Indian Journal of Animal Sciences 67 (12) : 1033-1035, December 1997

Comparison of competitive ELISA, indirect ELISA and agar-gel precipitation test for the detection of bluetongue virus antibodies in sheep

S C DAS[1], S MISHRA [2], N MISHRA[3] and P S K BHAGWAN[4]

Central Sheep and Wool Research Institute, Avikanagar, Rajasthan 304 501

Received : 22 February 1997

ABSTRACT

The present study was undertaken to compare 3 serological tests, viz. competitive enzyme linked immunosorbent assay (C-ELISA), indirect ELISA (I-ELISA) and agar-gel precipitation test (AGPT) for detection of bluetongue virus (BTV) antibodies in sheep. Out of 388 serum samples tested; AGPT could detect only 91 (23%), whereas ELISA could detect 247 (63.6%) positive samples. Testing of 85 samples by I-ELISA and C-ELISA showed that both the tests were more or less equally sensitive in detecting BTV antibodies. On analysing the breed distribution of positive samples tested by ELISA it was found that the Russian Merino, Bharat Merino, Rambouillet, Mutton cross, Avikalin and indigenous breeds of sheep had 92.8. 76.3, 73.07, 60.7, 61.4 and 53.6% of BTV antibodies respectively. The prevalence of higher rate of BTV antibodies in exotic breeds of sheep than crossbred and indigenous breeds showed their higher susceptibility of BTV. It was also found that the hoggets (85.7%) were more susceptible than the adults (62.4%) and both males and females were almost equally susceptible having per cent prevalance of 62.5 and 65.09 respectively.

Key words : Antibodies, Bluetongue virus, ELISA, I-ELISA

In India studies on the seroprevalence of bluetongue virus (BTV) antibodies have been based mainly on AGPT and the exotic breeds of sheep were reported to be more susceptible than crossbred and indigenous breeds (Sodhi *et al.* 1981, Prasad *et al.* 1987, Dubey *et al.* 1988, Mehrotra and Shukla 1990). To compare the sensitivity and specificity of C-ELISA, I-ELISA and AGPT, Afshar *et al.* (1989) analysed BTV antibodies in cattle and sheep. Due to their high specificity, monoclonal antibodies (Mabs) have been used in different ELISA to ensure better result. Afshar *et al.* (1987, 1989, 1992) and House *et al.* (1990) reported Mab based C-ELISA in which the test serum and the Mabs reacted simultaneously to compete for BTV antigen.

In the present study an attempt has been made to compare C-ELISA, I-ELISA and AGPT for detection of BTV antibodies in different breeds of sheep.

MATERIALS AND METHODS

The standard group specific antigen used in ELISA and AGPT, antigen coated plates, BTV strong positive, weak positive and negative sera used in C-ELISA and the hyperimmune sera used in AGPT were supplied by Veterinary Diagnostic Technology, Inc., USA.

Present address : [1,2,3]Scientist, Veterinary Bacteriology and Virology; [4] Principal Scientist and Head.

A total of 388 serum samples were collected during 1988-1996 from Sheep Breeding Farm (SBF), Fatehpur; SBF, Chittorgarh (Rajasthan), Operational Research Project (ORP) areas surrounding Avikanagar (Rajasthan) and from CSWRI, Avikanagar. All the samples were collected from animals suspected for bluetongue. Animals in none of the farms were vaccinated with BTV vaccine. Blood samples were collected from jugular vein for serum samples. Serum samples were dispensed in small aliquots and kept at –70°C until further use.

Ager gel precipitation test (AGPT)

All the test serum samples were tested by the method of Jochim and Chow (1969).

