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Indigenous Diagnostic Approach for Detection of Bluetongue Disease in West Bengal, India

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Abstract: Indigenous indirect enzyme linked immunosorbent assay (indirect-ELISA) has been developed for detection of anti-bluetongue antibody in sheep from different parts of West Bengal. BTV antibody was detected with a prepared horse radish peroxidase conjugated rabbit anti-sheep immunoglobulin. The indirect-ELISA was specific in detecting BTV antibody in sera of sheep. The results were in complete agreement with the commercial competitive ELISA results. The indirect-ELISA appears to have potential application, especially for indigenous and inexpensive laboratory diagnosis of bluetongue virus infection.

Key words: Horse radish peroxidase · Immune-conjugate · Indirect ELISA · Competitive ELISA

INTRODUCTION

Bluetongue virus (BTV) is a member of Orbivirus genus within the *Reoviridae* family. In India, the first report of the disease was from Maharashtra in 1963 that cause a heavy loss in sheep [1]. Since then the infection of the disease has been found in the states of Southern and Western India for several times. Out of the 25 distinct serotypes of BTV, 21 are prevalent in India [2]. Sheep may show distinct clinical signs with heavy losses in the form of mortality and morbidity. BT in India is endemic to Tamil Nadu andhra Pradesh, Karnataka, Maharashtra, Gujarat, Rajasthan, Haryana, Himachal Pradesh and Jammu and Kashmir [3].

According to the all-India livestock census of 2003, West Bengal state, situated in eastern India (latitude 21°38' N to 27°10' N, longitude 85°50' E to 89°50' E), has a population of 1.4 million sheep, susceptible to BT. Anti-BT antibodies have been detected in sheep, goats and cattle of West Bengal [4, 5].

West Bengal has not recorded any outbreak till-date. But two serotypes of the virus i.e. 15, 21 have been isolated from this area [6]. The vector for the disease viz. the culicoides midges are widely prevalent in this part of the country. For this mysterious un-affected area of the country random sero-surveillance is an utmost need to known the infection status. For the serological survey of the infection of Bluetongue in the different states including West Bengal the reliable method is the commercial competitive ELISA kit [7]. These tests have certain benefits as well as reliability, but are costly and have to be imported. The present investigation was based on the development of indigenous diagnostic tool to make it cost effective.

MATERIALS AND METHODS

Serum Samples: A total of 160 serum samples were collected from sheep of different agro-climatic zones of West Bengal state (India). Sera were collected aseptically in screw capped plastic vial and heat inactivated at 56°C for 30 min and stored at -20°C till further use.

Preparation of Anti-Sheep Rabbit Immune-Conjugate: Horse radish peroxidase (HRPO) conjugated rabbit anti-sheep immunoglobulin was prepared according to Wilson and Nakane [8]. Briefly, immunoglobulin, isolated from sheep serum, was used to prepare hyperimmune serum in rabbit. From this serum, rabbit immunoglobulin was purified and coupled with HRPO enzyme using the glutaraldehyde method as described elsewhere [9]. The specificity of the laboratory prepared immune-conjugate was assessed by a direct ELISA assay.

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Assessment of Anti-bluetongue Antibodies in Suspected Sheep Sera by Competitive-Elisa Using Commercial Kit: Competitive ELISA (cELISA) was done by using the bluetongue competitive serum ELISA kit version P00450/40 of Institute Pourquier, Montpllier, France (supplied by CADRAD, Indian Veterinary Research Institute, Izatnagar). Field sera were tested as per the protocol of the kit that was described elsewhere [4].

Assessment of Anti-Bluetongue Antibodies in Suspected Sheep Sera by Plate-Elisa (Indirect ELISA): The positive and negative sera (6 in no. each) were tested for the diagnosis of bluetongue disease on the basis of presence of antibody in the samples by indirect plate-ELISA with the prepared conjugate [8] The wells of a 96 well plate were coated with the bluetongue antigen, obtained from All India Network Programme on Bluetongue, Institute of Animal Health and Veterinary Biologicals. The plate was then blocked with 5% skimmed milk powder. Field sera were given separately on those wells at 1:1 dilution with PBS. The conjugate was added at a dilution of 1:500. TMB (3,3,5,5-tetramethylbenzidine) + H_2O_2 were used as substrate. The reading was taken after 10 min. at 492nm.

RESULTS AND DISCUSSION

For serodiagnosis, control and monitoring of BTV infection, a rapid, low cost, reliable, sensitive and specific test is a necessity. In recent years, several studies have clearly indicated the advantages of the cELISA for the detection of group-specific antibodies to BTV [10]. The results of several inter laboratory studies on the application of cELISA for serodiagnosis of bluetongue facilitated the recognition of this assay as an international test [10]. In this study, we developed an indigenous indirect ELISA kit and compared the performance of this indigenous indirect ELISA kit with commercial cELISA kit (version P00450/40 of Institute Pourquier, Montpllier, France).

Assessment of the Immune-Conjugate by Direct ELLSA to Determine the Specificity and Titer: The O.D. value of least diluted anti-sheep rabbit conjugate was 0.247 at concentration of 1:100 and its 50% O.D. value i.e. 0.123 was in between 0.135 and 0.111 (Fig 1). So, the titer required for the ELISA would be in between concentration of 1:400 and 1:800. For the present investigation 1:500 concentrations was taken as the required concentration for further indirect ELISA.

