Research Journal of Biological Sciences 3 (11): 1265-1270, 2008
ISSN: 1815-8846

Seroprevalence of Bluetongue Virus Infection in Sheep in East-Azarbajan Province in Iran

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Abstract: This study was conducted on 832 sheep blood samples from 90 sheep flocks in 17 cities of East-Azarbaijan province in the West-North of Iran. Total 644 sheep are ewe and 188 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. Total 76.44% were positive and 23.56% were negative. 78.26% of males and 70.21% females were positive. The difference prevalence of antibodies in serum between male and females was not significant. The highest prevalence of antibodies in serum was in Bonab, Charoimagh and Ahar (100%) and lower was in Tabriz (18.75%). The relationship between prevalence antibodies in serum and cities was significant (phi = 0.59) and ($p<0.05$).

Key words: Seroprevalence, bluetongue virus, sheep, competitive ELISA, Iran

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 (Darpel *et al*., 2007; Radostitis *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: vascular insult of several organ systems and 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

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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; Radostitis *et al*., 2007).

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 East-Azarbaijan province in the West- North of Iran which has a tropical climate.

MATERIALS AND METHODS

Sample population: This study was achieved on 832 sheep blood samples from 90 sheep flocks in 17 cities of East-Azarbaijan province in the West- North of Iran. Six hundred and forty four sheep are ewe and 188 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-Azarbajan province office.

Testing: Competitive ELISA was applied to detect antibodies against bluetongue virus in the Mabna Veterinary laboratory. For this study IDVET 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 450 nm.

The kit components: Microplate coated with vp7 (8 strips 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).

Testing procedure: Allow all the reagents to come to room temperature (21±5°C) before use. Homogenize all reagents by inversion or vortex.

- Add:
  - A 50 μL of dilution buffer 2 to each well.
  - A 50 μL of the positive control to wells A1 and B1.
  - A 50 μL of the negative control to wells C1 and D1.
  - A 50 μL of each sample to be tested to the remaining wells.
- Incubate 45±4 min at 21±5°C.
- Prepare anti-vp7 conjugate 1x by diluting the anti-vp7-po conjugate (10x) to 1/10 in dilution buffer 2.
- Add 100 μL of the anti-vp7-po conjugate to each well.
- Incubate 30±3 min at 21±5°C.
- Wash each well 3 times approximately 300 μL of the wash solution. Avoid drying of the wells between washing.
- Add 100 μL of the substrate solution to each well.
- Incubate 15±2 min at 21±5°C.
- Add 100 μL of the stop solution to each well in order to stop reaction.
- 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:

- Competition% = ODsample/ODnc×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 x^2 test. The difference of numbers of positive and negative between cities was analyzed by ANOVA. The difference of means of PP between 2 genders was analyzed by t-test. A p-value of 0.05 and 0.01 was considered significant.

RESULTS

Seroprevalence of bluetongue in different cities of East-Azarbaijan province was showed in Table 1. The highest PP was in Tabriz (93.61±7.48) and the lower PP was in Bonab city (8.91±0.34). The difference means of PP between cities was significant ($p<0.05$).

Samples presenting a competition percentage (PP) Greater than or equal 40% are considered negative and less than 40% are considered positive. In the Table 2 the numbers of positive and negative were showed. In this table the numbers of females and males were showed also. Six hundred and thiryt six samples (76.44%) were positive and 196 samples (23.56%) were negative (Fig. 1). Total 132 of males and 504 of females were positive (78.26 and 70.21%, respectively) (Fig. 2 and 3). The difference prevalence of disease between male and females was not significant. The highest prevalence of disease was in

Table 1: Seroprevalence of bluetongue in different cities of East-Azarbaijan province

City	N	Mean	SD	SE
Bonab	42	8.91	2.20	0.34
Bostan aba	56	12.38	18.48	2.47
Charomag	26	9.11	3.40	0.66
Shabestar	40	26.66	34.90	5.51
Ahar	52	11.96	6.45	0.89
Osco	28	17.58	19.41	3.66
Ajsab Shir	40	56.84	43.50	6.87
Tabriz	32	93.60	42.30	7.47
Kalaibar	80	17.47	17.51	1.95
Heris	40	65.06	36.71	5.80
Jolfa	54	62.08	46.10	6.27
Minae	72	30.42	35.21	4.14
Varzegan	56	50.96	32.88	4.39
Hashtrood	30	12.85	10.32	1.80
Maraghe	54	29.91	40.16	5.46
Azarshar	40	19.28	25.03	3.95
Marand	90	17.53	24.02	2.53
Total	832	30.65	36.48	1.26