Indirect ELISA

The optimum dilutions of antigen, sera and conjugate were ascertained by chequer board titration. The wells of the plates were coated with 50 µl of BTV antigen diluted to 1:500 in coating buffers and incubated overnight at 4°C. The wells were washed thrice in washing buffer (PBS with 0.05% Tween -20) and blocked with 1% BSA solution. Test sera diluted to 1 : 200 in PBST containing 1% BSA (50 µl) in duplicate were added to the wells and incubated for 2 hr at 37°C. The wells were then washed as before and 50 µl of antisheep-HRPO conjugate diluted to 1 : 8000 was added. After 1 hr of incubation, the plates were washed and the substrate (orthophenylenediamine) solution was added in 100 µl

quantities and incubated in dark for 30 min at 37°C. The optical density (OD) was recorded at 450 nm. The cut-off point was calculated using the tolerance factors as described by Hubschle *et al.* (1981) and the test sera showing OD values more than that were considered as positive.

Competitive ELISA

The test was performed as described by Afshar *et al.* (1987) with some modifications by using a C-ELISA kit. Antigen coated wells were washed once with washing buffer and the serum samples in 50 µl quantities were added in duplicate at a dilution of 1:5 followed by the addition of same quantity of BTV group specific Mab. The plates were shaken well for mixing and incubated for 2 hr at room temperature. The wells were washed thrice and 100 µl of antimouse-HRPO conjugate (Sigma) diluted to 1 : 300 was added and incubated for 1 hr. After washing the wells 5 times, the substrate (100 µl) was added to each well and incubated for 20 min. The reaction was stopped by the addition of stopping buffer and the OD was recorded at 492 nm. The different controls included diluent only, weak positive, strong positive and negative sera samples. The percentage of inhibition (PI) was calculated as per the method supported with the Kit.

$$PI = 100 - \frac{\text{Adjusted average OD of test sample}}{\text{Adjusted average OD of negative sample}} \times 100$$

Where adjusted average OD = Average OD of the serum - Average OD of the diluent.

The sera samples having the PI values < 50% were classified as negative, 50-80% as weak positive and 80% and above were strongly positive.

RESULTS AND DISCUSSION

Out of 388 serum samples tested, 247 samples (63.6%) were positive in I-ELISA and 91 samples (23%) were positive in AGPT. The higher sensitivity of I-ELISA over AGPT in detecting presence of BTV antibodies was also reported by Gupta *et al.* (1990). Unlike our observations, Lunt *et al.* (1988) reported the total number of cases detected by either AGPT or ELISA were more or less similar. However, they could attribute the AGPT - negative and ELISA - positive cases to the increased sensitivity of ELISA in detecting lower levels of antibody. For the comparison of I-ELISA and C-ELISA and for the confirmation of doubtful cases, a total of 85 samples were tested of which 47 samples were found to be positive in I-ELISA and 53 were positive in C-ELISA. This indicated more or less equal sensitivity of both the tests.

The prevalence of BTV antibodies in different breeds of sheep was analysed (Table 1). There was higher rate of seroprevalence exotic breeds (80.4%) than crossbreds (63.8%) and the indigenous breeds (53.6%). Russian Merino (92.8%) had higher rate of seroprevalence followed by Bharat Merino (76.3%) and Rambouillet (73.07%). Mutton cross and Avikalin had prevalence of 60.7 and 61.4% of BTV antibodies respectively. The results shows that exotic breeds were more susceptible than crossbreds and the indigenous breeds were least susceptible. The same observations have been made by various other workers using AGPT (Sodhi *et al.* 1981, Bandyopadhyaya and Mullick 1983, Lonkar *et al.* 1983, Prasad *et al.* 1987 and Dubey *et al.* 1988).

Table 1. Distribution of BTV antibodies in different breeds of sheep by ELISA

Name of the breed	Total no. of samples	No. of positive samples	% positive
Indigenous	82	44	53.6
Crossbred	260	166	63.8
Exotic	46	37	80.4
Total	388	247	63.6

Table 2. Sex-and age-wise distribution of BTV antibodies in sheep by ELISA

Attributes	Total no. of samples	No. of positive samples	% positive
Sex			
Male	219	137	62.50
Female	169	110	65.09
Age			
Adult	367	229	62.40
Hogget	21	18	85.70

BTV antibodies were equally prevalent in male and female and more in hoggets than adults (Table 2). The higher rate of seroprevalence in hoggets was also reported by Prasad *et al.* (1987). They reported that there was a higher percentage of antibodies in aborted ewes and rams showed a very less incidence. Contrary to their observations present study indicated that both the males and females had similar incidence. Lower prevalence of BTV antibodies in rams as reported by Prasad *et al.* (1987) might be due to the less sensitivity of AGPT in detecting lower levels of antibody.

