</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>127</td>
<td>yes</td>
<td>80</td>
<td>38.90</td>
<td>1.64</td>
<td>Positive</td>
<td>38</td>
<td>47.5</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>42</td>
<td>52.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>92</td>
<td>33.97</td>
<td>1.22</td>
<td>Positive</td>
<td>26</td>
<td>28.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>negative</td>
<td>66</td>
<td>71.74</td>
<td></td>
</tr>
</tbody>
</table>

In Table 7, the prevalence of blue tang infection and comparison of the mean positive percentage (PP) in understudied sheep of various age groups has been estimated. The difference between the percentage of positivity in different age groups was significant according to ANOVA test (P = 0.010). The blue tang infection rate was positive in 12, 16, 24 and 19 cases at ages under 2 years, 3, 4 and 5 years old, respectively.
rope, recent developments indicate

ty to BTV

- Ward and Thurmond

- Gloster

- Akhtar

ongue in

ging that C. imicola might have spread from Spain,

- e sheep sera

out of 380 tested sera (BTV group 2002

prevalence correlated with the probable distribution of the Culicoides vector (different agro

Competitive ELISA was applied to detect antibodies agai

in a Rambouillet sheep flock in Pakistan (2002

sheep in the Sudan was reported (1983

reported (1980

and cattle

2007

and Montenegro, and Bosnia and Herzegovina (Greece and Italy to some areas along the Croatian coast as well as to the coastal areas of Albania, Serbia

potential distribution of C. imicola in Europe, predicti

1.9% and 57.6% in sheep (1995

1.45

1.23±2.37

4

30

34.88±1.72

5 and older

50

Frequency of blue tang infection and comparison of the mean positivity percentage (PP) in

understudied sheep in different age groups

Table

Age group

number

PP mean

Infection result

Positive

negative

Positive

negative

Positive

negative

Positive

negative

53.33±2.45

28.30±1.46

53.00±3.13

29.16±0.77

50.58±2.07

26.27±1.57

48.32±1.09

26.65±1.17

Discussion

BT virus is present in much of the Americas, Africa, southern Asia and northern Australia. While the virus is occasionally present in some areas in the southern part of Europe, recent developments indicate that it may be extending its range northwards into areas of Europe that have never been affected before (Purse et al., 2005). The BTV is a vector born pathogen and hence meteorological and climatic conditions can affect the spread and establishment of this disease.

The results presented here record the first confirmation of BTV antibody in sheep from Tekab area in Iran. Occurrence of precipitating antibodies to bluetongue virus in sera of farm animals in Iran reported (Afshar and Kayvanfar, 1974). In a study in east Azerbaijain in Iran 23.56 % of samples were seropositive (Hasanpour et al., 2008). A similar situation has been reported in India, where the highest number of BT cases occurred in districts lying in close proximity to BTV affected areas of neighbouring states (Sreenivasulu et al., 1999). Reports in India have recorded BTV antibody prevalence levels of between 1.9% and 57.6% in sheep (Shringi and Shringi, 2005). Climatic factors play an important role in the occurrence of BTV infection in animals and also influence the size of vector populations and periods of their seasonal activity (Ward and Thurmond, 1995). An analysis of climatic data was used to model the potential distribution of C. imicola in Europe, predicting that C. imicola might have spread from Spain, Greece and Italy to some areas along the Croatian coast as well as to the coastal areas of Albania, Serbia

Montenegro, and Bosnia and Herzegovina (Gloster et al., 2002; Gubbins et al., 2002; Wilson et al., 2007; Wittmann et al., 2001). Culicoides from Western Turkey in relation to bluetongue disease of sheep and cattle was reported (Jennings et al., 1983). Oral susceptibility to bluetongue virus of Culicoides was reported (Carpenter et al., 2006). Serological studies of Australian and Papua New Guinean cattle and Australian sheep for the presence of antibodies against bluetongue group viruses have been achieved (Della-Porta et al., 1983; Flanagan et al., 1995; Flanagan et al. (1993). An outbreak of bluetongue in sheep in the Sudan was reported (Eisa et al., 1980). Prevalence of five serotypes of bluetongue virus was in a Rambouillet sheep flock in Pakistan (Akhtar et al., 1997; Akhtar et al., 1995). Competitive ELISA was applied to detect antibodies against bluetongue virus in sheep sera collected from different agro-climatic areas in Ethiopia. 46.67% were positive for bluetongue virus antibodies. The prevalence correlated with the probable distribution of the Culicoides vector (Woldemeskel et al., 200225). A competitive enzyme-linked immunosorbent assay was conducted to test the serum samples for BTV group-specific antibodies in Pakistan and BTV seropositive reactions were obtained in 184 (48.4%) out of 380 tested sera (Akhtar et al., 1997; Akhtar et al., 1995). Serologic data in Mexico were obtained by use of agar-gel immunodiffusion for identification of BTV group-reactive antibodies, with 35% seropositive (Stott et al., 1989). From this study it is concluded that the bluetongue antibodies presence in the sheep sera from Tekab area in West – Azerbaijain province in Iran and can to create a disease.
REFERENCES


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