Experiences on diagnosis and isolation of *Trypanosoma evansi* infection in cattle in field condition

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**ABSTRACT**

Two chronically infected cattle suspected for *Trypanosoma evansi* infection were studied for confirmative diagnosis and isolation of parasites. One zebu cattle with symptoms of intermittent rise of temperature (38.3°C to 41.1°C), bottle jaw and chronic emaciation, found positive for *T. evansi* in Giemsa stained blood smears, but found negative for *T. evansi* after mouse inoculation test (MIT). Other crossbred cattle with symptoms of intermittent rise of temperature (38.3°C to 41.7°C) and decreased milk yield, found negative for *T. evansi* infection in Giemsa stained blood smears, but found positive after repetition of MIT. In such MIT only 25% inoculated mice of batch showed positivity in MIT. The presence of anti-*T. evansi* antibody was detected in serum samples of both of these cattle, as observed in indirect ELISA test. It has been concluded that cattle positive for *T. evansi* infection in blood smears may not always positive in MIT and negative in blood smears for *T. evansi*, may be positive in MIT, which may need repeated inoculation of suspected blood to mice in different time intervals and in different batches to get positive result in MIT, in field condition. Repetition of MIT for successful result for isolation of *T. evansi* in field condition is reported.

**Keywords:** cattle; diagnosis; isolation; *Trypanosoma evansi*

**INTRODUCTION**

The hemoprotozoan parasite, *Trypanosoma evansi*, causes a disease in domestic animals, which is popularly known as ‘sura’. The disease is prevalent in cattle, buffaloes and horses of Eastern Region of India (Ray et al., 1992; Laha et. al., 2004; Laha and Sasmal, 2008a, b). Cattle are mostly suffers from chronic form of the disease although the outbreak due to *T. evansi* infection in cattle has also been reported from the Eastern Region of India. Diagnosis of *T. evansi* infection is difficult because of its varied and non-specific clinical manifestations in enzootic areas (Herrera et al., 2004). For various types of research works, particularly preparation of antigens for serodiagnosis of the disease, molecular characterization of the parasites and also for confirmative diagnosis of the infection, isolation of the parasite from cattle is needed. As cattle are mostly suffers from chronic form of the disease, hence, 2 chronically infected cattle, suspected for *T. evansi* infection were studied for confirmative diagnosis and isolation of parasites and the experiences gained during the study is reported in the present communication.

**MATERIALS AND METHODS**

One zebu cattle (ID No. 35C) of the area of Dogachia, Distt. North 24 Paragona, West Bengal, India, found to suffer from intermittent rise of temperature varied between 38.3°C to 41.1°C, bottle jaw and chronic emaciation (Fig. 1). Other crossbred cattle (ID No. 58C) of area of Garifa Bostompara, Naihati, Distt. North 24 Paragona, West Bengal, India, also found to suffer from intermittent rise of temperature
varied between 38.3°C to 41.7°C and decreased milk yield (Fig. 2) was taken for this study.

Fig. 1. Animal ID No. 35C suffering from intermittent rise of temp. (38.3°C to 41.1°C) bottle jaw and chronic emaciation

Blood smears of first cattle (ID No. 35C) were prepared at the height of temperature (41.1°C) and stained with Giemsa stain for detection of parasites. After a period of 24 hours of height of temperature, the animal showed afebrile stage (38.3°C) and at that time for isolation of parasite, MIT was performed by inoculating 0.5 ml of blood of this cattle to 4 mice intraperitoneally, directly at the spot, to observe the
multiplication of the parasites inside the inoculated mice. The inoculated mice were examined for the presence of the parasite by the examination of wet blood film (WBF) and examination of Giemsa stained blood smears at least twice in a week up to a period of 60 days animals (Holland et al., 2001), for detection of the parasite in inoculated mice and thereby in suspected cattle. During the time of MIT, blood smears of this cattle was also prepared and stained as described earlier. Blood smears of the second cattle (ID No. 58C) were prepared during height of temperature as well as during afebrile and moderate rise of temperature (38.3°C, 38.6°C and 39.4°C) and stained with Giemsa stain for detection of parasites. For diagnosis and isolation of parasites, MIT of this cattle was performed by inoculating 0.5 ml of blood of this cattle to 8 mice intraperitoneally, directly at the spot, in 3 times in 3 batches containing 8 mice in each batch of mice, at an interval of 7 days, when temperature was 38.6°C, 39.4°C and 38.3°C, respectively. Serum samples from these 2 animals were collected to detect antibodies against T. evansi infection and an indirect ELISA as described earlier (Luckins, 1977; Jithendran et al., 1997) was followed for detection of anti-T. evansi antibody in these 2 suspected cattle, using whole cell lysate crude T. evansi antigen, prepared from T. evansi isolated from buffalo origin (Laha and Sasmal, 2008b).

