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Detection of Bluetongue Virus Antigen from Livestock of Gujarat State

S.S. Patel*, N.M. Shah, H.C. Chauhan, B.S. Chandel, M.D. Shrimali, A.C. Patel, K.B. Patel, M.A. Patel, B.K Patel, M.G. Patel, J.K. Kala and Manish Rajgor

Department of Veterinary Microbiology, Veterinary College, S.D.A.U., Sardarkrushinagar, India.

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Bluetongue (BT) is an infectious, non-contagious disease of domestic and wild ruminants. Bluetongue virus (BTV) causes severe disease in sheep, which is transmitted by insect vector belonging to *Culicoides* spp. It is particularly a viral disease of sheep, occasionally affecting cattle, buffaloes, goats, camels and other wild ruminants. Out of 377 (364-blood, 5-spleen and 8-pooled *Culicoides*) samples 110 (29.18%) and 28 (7.42%) were found positive for BTV antigen by s-ELISA and BT-AGID respectively. Specieswise incidence by s-ELISA recorded was 48.20 per cent in sheep, 57.14 per cent in goats and 2.60 per cent in cattle however, none of the blood sample found positive from buffalo and camel. Specieswise incidence by BT-AGID recorded was 12.23 per cent in sheep and 15.71 per cent in goats however, none of the blood sample found positive for BTV antigen from cattle, buffalo and camel. Higher incidence seen in goats by both the test. s-ELISA proved to be the most sensitive in detecting BTV antigen than BT-AGID. Considering s-ELISA as the reference test, the relative sensitivity, specificity and overall agreement between both the tests were 25.45 per cent, 100 per cent and 78.24 per cent respectively.

Keywords: Bluetongue virus, antigen, s-ELISA, BT-AGID.

Bluetongue (BT) is an infectious, non-contagious, arthropod-borne viral disease, principally of sheep but many domestic and wild animals are also affected by this disease[3]. The severity of BT in sheep varies depending on the virus, the breed of sheep and environmental stress. Infection in cattle and goats is usually sub-clinical. Etiological agent of the disease belongs to the genus *Orbivirus* in the family *Reoviridae*. Twenty seven serotypes of BTV have so far been recognized worldwide and many more may be prevalent in regions where no survey has been made so far[10]. BTV infection occurs in most of the tropical, semitropical and temperate region of the world in parallel with the distribution of its vectors. The regions which are considered endemic areas of bluetongue virus are inhabited by 2/3 of sheep and cattle population around the globe between 40^0 North and 35^0 South[9]. The disease is characterized by high fever, excessive salivation, swollen lips and tongue, petechial hemorrhage, congestion and small ulcer in the mucous membrane of mouth and conjunctiva, coronitis and reproductive disorders leading to abortion or congenital deformities. The disease is a cause for serious concern to the livestock industry.

Studies on Bluetongue in Gujarat is concerned various workers have reported the existence of Bluetongue in Gujarat based on detection of group specific BTV antibodies, Serotypes specific antibodies, antigen detection by AGID, RT-PCR based detection and isolation of BTV[2,4,6]. However, no systemic studies based on surveillance on detection of BTV in wide host

* To whom all correspondence should be addressed.

ranges including sheep, goats, cattle, buffaloes and camels, using sandwich ELISA has been carried out.

MATERIAL AND METHODS

During the present investigation, s-ELISA and BT-AGID were used for the detection of BTV group specific antigen from the blood samples, Spleen samples and pooled *Culicoides.* A total of 364 blood, five spleen and eight pooled *Culicoides* samples were collected from different places of Gujarat for detection of BTV antigen.

