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Monoclonal Antibody Based Latex Agglutination Test for the Diagnosis of Trypanosomosis in Cattle

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Abstract

Trypanosomosis which is an arthropod borne disease had become the menace to the Indian farmers because of its significant impact on the productive status of the animals. Moreover, the zoonotic effect of this disease has also proved now. Research on newer techniques for the diagnosis of this important disease has been carried out for the past hundred years, and still this search is going on for finding a more sensitive and specific test. The parasitological examination which is used for the diagnosis at the field level misses about 80% of positive cases. Keeping in view the shortcomings of the conventional diagnostic methods we carried out the present investigation for the detection of active infection of Trypanosoma evansi by monoclonal antibody based latex agglutination test (MAb-LAT). About 88 blood samples collected from cattle of the karnal district of Haryana were screened initially by Wet Blood Film (WBF) immediately after collection and their corresponding serum samples were subjected to latex agglutination test. WBF could detect the presence of motile trypanosomes only in three samples (3.41%) where as MAb-LAT detected 53 samples (60.23%) positive for the circulating antigens of Trypanosoma evansi. Study found that MAb-LAT is much sensitive than the conventional parasitological examination. Moreover, MAb-LAT is simple to perform, rapid, and cost-effective and can be used in field-level.

Keywords: Diagnosis; MAb-LAT*; Trypanosoma evansi;* WBF

Introduction

Trypanosoma evansi, a blood protozoan parasite, causes a serious disease known as 'surra' in domestic and wild animals. It is a mechanically transmitted arthropod borne disease and Tabanus spp. has been implicated as the main vector. It is the most widely geographically distributed pathogenic trypanosome in Africa, South and Central America and Asia (Luckins, 1998, Pathak and Khanna, 1995). In India, *T. evansi* infection is widely prevalent in different parts and is of significant economic importance in livestock production (Juyal *et al*., 2007). Surra may occur in acute, sub-acute, chronic and in apparent forms. Acute and sub-acute forms of the disease are fatal. In buffaloes, cattle and camels, the disease is usually chronic, though acute cases have also been reported. Recently, a case of surra infection in a man has been reported in India (Joshi *et al*., 2005; Powar *et al*., 2006, Shah *et al*., 2011). This report assumes significance, for it indicates the possible zoonotic threat in future (Laha and Sasmal, 2007).

Trypanosomosis has been studied over the past several years; still, their definite diagnosis suffers from low sensitivity and specificity. The fluctuating nature of parasitaemia, makes it often difficult to be detected by the commonly used parasitological methods. Though, animal inoculation methods are more sensitive to diagnosis of surra, yet they are laborious, time-consuming and unsuitable for large-scale use in the field. Further, SPCA (Society for Prevention of Cruelty to Animals) does not permit the use of experimental animals, if alternatives are available.

The main limitation of parasitological diagnostic techniques is its low sensitivity, which had been a driving force for research into alternate techniques such as serological and DNA based methods, which have got a greater potential for unequivocal identification of the causative agent with higher sensitivity. Serological diagnosis using antibody detection is hampered by its inability to distinguish between current and past infections be-

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cause of persistent titres and occurrence of false-positive results. The identification of circulating variable antigen types (VAT) would be a great value in developing more sensitive diagnostic tests. Accurate diagnosis of 'surra' is extremely important to track the prevalence of the disease and to avoid misuse of the trypanocidal drugs. So development of cost-effective and field-oriented diagnostic test is required for large-scale screening of animals for effective control of disease. Monoclonal antibody based latex agglutination test was used in the present study for the detection of circulating antigen of T. evansi in the serum of infected cattle.

Materials and methods

Collection of blood and serum samples:

A total of 88 blood samples from naturally infected, suspected and healthy cattle from different places of Haryana state were collected during September, 2008 – January, 2009 in separate vials with and without anticoagulant. The blood samples with anticoagulant (heparin, 10 units/ml of blood) were used for detecting *T. evansi* in wet blood film (WBF) within three hours of collection. The blood samples without anticoagulant were collected to separate serum for use in latex agglutination test. The blood and serum samples were stored in sterilized vials at –70ºC until further use.

Wet blood film

A drop of blood from each sample was used for preparation of WBF in triplicate on a clean glass slide and examined for *T. evansi* at a magnification of 100x and 400x according to the method of Killick-Kendrik (1968).

Monoclonal antibody based latex agglutination test (MAb-LAT): Monoclonal antibody (MAb) used in the present study was produced in a larger study by Rayulu *et al*. (2007). For detection of *T. evansi* antigen in cattle sera samples by MAb-LAT, latex reagent which is a suspension of latex microbeads, 0.8 µm in diameter, coated with anti-T. evansi murine monoclonal antibody was used. MAb-LAT was performed according to the method of Rayulu *et al*. (2007). Latex reagent was prepared by mixing one part of polystyrene latex beads (Sigma, USA) with mean particle size 0.8 µm to nine parts of anti-T. evansi murine monoclonal antibody (IgA isotype) produced against cell membrane antigens of *T. evansi.* The suspension was stirred for two hours at room temperature (25°C). The suspension was centrifuged at 2400 x g for 10 minutes at 4ºC and the supernatant discarded. The MAb coated latex beads were resuspended in PBS, pH 8.0 and the centrifugation was repeated thrice. Finally, the pellet containing MAb coated beads was resuspended in PBS, pH 8.0 with 0.1% BSA. This reagent was used to screen the cattle sera samples collected for detection of T. evansi antigens. Twenty microliters of the latex reagent were taken in the cavity of the slide (Himedia, India) and an equal volume of cattle serum was added to it. The reagent and the serum sample were mixed by gentle swirling motion of the slide for five to ten minutes. In case the clumps or granular aggregates formed within five minutes, the sample was scored as strong positive and within ten minutes, the sample was scored as weak positive. The five and ten min. Reaction criteria had been established earlier in a larger study. Controls, including known positive and negative rat serum samples were also used in parallel.

