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Latex Agglutination Test for Detection of Epsilon Toxin

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Abstract

Latex agglutination test was developed and standardized for detection of epsilon toxin in culture supernatants and is rapid screening test in enterotoxaemia out breaks in sheep. Out of 160 sheep fecal samples tested, 21 (13%) were found to be positive and all nine culture supernatants were found to be positive showing 100% positivity which were also confirmed by mouse protection tests.
Key words : Latex agglutination test, Epsilon toxin, Detection, Sheep.

Enterotoxaemia is considered as one of the most important diseases of sheep caused by *Clostridium perfringens* type D, a normal inhabitant of intestines. Epsilon toxin is the major lethal toxin produced by this organism, which is activated by proteolytic enzymes present in the gut is responsible for acute toxemia. Mouse protection test (5) is recommended test for diagnosis of enterotoxaemia but the test is huge time taking. To overcome this problem,latex agglutination test was standardized for the detection of epsilon toxin from enterotoxaemia cases in sheep.

Methods

Preparation of antiepsilon IgG was done by separating IgG's from antiepsilon hyper immune rabbit serum by ammonium sulphate precipitation method (2). A total of 10ml antiepsilon (hyper immune) rabbit serum was taken and equal volume of saturated ammonium sulphate solution was added slowly drop by drop stirring for 20 min. Contents were centrifuged at 3000 rpm for 30 min, the pellet was resuspended in 10 ml of PBS pH 7.2. Again equal volume of saturated ammonium sulphate solution was added and the entire procedure was repeated three times. The pellet was finally suspended in 5 ml of PBS, pH 7.2. The suspension was dialyzed against three changes of PBS (pH 7.2) for 24—30 h. After dialysis, the suspension was loaded in DEAE cellulose column. Elution was done with phosphate buffer. Three ml fractions were collected in test tubes till no protein was eluted. The OD 280 of each fraction was measured. The fractions in the first peak of the curve were pooled and concentrated using PEG-6000. Finally the OD 280 values of the concentrate sample were measured, protein estimated and stored at -20C until used.

Conjugation of latex beads with IgG (3) by mixing equal volumes of diluted latex beads (1/15) and IgG's (2mg/ml) and left at room temperature (28—35 C) for one hour with intermittent shaking. Then the contents were removed carefully and the pellet was washed for sensitizing these beads, twice with 0.02% PVP in 0.1M Tris buffer pH 7.4 containing 0.02% sodium azide. Then the final volume of the reagent was made up to the volume of the IgG's added at one percent to the sensitized latex beads. The reagent was stored at 4C until used. Epsilon toxin having 15, 000 MLD/ml and nine known positive *Clostridium perfringens* culture supernatants were used as positive controls in LAT.

A total of 160 fecal samples from sheep were collected and received from Chittoor (52) and the remaining samples (102) were obtained from VBRI were examined for the presence of epsilon toxin in the present study. Out of which 15 enterotoxaemia positive fecal samples and 20 enterotoxaemia negative fecal samples tested with mouse protection test were used as positive and negative controls.

Latex agglutination test was performed with these samples from different sources for the presence of epsilon toxin. These samples were suspended in equal volume of PBS pH 7.2 and centrifuged to get clear supernatant. The clear supernatant was tested for the presence of epsilon toxin. One drop of latex reagent

Table 1. Titration of epsilon toxin by latex agglutination test. ++++, Strongly positive; +++, Positive; ++ Weakly positive.

	Epsilon toxin MLD/ml	LAT
1	15000	++++
2	7,500	++++
3	5,000	++++
4	2,500	++++
5	1,000	++++
6	500	++++
7	250	+++
8	150	++

mixed with one drop of toxin on a clean microscopic slide and mix gently with tooth pick. Distinct clumping or agglutination was taken as positive while the suspension remained uniformly milky was considered as negative for the test. The test was also used to detect the toxin in *C. perfringens* type D culture supernatants. Epsilon toxin diluted to 250 MLD/ml showed clear agglutination with in one minute. However toxin with 150 MLD/ml reacted and slowly and agglutination of latex beads was noticed after two minutes. The LAT was used to detect the presence of epsilon toxin in the fecal samples collected from the sheep died and suspected for enterotoxaemia from different places. The test fecal samples (160) were individually screened for the detection of epsilon toxin. All the samples showing positve reaction were also tested with mouse protection assay for confirmation.

Results and Discussion

LAT was standardized as per the method described by Martin and Naylor (2). Two mg/ml of antiepsilon rabbit Ig was found to be optimum to sensitize latex beads suspension of 0.1M tris buffer with 0.02% sodium azide and 0.02% PVP to give visible agglutination. In the present study LAT was able to detect about 250MLD/ml of epsilon toxin clearly. The results are shown in the Table 1. Interpretation of the reaction was based on the visible agglutination rather than microscopic agglutination. The test was found to be rapid as the agglutination reaction can be read visually similar advantages were reported previously for detection of other agents (2, 4). The results of evaluation of LAT for its suitability to detect epsilon toxin in fecal samples indicated that 21 out of 160 suspected samples were found LAT positive. The samples both positive and negative for epsilon toxin were further confirmed by mouse protection test. Martin and Naylor (2) reported that ELISA was more sensitive than LAT in detecting epsilon toxin. In the present study a total of 160 fecal samples and 9 culture supernatants were screened using latex agglutination test. Out of 160 fecal samples 21 were positive (13%) and all nine culture supernatants were positive with 100% positivity (1) whereas all these positive samples showed toxicity in mouse when tested by mouse protection test (6).

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