Comparison of Standard tube agglutination test and indirect haemagglutination test in the detection of Listerial antibodies in animals

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Abstract

Efficacy of standard tube agglutination test (STAT) & indirect haemagglutination test (IHA) was compared for detecting listerial antibodies in cattle and buffaloes. Out of 530 serum samples (263 cattle and 267 buffaloes) 165 serum samples (31.13 %) gave positive reaction by STAT while 151 serum samples (28.49 %) gave positive reaction by IHA. It was observed that both the tests are equally efficient in detecting listerial antibodies.

Keywords: Standard tube agglutination test, Indirect Haemagglutination test, Antibodies, Listeria, Laboratory Technique, Detection.

Introduction

Listeriosis is an important bacterial zoonosis caused by pathogenic strains of Listeria mono-cytogenes that occurs world wide and reported in variety of animal species and man (Gray and Killinger, 1966).Listeria monocytogenes has gained a great deal of attention due to not only increased report of clinical disease, manifested by septicaemia, abortion, stillbirth, meningitis and meningio-encephalitis in man and animals but also for it's implication as a food borne pat- hogen (Low and Donachie, 1997).

Occasionally it also causes mastitis, spinal myelitis or keratoconjunctivitis and opthalmitis (Radostits et al., 1995). Detection of listerial antibodies has been used as the indirect measures of infection in various domestic animals with the advantage of screening large population in comparatively short period (Teruya et al., 1977). In present study the detection of listerial antibodies in serum samples was compared by tube agglutination test and indirect haemagglutination test.

Materials and Methods

Collection of serum samples:

A total of 530 serum samples comprising 263 of cattle and 267 of buffaloes were collected from different regions of Gujarat viz.., North Gujarat, Central Gujarat, South Gujarat, Saurastra and Kuchchh. All the serum samples were tested for detecting listerial antibodies by standarad tube agglutination test and indirect haemagglutination test.

Preparation of antigen:

Trypsinised antigen for tube agglutination test and flagellar antigen for indirect haemagglutination test were prepared from standard reference culture strain of Listeria monocytogenes (MTCC-1143) obtained from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh. Confirmation of culture was done by cultural isolation by streaking on blood agar and tryptone soya agar, morphological confirmation and motility examination.

(a) Preparation of trypsinised antigen:

The trypsinised antigen for tube agglutination test was prepared by method of Osebold et al. (1965) with some modifications. A 5 ml of Tryptone Soya Broth tube was inoculated with a typical colony and was incubated at 37 oC for 18 hrs. The tube growth was then used to inoculate the Roux flask containing Tryptone Soya Agar. The inoculated flask was incubated 37 °C for 48 hrs.

The growth was harvested with sterile normal saline solution and heat killed at 100 °C for 1 hr. The suspension was then washed thrice with NSS and preserved after dilution to 1: 20 equal to McFarland opacity tube No.4. Then cell suspension was trypsinised using 1 per cent trypsin solution. One-part of 1 per cent trypsin was mixed with nine parts of cell suspension and then incubated at 37 °C for 15 mins.

The cells were then centrifuged and washed twice with NSS and preserved with 0.25 per cent formal saline. The treated antigen was diluted to its original dilution of 1:20 equal to McFarland opacity tube No. 4.

(b) Preparation of flagellar antigen:

Listeria monocytogenes suspension in PBS to match Brown's opacity tube No.3 was heated at 93°C for 30 minutes. Supernatant collected after centrifugation at 1000 rpm for 30 minutes was used as flagellar antigen.

Preparation of antigen sensitized cells:

Sheep RBCs (2.5%) and the flagellar antigen as prepared above in the ratio of 2:1 were incubated at 37oC for 1 hour for sensitization. After that the sensitized sheep RBCs were washed twice with the PBS and finally suspended in PBS at the concentration of 0.5 %.

Test procedure:

Tube agglutination test:

All the serum samples were tested as per the method described by Vishwanathan (1978). Two fold serial dilution of serum samples were made in NSS in test tubes.o.5 ml of trypsinised antigen was added in all the test tubes. The whole set was incubated in water bath at 50-52 °C for 2 hrs followed by holding at 4 °C for 24 hrs. The sample showing titre of 1:80 or above with at least 50 % clarity considered positive.

Indirect haemagglutination test:

0.02 ml of N. S. S. was pipetted in each well. Inactivated serum under test was added in 0.02 ml quantity in the first well.

Serum in first well was mixed and 0.02 ml of the diluted serum was transferred to the next well. The process was repeated for making two fold dilutions in the remaining wells. 0.02 ml-diluted serums from last well was discarded. 0.02 ml sensitized cell were added to each well. The plate was incubated at room temperature for two hours and then read. Positive reaction was indicated by mat formation while the negative reaction by button formation. A titre of 1.8 and above was considered as positive.

Results and Discussion

Out of 530 serum samples 165 serum samples were found positive by Tube agglutination test giving31.13 % seroprevalence while 151 samples were found positive by IHA giving 28.49 % seroprevalence.

Tests	Total No.	Positive samples	% positive
STAT	530	165	31.13
IHA	530	149	28.49

X2 test of independent of two factors was carried out to test efficacy of both tests showed that both are equal effective in detecting listerial antibodies.

Listeria monocytogenes causes economic losses as it causes disease in a wide variety of animals (sheep, goats, cattle, buffaloes, dogs, horses, chickens and rabbits) including man (Katiyar, 1960). It is zoonosis with a broad distribution. It is considered as a potentially pathogenic species that has diverse distribution in nature. It's role in causation of meningo-encephalitis, septicemia, abortion, endometritis, cervicitis, mastitis, kerato-conjunctivitis, local purulent lesions etc. has been established beyond doubt (Gitter, 1980). Association of bovine infertility with seropositivity against *L. monocytogenes* antigens was reported from various parts of the world (Protin et al., 1979, Schweighardt et al., 1984) including India (Srivastva et al., 1985) with variable agglutinin titers. The interpretation of serological tests for antibodies against *L. monocytogenes* is made difficult by the presence of positive reactions of upto 1:200 in clinically normal animals. This is because of *L. monocytogenes* is widespread in the environment and a significant proportion of animals sporadically shed the organisms in their feces or milk. (Skovgaard and Morgen, 1988). Thus, many animals in the dairy cattle population are expected to present antibodies towards *L. monocytogenes*. Hence Osebold et al have demonstrated that treatment of heat killed listerial antigen with trypsin increases the sensitivity of the antigen and eliminates cross reaction problem to some extent. In the present study it is concluded that both the tests are equally applicable in detecting listerial antibodies as there is no significant difference between these two tests.

Acknowledgement

Authors are thankful to Dr. V.P.Vadodaria, Dean, College of Veterinary Science and A.H., for providing the necessary facilities. We also gratefully acknowledge the help of field veterinarians for collection of serum samples.

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