J. Vet. Sci. (2005), **6**(1), 77–79

JOURNAL OF
Veterinary
Science

Short Communication

Comparative efficacy of standard AGID, CCIE and competitive ELISA for detecting bluetongue virus antibodies in indigenous breeds of sheep and goats in Rajasthan, India

Smriti Shringi*, B. N. Shringi

Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Rajasthan Agricultural University, Bikaner-334 001, Rajasthan, India

The sero-prevalence of antibodies against blue tongue virus (BTV) in 408 local breeds of sheep in Rajasthan state in India was investigated using standard agar gel immunodiffusion (AGID) test. Maximum seropositivities of 11.3% (13/115), 10.7% (13/121), 7.1% (11/155) and 5.9% (1/17) were recorded in the Chokla, Magra, Nali and Pugal breeds, respectively. Out of 107 goat serum samples, 6 (5.6%) were AGID positive. The performance of the standard AGID, counter current immuno-electrophoresis (CCIE) and the competitive enzyme-linked immunosorbent assay (cELISA) for the detection of serum antibody against BTV in indigenous breeds of sheep were compared. Out of 178 sheep serum samples tested, 17 (9.5%), 22 (12.3%) and 54 (30.3%) were positive for group-specific bluetongue antibodies by AGID, CCIE and cELISA, respectively. There was appreciable difference in the seroprevalence detected by AGID, CCIE and cELISA in clinically healthy and diseased sheep with regard to relative sensitivities and specificities of the tests with cELISA being highly sensitive and specific followed by CCIE and AGID test. It was concluded that these indigenous breeds of sheep may be a potential reservoir of BTV infection and cELISA should be routinely used for the detection of antibodies against BTV in these local breeds of sheep.

Key words: Blue tongue, seroprevalence, AGID, CCIE, competitive ELISA

Bluetongue infection caused by blue tongue virus (BTV) belonging to family *reoviridae* (genus *Orbivirus*) is considered as one of the most important diseases of domestic livestock. BT exists around the world in a broad band covering much of the Americas, Africa, southern Asia, northern Australia and, occasionally, the southern fringe of Europe [7]. Twenty four serotypes of BTV have so far been recognized worldwide and 18 have been reported from various states in India [9]. This genetic diversity of bluetongue virus is a consequence of both drift and re-assortment of individual gene segments [8]. BT infection has been categorized under list A disease by *Office International des Epizooties* (OIE) and poses one of the major impediment in trade and free international movement of livestock, their products and germplasm. This warrants continuous monitoring of the disease in the regions where the disease is endemic.

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 [1]. 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 is now recommended as an official test by OIE for serological monitoring of BTV antibodies in small ruminants like sheep and goats [8].

In India, sheep population totals 51 million, accounting to 5 per cent of world's sheep population and 123 million goats

*Corresponding author
Tel: +91-744-2421331; Fax: +82-63-278-3884
E-mail: smriti_vet@yahoo.com

Fig. 1. Map of India showing location of Rajasthan State from where the serum samples were collected.

accounting for 20 per cent of the total global livestock (3). The Rajasthan State alone accounts to 25% and 13% of the total Indian population of sheep and goats, respectively. Most of the sheep are of indigenous types and there is a general understanding that the indigenous breeds of sheep are less susceptible to the BTV infection. Therefore, the purpose of this study was the serological surveillance of BTV in sheep and goats and in particular the indigenous breeds of sheep that are generally considered as resistant to BTV infection, in the Rajasthan State of India. Since, the serological testing of small ruminants for anti-BTV antibodies is still mainly based on AGID test, we also investigated the comparative efficacy of the presently available standard AGID test with that of counter current immunoelectrophoresis (CCIE) and recently introduced cELISA for the efficient detection of serogroup specific anti-BTV antibodies among local breeds of small ruminants in India. Our results suggest that cELISA has a better sensitivity and specificity over AGID and should be used for the routine monitoring of anti-BTV antibodies in local breeds of sheep and goats in India.

During the year 2003, we collected a total of 408 sheep (mainly from local breeds) and 107 goat blood serum samples from different district headquarters of Rajasthan state in India (Fig. 1). Serum sample from each animal was then stored at –20°C until further use. The details of local breeds of sheep screened during this study are given in Table 1. All the serum samples were subjected to initial screening for the presence of BTV antibodies using standard AGID

Table 1. Details of local breeds of sheep screened for presence of antibodies against BTV using standard AGID test