Preparation of samples

The blood samples mixed in lysis buffer in 2:1 proportion were kept at 4^0C for overnight and used in the test. The tissue materials were ground in mortar and pestle in sterile 1X PBS (pH 7.4) to make 10 per cent (w/v) tissue suspension followed by sonication and centrifuged at 10,000X g for 30 minutes and the resulting supernatant was used. The *Culicoides* were ground in mortar and pestle in sterile 1X PBS (pH 7.4) to make suspension and sonication and used for the test.

s-ELISA

The test was performed as per the standard protocol developed at BTV Lab, IVRI, Mukteswar[8] for the detection of BTV antigen from blood, *Culicoides* and spleen samples.

1. Capture antibody (50 ml rabbit HIS to whole BTV-23 purified) was coated on to wells of ELISA plate at 1:2000 dilutions in carbonate bicarbonate buffer, pH 9.6 for 4^0C overnight.
2. The unbound capture antibodies were removed by three washing with washing buffer.
3. Un-occupied places in the wells were blocked by adding 100 ml of blocking buffer. The plated was incubated at 37^0C for 1h with continuous shaking.
4. Following incubation and washing, 50 ml positive antigen (1:10 dilution), negative antigen (1:10 dilution) and test samples (1:2 dilution) were added in respective wells and incubated at 37^0C for 1h with continuous shaking. No antigen was added in the blank control wells.
5. After washing (as described above), 50 ml of detection antibody diluted 1:400 in blocking buffer, was added in wells. Plate was incubated at 37^0C for 1h after which washing was done as mentioned above. No detection antibody was added in the conjugate control wells.
6. Fifty ml conjugate (rabbit anti-guinea pig immunoglobin conjugated to HRPO, Dakopad Corp., USA), diluted to 1:2000 in blocking buffer was added and the plate was incubated at 37^0C for 1h with continuous shaking.

Table 1. Specieswise detection of BTV antigen from blood samples by s-ELISA and BT-AGID

Species of animal	No. of blood sample Tested	Sample found positive by s-ELISA	Percentage (%)	Sample found positive by BT-AGID	Percentage (%)
Sheep	139	67	48.20	17	12.23
Goats	70	40	57.14	11	15.71
Cattle	77	02	2.60	00	00.00
Buffaloes	40	00	00.00	00	00.00
Camels	38	00	00.00	00	00.00
Total	364	109	29.95	28	7.69

Table 2. Comparative evaluation of s-ELISA and BT-AGID for detection of BTV antigen

Test		BT-AGID	
		Positive	Negative
s-ELISA	Positive	28	82
	Negative	00	267
Sensitivity (%)		25.45	
Specificity (%)		100.00	
Overall agreement (%)		78.24	

$$\text{Sensitivity (\%)} = \frac{28}{110} \times 100 = 25.45, \quad \text{Specificity (\%)} = \frac{267}{267} \times 100 = 100.00$$

$$\text{Overall agreement (\%)} = \frac{295}{377} \times 100 = 78.24$$

7. After washing, 50 ml of freshly prepared substrate/chromogen mixture was added to wells and plate was kept at 37^{0}C for 10-15 min for color development.

8. The color reaction was stopped by adding 50 ml of 1M H_2SO_4 in all wells and optical density (OD) was measured at a wavelength of 492 nm on an ELISA reader.

9. Test samples showing double or more than double OD492 of the mean negative control value are considered as positive.

BT-AGID:

The BTV antibody test kits used for the present study were made available by courtesy of Dr. M. M. Jochim, President, Veterinary Diagnostic Technology Incorporation, USA. All the sonicated blood cells, spleen and *Culicoides* suspensions were tested for the presence of BTV antigen by AGID Test. 0.9% Agarose was prepared and add six ml of molten gel was poured in each petridish (60×15mm) and allowed to solidify on a horizontal plane. A pattern consisting of a center well surrounded by six well was made using an immunodiffusion template. Each well had a diameter of four mm and the center to center distance between wells was 6.4 mm. Each well was sealed with one drop of molten gel. The central well was charged with sonicated sample as an antigen and all the peripheral wells were charged with different dilution of BTV antiserum (1:1, 1:2, 1:4, 1:8, 1:16 and 1:32). The charged petridishes were incubated at room temperature under humid condition for 72 hours before pronouncing the sample as negative or positive. The Positive sample petridishes were washed in normal saline for 24 hours followed by distilled water for 24 hours. The gel was pressed and dried under five layer thick whatman No.1 filter paper cover at room temperature for 3 days. The petridishes were stained with coomassie brilliant blue for 10 minutes, destaining with destaining solution for 10 minutes, air dried and examined for precipitation lines.