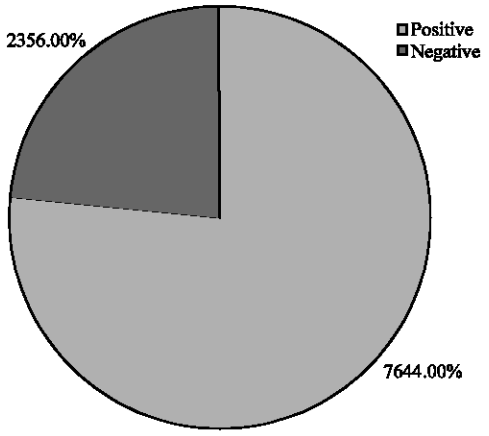

Fig. 1: Percent of bluetongue positives in all sheep of East-Azarbaijan province

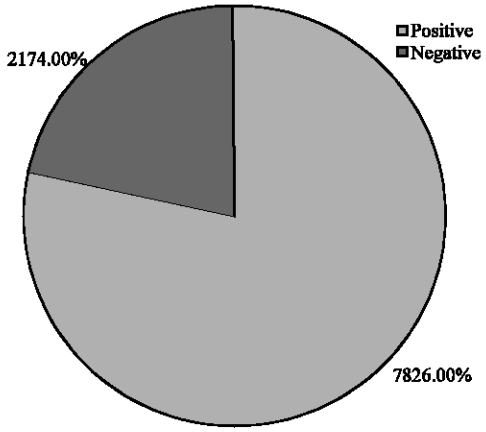

Fig. 2: Percent of bluetongue positives in male sheep of East-Azarbaijan province

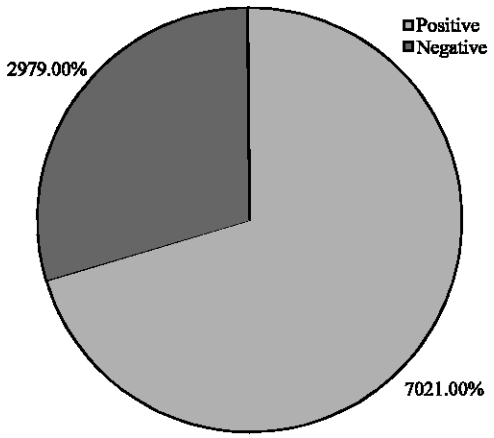

Fig. 3: Percent of bluetongue positives in female sheep of East-Azarbaijan province

Tabe 2: Frequency of bluetongue positives in different cities of East-Azarbaijan province

		Bluetonge (N)		Female (N)		Male (N)	
City	N	Negative	Positive	Negative	Positive	Negative	Positive
Bonab	42	0	42	0	32	0	10
Bostan aba	56	4	52	4	36	0	16
Charoimagh	26	0	26	0	14	0	12
Shabestar	40	8	32	2	26	6	6
Ahar	52	0	52	0	42	0	10
Osco	28	2	26	2	14	0	12
Ajsab Shir	40	22	18	22	10	0	8
Tabriz	32	26	6	16	6	10	0
Kalaibar	80	6	74	4	68	2	6
Heris	40	28	12	18	12	10	0
Jolfa	54	30	24	14	22	16	2
Minae	72	14	58	8	52	6	6
Varzegan	56	34	22	30	16	4	6
Hashtrood	30	2	28	2	22	0	6
Maraghe	54	10	44	8	36	2	8
Azarshar	40	4	36	4	28	0	8
Marand	90	6	84	6	68	0	16
Total	832	196	636	140	504	56	132

Bonab, Charoimagh and Ahar (100%) and lower was in Tabriz (18.75%). The relationship between prevalence the disease and cities was significant (phi = 0.59) and ($p<0.05$).

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 East-Azarbaijan province in Iran. The highest PP was in Tabriz (93.61±7.48) and the lower PP was in Bonab city (8.91±0.34). The difference means of PP between cities was significant ($p<0.05$). Samples presenting a competition percentage (PP) less than 40% are considered positive. The overall prevalence of the BTV antibodies in sheep in this state was found to be 76.44% and sero-positive animals were detected in 17 of the 17 districts sampled.

The highest proportion of seropositive sheep came from Bonab, Charoimagh and Ahar (100%) district and the second highest rate was from the Marand (93.33%) district. This may be attributed to the presence of many insects in these states. The relationship between prevalence the disease and cities was significant (phi = 0.59) and ($p<0.05$). Most of the districts East-Azarbaijan province that had a lower seroprevalence had letter insects. The prevalence of BTV antibodies in female and male sheep was 70.21 and 78.26%, respectively. The difference prevalence of disease between male and females was not significant. Occurrence of precipitating antibodies to bluetongue virus in sera of farm animals in Iran reported (Afshar and Kayvanfar, 1974). 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 *et al*., 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 and Montenegro and Bosnia and Herzegovina (Gloster *et al*., 2007; Gubbins *et al*., 2007; 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 (Carpente *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. Total 46.67% were positive for bluetongue virus antibodies. The prevalence correlated with the probable distribution of the Culicoides vector (Woldemeskel *et al*., 2002). 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 East-Azarbaijan province in Iran and can to create a disease.

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