Results

Examination of blood samples collected from cattle by WBF revealed the presence of *T. evansi* parasite in only three samples (3.41%). These samples were showing mild parasitaemia in wet film.

Out of 88 cattle sera samples examined, 53 samples (60.23%) showed positive for the circulating antigens of *Trypanosoma evansi*. by MAb-LAT. Among the positive samples 44 (50%) were strong positive and 9 (10.23%) samples showed weak agglutination reaction. All the samples which were positive by WBF showed strong reaction by MAb-LAT.

Discussion

Diagnosis of trypanosomosis has been extremely difficult due to its switching nature of variable surface glycoprotein (VSG) to another antigenic form within the infected host causing fluctuating parasitaemia, in order to escape from host's immune attack. In the present study, we could detect only a

few samples positive for T. evansi by WBF. We expected more samples to be positive since most of the samples were collected from Karnal district of Haryana- a high vector density region in the state. Therefore, the number of animals infected in the region should be higher than those detected by parasitological examination. Wet film examination for the parasite in the infected animals is often the only test used in the field, but it is probably the least sensitive test missing 50-80% of positive cases. So, one obvious reason for the low number of positive samples by WBF is the inherent low sensitivity of the test. Similar observations have been made by numerous workers during the past two decades in India (Swarnkar *et al*., 1993, Pathak *et al*., 1993, Singh *et al*., 1995; Rayulu *et al*., 2007) and in other countries (Masake and Nantulya, 1991, Olaho-Mukani *et al*., 1993, Davison *et al*., 2000, Ngaira *et al*., 2003). Latent infections with low parasitaemia are also common in cattle (Woo, 1974; Losos, 1980, Pathak and Singh, 2005), it was highly probable that many samples were missed by WBF. Another reason for this low number of cases could be probably due to the treatment of animals for trypanosomosis on symptomatological basis-quite common practice in the field in India, including the state of Haryana. It was difficult to extract history of treatment of the substantial number of cases, if not all from which the samples had been taken.

Monoclonal antibody based latex agglutination test detected far more samples positive than WBF. The monoclonal antibody (IgA isotype) used in the present study were produced against an invariant region of surface glycoprotein (Rayulu *et al*., 2007). The VSGs have variable and conserved parts carrying variant and invariant epitopes, respectively. Similar observations of MAb-LAT have been reported by Nantulya (1994) using monoclonal antibody-based latex agglutination test (Suratex®), detected the antigens in 53 (88.3%) of 60 blood samples collected from experimentally infected rabbits in comparison to 22 (36.7%) and 2 (2.3%) by buffy coat and WBF, respectively. Olaho-Mukani *et al*. (1996) screened 549 camels by Suratex® and found T. evansi antigens in 254 (46.3%) camels. Rayulu *et al*. (2007) using latex agglutination test (LAT) declared an overall of 42.59% positive out of 1538 field samples. Overall, both MAb-LAT reagents (Nantulya's and Rayulu's) could detect far more samples positive than those detected by WBF, indicating thereby higher sensitivity of the LAT than that of WBF.

Specificity of the MAb-LAT was determined using Babesia bigemina and Theileria annulata infected cattle serum samples collected from the field. These samples did not show any agglutination reaction in MAb-LAT which excludes the cross reactivity of monoclonal antibodies with other haemoprotozoan parasites like Babesia and Theileria to certain extend. The *T. evansi* infected rat serum as well as the cattle serum sample which was found positive by WBF was used as positive control for MAb-LAT.

Ag-detecting serological test like LAT has an inherent limitation of declaring the recently-treated animals as positive, since the antigens released from the killed parasites remains in blood circulation up to nearly four weeks after treatment, as observed previously in other studies (Olaho-Mukani *et al*., 1996, Thammasart *et al*., 2001, Wernery *et al*., 2001, Singh and Chaudhri, 2002). Therefore, this necessitates getting reliable history of the animal that receives anti-trypanosome treatment during past few weeks before sample collection to make LAT more dependable. In the present study, we could get the history of treatment of cattle from few cases but not from all.

MAb-LAT detected more samples positive for T. evansi than WBF. Moreover, the test is simple to perform neither requiring multiple and complex procedural steps, nor the use of sophisticated equipment for reading the results. MAb-LAT was found to be a rapid, convenient, cost-effective and field adaptable test. The merits of MAb-LAT make the test suitable as a field-level test for screening of *T. evansi* infected cattle, thereby helping in effective control of the disease.

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