Detection of *Trypanosoma evansi* infection among parasitologically and immunologically negative animals by polymerase chain reaction

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ABSTRACT

A study was undertaken to detect *Trypanosoma evansi* infection by polymerase chain reaction (PCR) among clinically ill cattle, buffaloes and horses which were suspected for ‘surra’, but found negative by parasitological and immunological tests. Detection of trypanosomal DNA based on PCR have been found more useful tool for diagnosis of the infection in clinically ill animals which were declared as negative by examination of Giemsa stained blood smears, mouse inoculation test and antibody detection enzyme linked immunosorbent assay (ELISA) test.

Keywords: animals; detection; polymerase chain reaction; *Trypanosoma evansi*

INTRODUCTION

The hemoprotozoan parasite, *Trypanosoma evansi*, is the causal agent of the disease trypanosomosis in domestic animals and is popularly known as ‘surra’. Nowadays, the disease is not only restricted to animals. Recently the infection has been reported for the first time in the world from human being also from India (Joshi *et al.*, 2005). The infection in human by *T. evansi* could be considered as an alert for the persons working on trypanosomosis in Southeast Asia (Juyal, 2005). But, the diagnosis of *T. evansi* infection is difficult because of its varied and non-specific clinical manifestations in enzootic areas (Herrera *et al.*, 2004) and most of the cases field diagnosis of *T. evansi* in eastern region of India has been done either by parasitological or immunological techniques (Laha *et al.*, 1989, 2004; Ray *et al.*, 1992; Laha and Sasmal, 2008a, 2008b). Demonstration of the parasite in the blood of infected animal is the confirmative diagnosis of the infection. But, parasite detection techniques by examination of Giemsa stained blood smears, can not always be able to detect current infections when the level of parasitemia remains low and fluctuates, particularly during the chronic stage. As an alternative method of diagnosis of such suspected cases of ‘surra’, mouse inoculation test (MIT), antigen and antibody detection enzyme-linked immunosorbent assay (ELISA) tests have been done. But they also have their limitations. As an alternative to parasitological and immunological techniques, detection of trypanosomal DNA based on polymerase chain reaction (PCR) has been undertaken in the present study, amongst animals which were clinically ill and suspected for ‘surra’, but declared as negative by blood smear examination, MIT and antibody detection ELISA.
MATERIALS AND METHODS

The study was conducted on buffaloes, cattle and horses which were found clinically ill and suspected for *T. evansi* infection as observed by clinical symptoms like persisting temperature or intermittent rise of temperature, anorexia, emaciation, anemia, decreased hemoglobin value in blood, conjunctivitis, nervous symptoms, decreased milk yield, abortion etc. as reported by the local veterinarians or owners of such animals. Blood samples from these clinically ill cattle, buffaloes and horses, suspected for ‘surra’, were collected aseptically and each sample was processed for preparation of blood smears for staining, preparation of serum, MIT and processed for extraction of genomic DNA of *T. evansi*. Examination of Giemsa stained blood smears and MIT were performed as per standard methods. For detection of anti-*T. evansi* antibody in suspected buffaloes, cattle and horses, an indirect ELISA as described earlier (Luckins, 1977) was followed.

The genomic DNA of samples (1) containing DEAE purified *T. evansi* of cattle origin (Sample No.58C), buffalo origin (Sample No.3B) and horse origin (Sample No.215) were taken as known positive control, (2) containing *T. evansi* infected blood of horses (Sample Nos.211, 210, 206), buffalo (Sample No.3B) and cattle (Sample No.58C), were taken as known positive control of blood samples (3) containing blood of horse (Sample No.H1KMP), cattle (Sample No.46C) and buffalo (Sample No.48B), which were collected from non-enzootic area and also found negative for *T. evansi* infection by examination of Giemsa stained blood smears, MIT and Ab-ELISA, were taken as known negative control. One sample containing no template DNA and only distilled water was also used as negative control during PCR.

Genomic DNA from DEAE purified *T. evansi*, known positive control, known negative control and suspected blood samples were extracted as per standard method (Sambrook et al., 1989) by proteinase K digestion and using phenol chloroform and isoamylalcohol extraction method.

Two primers ESAG 6/7 forward 5' ACA TTC CAG CAG GAG TTG GAG 3' Primer and ESAG 6/7 reverse 5' CAC GTG AAT CCT CAA TTT TGT 3' primers, which are *Trypanozoon* specific (Holland et al., 2001) were used for amplification of 237 bp fragment from *T. evansi* genomic DNA. Amplification was carried out in a Thermal cycler machine (Eppendorf, Hamburg, Germany). Positive and negative control of DNAs was run in each experiment. The reaction in a final volume of 50 μl was carried out according to following protocol: Template DNA, 5 μl; Forward primer (20 pmol), 2 μl; Reverse primer (20 pmol), 2 μl; 10x PCR buffer, 5 μl; 10 mM dNTPs, 1 μl; 15 mM MgCl2, 1.5 μl; Taq DNA polymerase (1.5 U), 0.5 μl; nuclease free water, 33 μl. Thermocyclic conditions were set as follows: initial denaturation step of 4 min at 94ºC followed by 35 cycles consisting of 1 min denaturation at 94ºC, primer-template annealing for 1 min at 55ºC and primer extension at 72ºC for 1 min. A final extension step at 72ºC for 5 min was carried out.