RESULTS AND DISCUSSION

Blood smears of cattle No. 35C showed the presence of T. evansi during the height of temperature only (Fig. 3), but the MIT of this cattle remained negative throughout the period of observation (60 days) in inoculated mice. Blood smears of cattle No. 58C showed negative for T. evansi during the height of temperature as well as afebrile and moderately raised temperature condition. After a period of 10 days post inoculation, only 2 mice (25%) of second batch which were inoculated with suspected blood of cattle No. 58C showed positive for the presence of T. evansi in wet blood smears as well as Giemsa stained blood smears. Other 2 batches of mice, which were inoculated with suspected blood from this cattle, showed negative for T. evansi throughout the period of observation (60 days).

![Fig. 3. Trypanosoma evansi in the Giemsa stained blood of cattle (x1,000)](image-url)
The result of this experiment showed that MIT is not a sensitive test for detection of *T. evansi* infection although blood smear showed positive as observed in case of first cattle. As diagnosis of the infection already has been done after examination of blood smears of this cattle, so the cattle was treated immediately after diagnosis with single injection of a mixture of quinapyramine sulphate and chloride at 7.4 mg/kg body weight 4 subcutaneously. During the period of post treatment, examination of Giemsa stained blood smears and MIT showed negative for the *T. evansi* infection along with normal temperature. In case of second cattle, the result indicates that negative for *T. evansi* in blood smears does not indicate negative for *T. evansi* infection in suspected animals. Besides, the result also indicates that it needs to inoculate different batches of mice in different days for isolation of parasite from a suspected cattle and inoculation of blood during febrile condition (39.4°C) is needed to get successful result in MIT as well as to isolate *T. evansi* from suspected cattle. The most interesting finding of this experiment is that only 2 inoculated mice (25%) showed the presence of *T. evansi* infection, although 8 mice were inoculated from the same inoculum, prepared from the blood collected during febrile condition (39.4°C) of the suspected cattle.

In case of first cattle, blood smears found positive for *T. evansi*, but MIT was negative for *T. evansi* might have occurred as MIT is not 100% sensitive (Monzon *et al.*, 1995). In case of second cattle examination of blood smears throughout the experiment and MIT done in first and third batches of mice showed negative for *T. evansi*, might be due to fluctuating level of parasitaemia (Nantulya, 1990). For the same reason and due to superiority of MIT than examination of blood smears for diagnosis of *T. evansi* infection (Ray *et al.*, 1992; Gutierrez *et al.*, 2004), second batch of mice showed positive for *T. evansi* infection. It can be presumed that very low level of parasitemia was present in the second cattle during collection of blood and also subsequently in the inoculum prepared for inoculation of second batch of mice. Some portion of inoculated blood might have deprived from any parasites, may be the reason of showing negative for *T. evansi* in rest of the 6 mice and might be the reason to attain 25% successful in MIT. The portion of blood inoculated to 2 mice which showed positive in MIT, might have contained a very minimum numbers of parasites, which made them to show positive in MIT with longer incubation period (10 days). Due to very low level of parasitemia, examination of blood smears prepared during MIT, showed negative for *T. evansi* in Giemsa stained smears. This type of report of repetition of MIT for diagnosis and isolation of *T. evansi* in of chronically ill cattle, in field condition, with a probability of success in 25% inoculated mice, never been reported earlier. In the present study these 2 cattle might have suffered since a long time which enabled them for production of Abs against *T. evansi* by giving sufficient time after infection. This might be the reason for presence of antibody of *T. evansi* in these 2 animals as detected in the indirect ELISA. It can be concluded from the present study that positive for *T. evansi* in blood smears may not always positive in MIT and negative in blood smear may be positive in MIT which may need repeated inoculation of suspected blood to mice in different days in different batches to get positive result in MIT in field condition. In such cases also there is a probability of success in 25% inoculated mice.

REFERENCES