Breed	Number tested	Number Positive	Percent Positive
Magra	121	13	10.7
Nali	155	11	7.1
Pugal	017	01	5.9
Chokla	115	13	11.3

test (VRC, Center for Animal Health Studies, Chennai, India) as per the procedure described earlier [5]. Interestingly, out of 408 (sheep sera) and 107 (goats sera) serum samples tested, 38 (9.31%) and 6 (5.61%), respectively were found sero-positive for BTV antibodies. The percent sero-positivity among different breeds of sheep varied from 11.3% (Chokla), 10.7% (Magra), 7.1% (Nali) to 5.9% (Pugal) (Table 1). The results clearly showed appreciably higher sero-positivity among indigenous breeds indicating that the local breeds of sheep may serve as a potential reservoir of BTV infection and may also transmit the infection to cattle and other breeds of sheep reared in the same area. Comparative analysis of percent sero-positivity among male and female sheep was 9.76% (8/82) and 9.2% (30/326), respectively, and 6.06% (2/33) and 5.41% (4/74), respectively in goats, indicating that prevalence of BTV was not affected markedly by the sex of these indigenous animals (data not shown). Interestingly, when we compared two age groups, less than 2 years and above 2 years of age for susceptibility to BTV infection, the percent sero-positivity was 5.97% (<2 years) and 9.97% (>2 years) (data not shown). The higher sero-positivity among the indigenous sheep above 2 years of age indicates that BTV infection was common among adult animals. These adult animals may be a potential source for spread of BTV in this region. Although, the samples were collected from the similar agro-climatic zones, the variation in the sero-positivity among different breeds of sheep (Table 1) is likely to be due to the genetic differences as well as the age of the animal at the time of collection of serum samples.

Subsequently, we determined the comparative efficacy of different serological tests for detection of antibodies against BTV. In all, 178 out of 408 serum samples mainly from local breeds of sheep were randomly selected and subjected to screening by standard AGID, CCIE and c-ELISA. The CCIE test was performed as described previously [4] while, the cELISA test was performed as per the procedure described by OIE [8]. The results of comparative study of AGID, CCIE and cELISA are shown in Table 2. Interestingly, the highest numbers of samples were found sero-positive with cELISA (54/178-30.3%) followed by CCIE (22/178-12.3%) and AGID (17/178-9.5%) indicating that cELISA was most sensitive test among all the three tests studied. We compared the relative performance of cELISA

Table 2. Comparative efficacy of serological tests used for detection of antibodies against BTV in indigenous breeds of sheep

Test	Number tested	Number positive	Percent positive
AGID	178	17	9.5%
CCIE	178	22	12.3%
CELISA	178	54	30.3%

Table 3. Relative performance of cELISA to standard AGID test

	Method	AGID (reference test)		
	Results	Positive	Negative	Total results
cELISA*	Positive	17	37	54
	Negative	0	124	124
	Total results	17	161	178

*Relative sensitivity: 100%; Relative specificity: 77%

Table 4. Relative performance of cELISA to CCIE

Method	CCIE (reference test)			
	Results	Positive	Negative	Total results
cELISA*	Positive	22	32	54
	Negative	0	124	124
	Total results	22	156	178

*Relative sensitivity: 100%; Relative specificity:>79%

to that of AGID (Table 3) and CCIE (Table 4). The cELISA detected 100% of the AGID and CCIE positive samples. In addition to this, cELISA could disclose additional 37 and 32 samples that were found negative by AGID and CCIE test, respectively, indicating that cELISA was more reliable test than AGID or CCIE. The relative sensitivity and specificity of cELISA was 100% and 77%, respectively when compared to AGID as a reference test. The relative sensitivity and specificity of cELISA was 100% and >79% when cELISA was compared to CCIE as a reference test. These results indicate that standard AGID may generate false negative results thereby increasing the chances that the animals with previous exposure to BTV infection may be detected as negative reactors. On the contrary, the cELISA has an advantage of being 100% sensitive as well as specific because it measures BTV-specific antibody without detecting cross-reacting antibody to other orbiviruses [1,2,6]. The specificity of cELISA is the result of using one of a number of BT serogroup-reactive monoclonal antibodies (Mabs), such as MAb 3-17-A3 [2] or MAb 20E9 [6]. The antibodies were derived in a number of laboratories, and although different, all appear to bind to the amino-terminal region of the major core protein VP7 [8]. Therefore, from the results obtained in this study, it appears to us that cELISA is highly specific and sensitive test and should be used for routine monitoring status of BTV infection in local breeds of small ruminants in India.

In conclusion, the present study revealed that there was widespread exposure to BTV infection among the indigenous breeds of small ruminants encompassing a large area of Rajasthan state in India. These animals can serve as a potential source of infection to other domestic animals in the region. The c-ELISA was more sensitive and reliable test and should be used for routine monitoring for presence of BTV antibodies in order to keep track on the status of BTV infection among the small ruminants in this part of the world.

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