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Short Communication

COMPARISON OF COMPETITIVE ELISA AND AGAR GEL IMMUNODIFFUSION TEST FOR DETECTION OF ANTIBODIES TO BLUE TONGUE VIRUS

The agar gel immunodiffusion (AGID) test is the widely used test for detection of group specific Blue Tongue Virus antibodies (Jochim, 1985). Though AGID is simple and rapid to perform, it is difficult to interpret, lacks sensitivity, is not quantitative and is complicated by cross reaction with other orbi viruses like epizootic haemorrhagic disease virus (Della-Porta *et al.,* 1985). To over come these problems enzyme linked immunosorbent assay (ELISA) was introduced. Two types of ELISA have been described, an indirect test (Manning and Chen, 1980; Afsher *et al.,* 1987) and a blocking test (Anderson, 1984) or its variant, the competitive assay using monoclonal antibody (Afsher *et.al.*, 1987). Competitive ELISA was found to be more specific and sensitive than AGID (Afsher *et al.,* 1992). In the present study, competitive ELISA and AGID were used to screen the serum samples collected from cattle, sheep and goats for BTV antibodies and the paper summarises the results of these tests.

Serum samples from 744 animals (495 samples from sheep, 95 from goats, 154 from cattle) collected from different districts of Andhra Pradesh were used in this study.

Competitive ELISA was used for detection of antibody to BTV as per the method of Lunt *et al.* (1988) using VP7 protein, monoclonal antibodies to VP7 (20E9/B7/G2) and anti mouse goat IgG-HRPO conjugate supplied from Australian Animal Health Laboratories (AAHL), Australia. A high titred positive serum and low titred positive serum and a negative serum were used as controls. Results of the test sera were expressed as per cent inhibition, where this value was 50 per cent, the serum was considered positive. The test was repeated if the positive control showed less than 40 per cent inhibition or the negative controls fell outside of the 1.1 to 1.6 OD range.

AGID was performed using 0.9 per cent agarose in normal saline (pH 7.2) in plastic petri dishes of 60 mm diameter. A pattern consisting of one central well and six peripheral wells was used. The size of the well was 4 mm and the distance between the central and peripheral wells was 2.4 mm. The bluetongue antibody test kit was obtained from M/S. Veterinary Diagnostic Technology. Inc., USA. The BTV antigen was placed in central well and 25 μl of test and reference serum

samples were placed in peripheral wells alternating with each other. The plate was incubated at 4°C in a refrigerator and observed for three days for precipitation lines.

BTV antibodies detected in different samples using competitive ELISA and AGID employing VP7 (Yeast expressed) antigen and monoclonal antibodies (20 E9 B7 G2) are shown in the Table. It can be seen from the Table that 312 samples (41.93%) were positive for both competitive ELISA and AGID. Seven samples were competitive ELISA negative and AGID positive. Three hundred and eighty seven samples (52.01%) were negative for both competitive ELISA and AGID. Thirty eight samples (5.10%) were detected as negative by AGID but, were found positive by competitive ELISA. Overall sensitivity of 97.80 per cent and specificity 91.05 per cent of competitive ELISA was recorded when compared with AGID. The level of agreement between competitive ELISA and AGID test for 744 serum samples of cattle, sheep and goat was calculated. Seven of 319 AGID positive serum sample were negative in the competitive ELISA, while 38 AGID negative serum samples reacted with competitive ELISA test resulting in a level of agreement of 95.83 per cent. The level of agreement between competitive ELISA and AGID test for 495 sheep serum samples was 95.32 per cent and 154 cattle serum samples was 98.05 per cent. Afshar *et al.* (1989) demonstrated a relatively low level of agreement (92.04%) with AGID results when tested the bovine sera samples collected from endemic areas. During the investigation three sheep and four goat serum samples which were AGID positive did not react in competitive ELISA. The positive AGID results for these samples could be due to presence of cross reactive epizootic haemarrhagic disease virus or other

Table Comparison of competitive ELISA and agar gel immuno diffusion test for detection of antibodies to bluetongue virus

Species	No. of serum samples tested	C. ELISA + AGID +	C. ELISA - AGID +	C. ELISA - AGID -	C. ELISA + AGID -
Sheep	495	224(45.25)	3(0.60)	242(48.88)	26 (5.25)
Goat	95	41(43.15)	0(0)	49(51.7)	5(5.26)
Cattle	154	47(30.51)	4(2.59)	96(62.23)	7(4.54)
Total	744	312(41.93)	7(0.94)	387(52.01)	38(5.10)

Parenthesis: Per cent

orbi virus antibodies which did not react with BTV specific VP7 protein.

Overall sensitivity of 97.80 per cent and specificity 91.05 per cent competitive ELISA was recorded during the study.

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