Indian Journal of Animal Sciences **80** (11): 1059–61, November 2010

Serological detection of bluetongue: comparison of competitive ELISA with other routinely used immunoassays

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Received: 23 August 2009; Accepted: 23 July 2010

ABSTRACT

The performance of 2 conventional serological assays, viz. the standard agar gel immunodiffusion (AGID) and counter current immunoelectrophoresis (CCIE) was compared with commercially available competitive enzyme-linked immunosorbent assay (cELISA) for the detection of group specific bluetongue virus antibodies in sheep and goats. The comparative efficacy was evaluated on 705 sera samples from sheep and goats. Differences were observed in the relative sensitivities and specificities of the tests and seroprevalence using AGID, CCIE and cELISA. The highest seropositivity of 62.69% was obtained with cELISA followed by AGID (29.92%) and CCIE (22.14%). The relative sensitivity and specificity of AGID was 38.23 and 84.83% whereas CCIE was 28.95 and 88.59% using cELISA as the reference test. Among all the assays cELISA was highly sensitive followed by AGID and CCIE. Based on the results, it is proposed that cELISA should be preferred over other assays for the detection of antibodies against bluetongue virus.

Key words: AGID, Bluetongue virus, cELISA, CCIEP, Sensitivity, Serodetection, Specificity

Bluetongue (BT), a non contagious viral disease, is considered as one of the important diseases of ruminants throughout the world. Bluetongue virus (BTV) belongs to the family *Reoviridae* and genus *Orbivirus* (Mertens *et al*. 2005). Till date, 24 serotypes of BTV have been recognized worldwide (OIE 2009). Because of the tremendous impact of BT disease on economy it has been classified as notifiable disease by *Office International des Epizooties* (OIE 2009). Identification of the virus or antibodies is an essential part of the diagnostic laboratories for confirmation of BT infection (Afshar 1994). A wide variety of tests have been developed for the diagnosis of BT infection (Gupta *et al*. 1990, Wade-Evans *et al*. 1990 and OIE 2009). Though, in the past significant progress has been made in molecular techniques, the serological assays are still the mainstay for laboratory confirmation, monitoring and diagnosis of BT infection (Afshar 1994). For seromonitoring mainly serogroup specific assays are in use. The present study focuses on the comparative evaluation of 2 conventional serological assays, viz. the standard agar gel immunodiffusion (AGID) and counter current immunoelectrophoresis (CCIE) with competitive enzyme-linked immunosorbent assay (cELISA).

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MATERIALS AND METHODS

Serum samples: Blood samples (705) were collected aseptically from sheep (248) and goats (457). Blood (5 ml) was collected randomly from each animal and processed for separation of serum. Serum was harvested from clotted blood within 1 h by centrifugation and immediately stored at –20°C prior to analysis.

Detection of BTV antibodies

Agar gel immunodiffusion (AGID): The AGID kit (VRC, Centre for Animal Health Studies, Chennai) was used and test was performed as per the instructions. The antigen and serum samples loaded gel slides were kept in humid chamber at 37ºC in incubator for 48 h. Afterwards slides were observed for appearance of precipitation line. After washing the gel with normal saline to remove un-precipitated protein, slide was dried by keeping Whatman-3 filter paper and stained with coommassie brilliant blue R250 stain (0.075%). After de-staining slides were kept for recording the results.

Counter current immunoelectrophoresis (CCIE): The test was carried out following the protocol of Gupta (1988). Briefly, 5 ml agarose prepared in Tris-barbitone buffer (TBB) was over laid on a clean glass slide and allowed to solidify. Two parallel rows of the wells were cut using 'gel punch'

with a distance of 5 mm. Then gel plate was placed carefully in electrophoresis unit and its connection was made with buffer tank through filter wicks (Whatman-3 G) and was pre run for 15 min before loading the samples. The wells were filled with BTV antigen and known positive antibody/test serum samples and electrophorosed at 15 mA for 1 h. After run, gel was kept for 45 min in refrigerator and washed with normal saline for 30 min for development of precipitation line. Gel was dried and stained with 0.075% coommassie brilliant blue R–250. After de-staining, slide was kept for recording results.

Competitive-enzyme-linked immunosorbent assay: The cELISA kit was used in the present study. The results of the test sera were determined by expressing them as inhibition percentage (IP) values which were calculated as given below. Inhibition percentage value >60% was counted as negative, 50–60% considered to be suspicious, while below 50% was positive.

$$\text{Inhibition \% (IP)} = \frac{\text{Mean absorbance of test serum sample}}{\text{Mean absorbance of negative control serum}}$$

RESULTS AND DISCUSSION

Serodetection of bluetongue infection: Serum samples from sheep and goats subjected to cELISA, AGID and CCIE for the detection of BTV antibodies revealed seropositivity of 62.69, 29.92 and 22.14% (Table 1). In sheep, 64.91, 28.62, 20.96% were positive in cELISA, AGID and CCIE, respectively, whereas in goats, 61.48, 30.63 and 23.19% were detected positive for BTV antibodies (Table 1).