Assessment of the Field Serum Samples Using Competitive Elisa (cELISA): Out of 160 field serum samples tested, 54 were found positive (33.75%) by cELISA. Earlier we got similar sero-prevalence of BT in sheep of West Bengal state [4]. Shiringi and Shiringi [11] reported that out of 178 sheep sera tested, 54 (30.3%) were positive for group specific BTV antibodies. However, Prabhakar *et al*. [12] reported much higher sero-prevalance of BT in sheep i.e. 87% in Tamil Nadu.

After the assessment, out of 160 field serum samples, 12 samples were selected among which 6 (Samples no. 302, 305, 308, 314, 329 and 342) were positive for Bluetongue antibody and another 6 (Samples no. 312, 322, 333, 345, Mo.-1, Mo.-18) were negative for the Bluetongue antibody (Fig. 2).

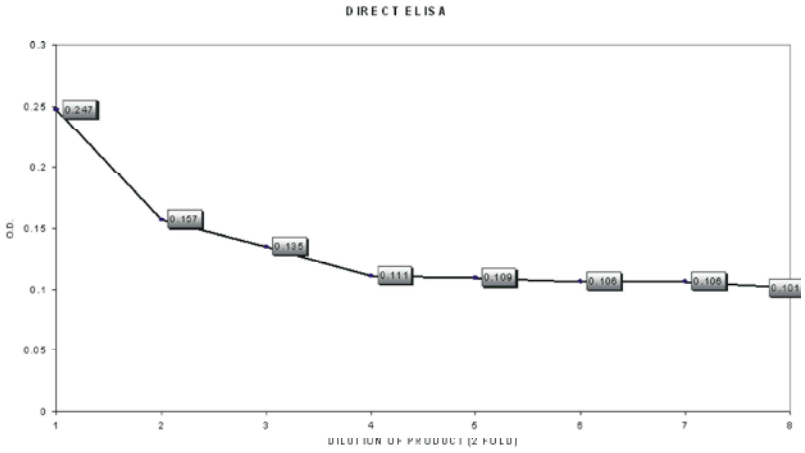

Fig. 1: Assessment of the immune-conjugate by direct ELISA.

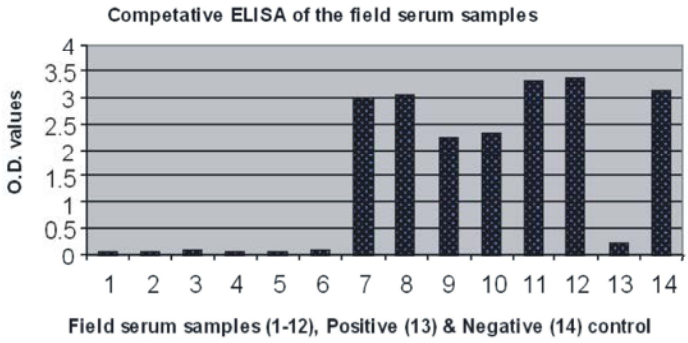

Fig. 2: Assessment of the anti-bluetongue antibodies in sheep sera by the commercial kit.

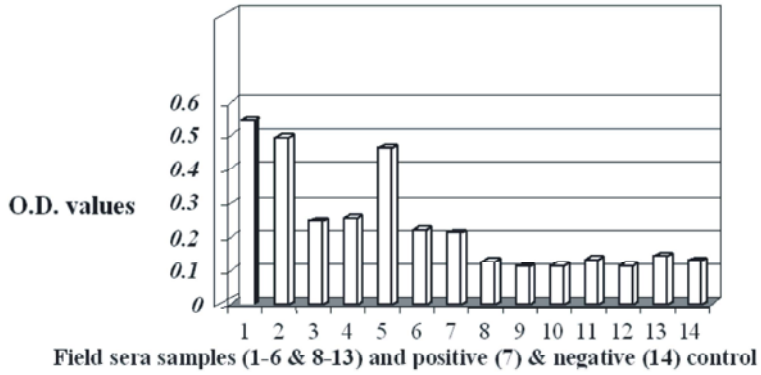

Fig 3: Assessment of anti-bluetongue antibodies of the field sera samples by indirect plate ELISA.

Assessment of Anti-Bluetongue Antibodies of Selected Field Sera by Indirect ELISA: The present study showed that the first 6 field sera samples (Sample no. 302, 305, 308, 314, 329 and 342) showed O.D. values more than 0.21 (O.D. value of the positive control at 14th well) and the last 6 field sera samples (Sample no. 312, 322, 333, 345, Mo.-1, Mo.-18) showed O.D. values less or around 0.129 (O.D. value of the negative control at 7th well) (Fig. 3). So, it was proved that the first 6 samples which were previously determined to be positive by cELISA were again determined to be positive by indirect ELISA. Similarly, the last 6 samples which were previously determined to be negative by cELISA were again determined to be negative by indirect ELISA. So, the result of the indirect ELISA with the prepared conjugate was comparable with that of cELISA kit.

The indigenously developed tool for diagnosis of anti-bluetongue antibody in sheep showed a comparable data when compared with that of the commercially available competitive ELISA kit. So, it can be concluded that the indigenous tool can reduce the cost of diagnosis and it may prove to be a good diagnostic for the random sero-surveillance of suspected sheep sera.

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