Wells A1, A2, B1, B2: Positive control, Wells C1, C2, D1, D2: Antigen blank; Wells E1, E2, F1, F2: Negative control, Wells G1, G2, H1, H2: Conjugate control; Rest of the Wells from A3-H12: test samples in vertical duplicate.; Wells A3, B3; A4, B4 etc.:- BTV antigen positive samples; Wells E8, F8; E12, F12 etc.:- BTV antigen negative samples

Fig. 1. Microtitre ELISA Plate showing results of s-ELISA

RESULTS

Overall incidence

A total of 377 samples (364-blood, 5-spleen and 8-pooled *Culicoides*) screened for detection of BTV antigen by s-ELISA and BT-AGID. Of these, 110 (29.18%) and 28 (7.42%) samples were found positive respectively (Fig 1, 2)**.** Among the 364

Placement of samples

Central well (S): Blood sample
Peripheral wells (1 to 6): Known antiserum

Well No.	Dilution of antiserum
1:	1:1
2:	1:2
3:	1:4
4:	1:8
5:	1:16
6:	1:32

Fig. 2. Agar gel immunodiffusion pattern of the blood sample positive for BTV antigen showing the precipitating line against log2 dilution of BTV reference antiserum

blood samples screened for BTV antigen by s-ELISA and BT-AGID. Of these, 109 (29.94%) and 28 (7.69%) samples were found positive respectively and out of five spleen samples, one calf spleen sample (20%) was found positive by s-ELISA and none of the spleen sample found positive by BT-AGID. where as none of the samples found positive from eight pooled *Culicoides* samples by both the test. Present finding was in agreement with earlier studies which detected 5 to 9 % BTV antigen by BT-AGID[1, 5]. However, In contrast to the present findings, reported 92.86 per cent and 100.00 per cent BTV antigen by BTID[12,13].

Specieswise incidence of BTV

Of 364 blood samples were screened and Specieswise incidence by s-ELISA recorded was 67 (48.20%) in sheep, 40 (57.14%) in goats and 2 (2.60%) in cattle however, none of the blood sample found positive from buffalo and camel. Specieswise incidence by BT-AGID recorded was 17 (12.23%) in sheep and 11 (15.71%) in goats however, none of the blood sample found positive from cattle, buffalo and camel (Table-1). Similarly, none of the blood sample positive from camel[7] and cattle[5] and 11.11 per cent incidence reported in sheep[2]. While in contrary to the present findings 9.09 per cent and Zero per cent incidence reported in sheep and goats respectively[5] and higher incidence rate 9.21% reported in buffaloes[2].

Comparison of s-ELISA and BT-AGID for detection of BTV antigen

Performance of the s-ELISA and BT-AGID for the detection of BTV antigen was compared. Cross tabulation of s-ELISA and BT-AGID considering s-ELISA as reference test was recorded as per method described by[7] to determine relative sensitivity and specificity of BT-AGID. Considering s-ELISA as the reference test, the relative sensitivity, specificity and overall agreement between both the tests were 25.45 per cent, 100 per cent and 78.24 per cent respectively (Table-2).

CONCLUSIONS

1. Specieswise incidence of BTV antigen indicated that the goats were more susceptible to BTV infection than sheep, cattle, buffaloes and camels.
2. None of the buffaloes and camels blood and pooled *Culicoides* yielded positive results in s-ELISA and BT-AGID.
3. s-ELISA is more sensitive then BT-AGID for detection of BTV antigen.

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