Comparative efficacy of serological tests: The relative performance of AGID and CCIE to cELISA was evaluated on 705 serum samples (Tables 2, 3). The standard AGID assay detected 38.23% of the cELISA positive samples, whereas CCIE detected 28.95% of the cELISA positive samples. In addition 42 and 30 samples were detected positive in AGID and CCIE tests which were negative by cELISA. The relative sensitivity and specificity of AGID was 38.23% and 84.03% and CCIE was 28.95% and 88.59%, using cELISA as reference test (Table 2).

The relative performance of AGID and CCIE was also compared taking one test as reference. The CCIE detected 63.03% of the AGID positive samples and also detected 25 samples, which were negative in AGID. Relative sensitivity and specificity of CCIE using AGID as reference test was 63.03% and 94.93% (Table 3). While the AGID detected 78.48% of the CCIE positive samples and also detected 87 samples, which were negative by CCIE. Relative sensitivity and specificity of AGID using CCIE as reference test was 78.48 and 84.09% (Table 3).

Bluetongue, since its first report in Maharashtra by Sapre (1964), has spread over the years and presently being recorded in number of states of India. Since then, higher isolations, endemicity of BT has been reported in Maharashtra (Harbola *et al.* 1982), Haryana (Prasad *et al*. 1987), Tamil Nadu, Andhra Pradesh (Sreenivasulu *et al*. 1999), Kerala (Ravishankar *et al*. 2005), Karnataka (Doddamani and Haribabu 2006), and Rajasthan (Shringi and Shringi 2005). In field generally for detection of bluetongue, a simple, economic, less cumbersome and less time consuming test is preferred. Our results showed that sensitivity of AGID and CCIE tests was less than cELISA, as out of 442 cELISA positive sera, only 38.23% were detected positive by AGID and 28.95% by CCIE. Similar results were reported by Shringi and Shringi (2005), in which they recorded

Table 1. Comparison of 3 serological assays for detecting BTV antibodies

Species	Samples tested	Positive by		
		cELISA (%)	AGID (%)	CCIE (%)
Sheep	248	161 (64.91)	71 (28.62)	52 (20.96)
Goat	457	281 (61.48)	140 (30.63)	106 (23.19)
Total	705	442 (62.69)	211 (29.92)	158 (22.14)

Table 2. Relative performance of AGID and CCIE (cELISA reference test)

Test	cELISA (reference test)			
AGID	Results	Positive	Negative	Total results
	Positive	169	42	211
	Negative	273	221	494
	Total	442	263	705
Relative sensitivity was 38.23% and relative specificity 84.03%				
CCIE	Positive	128	30	158
	Negative	314	233	547
	Total	442	263	705
Relative sensitivity was 28.95% and relative specificity 88.59%.				

Table 3. Relative performance of CCIE and AGID taking one test as reference

Test	AGID (reference test)			
	Results	Positive	Negative	Total results
CCIE	Positive	133	25	158
	Negative	78	469	547
	Total results	211	494	705
Relative sensitivity was 63.03% and relative specificity 94.93%.				
	CCIE (reference test)			
AGID	Positive	124	87	211
	Negative	34	460	494
	Total results	158	547	705
Relative sensitivity was 78.48 and relative specificity 84.09%.				

prevalence of 30.03, 12.3, and 9.5% by cELISA, CCIE, AGID, respectively. Though higher positivity was detected in present study than above study, which could be due to more number of samples tested in the present study or higher endemicity of BT infection in Madhya Pradesh than Rajasthan. The CCIE test was reported comparatively more sensitive and specific than AGID (Shringi and Shringi 2005) however, the results of present study are in contrary. During the study number of samples detected negative were positive in AGID and CCIE, which could be due to cross reactivity. There is still need to work on this aspect with more number of samples.

Comparison of relative performance of 3 immunological assays revealed that the cELISA was more sensitive than AGID and CCIE, as it detected 62.69% positive cases, whereas AGID and CCIE detected 29.92 and 22.41%, respectively. The relative sensitivity and specificity of AGID was 38.23 and 84.03%, respectively, whereas CCIE had shown 28.95% sensitivity and 88.59% specificity taking cELISA as a reference test. Similarly, Shringi and Shringi (2005) reported the highest number of samples seropositive with cELISA (30.3%) followed by CCIE (12.3%) and AGID (9.5%) and relative sensitivity and specificity of cELISA was 100% and 77% respectively when AGID used as reference test whereas sensitivity and specificity of cELISA was 100% and 79%, respectively, when CCIE was used as a reference test. The results of present study are slightly in variance to the findings of Shringi and Shringi (2005) for sensitivity of CCIE, as in present study AGID showed improved sensitivity over CCIE for both sheep and goats sera samples. However, the relative specificity of CCIE (94.93%) was better than AGID (84.09%).

Our results are consistent with previous reports that AGID and CCIE tests are neither sufficiently sensitive nor specific to justify their use as the tests for serological detection of BT infection of domestic ruminants. Though, limited numbers of serum samples were evaluated in the present study, based on findings we propose that cELISA is more useful out of the 3 tests evaluated and recommend its use in seromonitoring programmes for BTV infection in our country.

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