To conclude, comparison of AGPT, I-ELISA and C-ELISA tests in assessing prevalence of bluetongue virus antibodies showed I-ELISA to be more sensitive than AGPT and C-ELISA and I-ELISA to be more or less equally sensitive. The prevalence of BTV antibodies was higher in exotic breeds than crossbreeds and indigenous breeds.

ACKNOWLEDGEMENTS

We are highly grateful to the Director, CSWRI, for providing the facilities. Technical assistance of J K Achtani, T-I-3, and Gulab Chand, T-4, is thankfully acknowledged.

REFERENCES

Afshar A, Thomas F C, Wright P F, Shapiro J L, Shettigara P T and Anderson J. 1987. Comparison of competitive and indirect enzyme linked immunosorbent assays for detection of bluetongue

virus antibodies in serum and whole blood. *Journal of Clinical Mircrobiology* **25** (9) : 1705-10.

Afshar A, Thomas F C, Wright P F, Shapiro J L and Anderson J. 1989. Comparison of competitive ELISA, indirect ELISA and standard AGID tests for detecting BTV antibodies in cattle and sheep. *Veterinary Record* **124** : 136-41.

Afshar A, Dulac G C and Riva J. 1992. Comparison of blocking dot ELISA and competitive ELISA, using a monoclonal antibody for detection of BTV antibodies in cattle. *Veterinary Microbiology* **31** : 33-39.

Bandyopadhyay S K and Mullick B B. 1983. Serological prevalence of bluetongue antibodies in India. *Indian Journal of Animal Sciences* **53** : 1355-56.

Dubey S C, MohanRao T R, Srivastava C P, Lonkar P S and Maru A. 1988. Seroprevalence of bluetongue disease in Rajasthan. *Indian Journal of Animal Sciences* **58** : 66-68.

Gupta Y, Chand P, Singh A and Jain N C. 1990. Dot immuno binding assay in comparison with enzyme linked immunosorbent assay for detection of BTV antibodies in sheep. *Veterinary Microbiology* **22** : 365-71.

House C, House J A and Berninger M L. 1990. Detection of bluetongue group-specific antibody by competitive ELISA. *Journal of Veterinary Diagnostic Investigation* **2** : 137-39.

Hubschle O J B, Lorenz R J and Matheka H D. 1981. Enzyme-linked immunosorbent assay for detection of bluetongue virus antibodies. *American Journal of Veterinary Research* **42** : 61-65.

Jochim M M and Chow T L. 1989. Immunodiffusion of bluetongue virus. *American Journal of Veterinary Research* **30** : 33-41.

Lonkar P S, Uppal P K, Belwal L M and Mathur P B. 1983. Bluetongue in sheep in India. *Tropical Animal Health Production* **15** : 83.

Lunt R A, White J R and Della-Porta A J. 1988. Studies with enzyme-linked immunosorbent assays for the serodiagnosis of bluetongue and epizootic haemorrhagic disease of deer. *Veterinary Microbiology* **16** : 323-38.

Mehrotra M L and Shukla D C. 1990. Seroprevalence, diagnosis and differential diagnosis of BTV disease in India. *Indian Journal of Virology* **12** : 98-103.

Prasad G, Jain N C, Mahajan N K and Vasudevan B. 1987. Prevalence of bluetongue precipitating antibodies in different domestic animals. *Indian Journal of Animal Sciences* **57** : 522-24.

Sodhi S S, Oberoi M S, Sharma S N and Baxi K K. 1981. Prevalence of bluetongue precipitating antibodies in sheep and goats in Punjab. India. *Zentralblatt fur Veterinary Medizine B* **28** : 421-23.