Seven microliters of the PCR product were electrophoresed (Horizon 58, Submarine Gel Electrophoresis, Gibco BRL, Gaithersburg, USA) on a 1.5% agarose gel in Tris Boric acid EDTA (TBE) as running buffer (2 hours at 80 V) with 100 bp DNA size marker. The gels were stained with ethidium bromide (2 μl / 50 ml gel) and analyzed on a UV transilluminator to visualize the expected size (237 bp) product and taken the photographs (GENE GENIUS, Bio imaging system, SYNGENE).

A total of seven blood samples, three from horses (Sample Nos. 094, 177 and 185), two from buffaloes (Sample Nos. 22B and 41B) and two from cattle (Sample Nos. 2C and 47C), were found clinically ill and also found as negative by blood smears, MIT and Ab detection ELISA tests, were tested for detection of infection by PCR specific for ESAG6/7.
RESULTS AND DISCUSSION

The expected PCR product of 237 bp was detected (Fig. 1) in DEAE purified *T. evansi* populations isolated from cattle (Sample No. 58C, shown in Lane 2), buffalo (Sample No. 3B, shown in Lane 3) and horse (Sample No. 215, shown in Lane 4) and also in known *T. evansi* positive blood samples of horse (Sample Nos. 211, 210 and 206; shown in Lane 5, 6 and 7 respectively), buffalo (Sample No. 3B, shown in Lane 8) and cattle (Sample No. 58C, shown in Lane 9) isolates, which were considered as known positive samples. The lane 10 and 11 of figure 1 were devoid of any band which contains PCR products from genomic DNA extracted from clinically ill and suspected horse blood (Sample No. 094) and negative control contains water and no template DNA respectively.

![Electrophoresis gel](image)

**Fig. 1:** Electrophoresis gel (1.5% agarose, stained with ethidium bromide), showing lanes from left to right: Lane 1- 100 bp DNA ladder. Lanes 2 to 4- DEAE purified known positive *T. evansi* of following origin: Lane 2- cattle (No. 58C), Lane 3- buffalo (No. 3B), Lane 4- horse (No. 215). Lanes 5 to 9 - Known positive blood samples of *T. evansi* of following origin: Lanes 5 to 7- horse (Nos. 211, 210 and 206), Lane 8 - buffalo (No. 3B), Lane 9- cattle (No. 58C). Lane 10- Clinically ill and suspected horse blood (No. 094), Lane 11 - Negative control sample contains water and no template DNA.

The PCR products of known negative blood samples (Sample Nos. H1KMP, .46C and 48B) did not show any amplification. So, these samples were found as negative. Out of 7 suspected samples examined by PCR, one blood sample of a horse (Sample No. 177) was found positive by PCR and hence detected as positive for *T. evansi* infection.

Out of 7 blood samples, 3 from horses, 2 from cattle and 2 from buffaloes those were declared negative by examination of Giemsa stained blood smears, MIT and Ab-ELISA, one horse (Sample No. 177) became positive by PCR and thus it has been found that PCR could detect more number of animals than other three tests. The genomic DNA extracted from known negative blood samples of horse (Sample No. H1KMP), cattle (Sample No. 46C) and buffalo (Sample No. 48B) and their PCR products did not show any band.
Detection of *T. evansi* by PCR

This ESAG 6/7 primers are *Trypanozoon* specific and used for amplification of a 237 bp fragment from *T. evansi* genomic DNA. The result of the present study suggests that the known positive cattle, buffalo and horse isolates had this sequence in their genomic DNA and hence an expected PCR product of 237 bp were detected in the lanes 2-9 of Fig.1. The genomic DNA which was extracted from the blood sample of horse (Sample No. 177) had the sequence corresponding to these *Trypanozoon* specific primers and thus showed positive by PCR. Horse No. 177 was harbouring the infection at levels which were undetectable parasitologically and immunologically and more sensitive PCR assays provided evidence of infection in this animal. PCR has been reported as more sensitive for detection of *T. evansi* infection than conventional parasitological techniques (Wuys et al., 1995, Holland et al., 2001). The genomic DNA extracted from known negative blood samples of horse (Sample No. H1KMP), cattle (Sample No. 46C) and buffalo (Sample No. 48B) and their PCR products did not show any band indicating the absence of genomic DNA, thereby found negative by PCR.

In this study based on representative number of samples, it has been observed that PCR could detect positive animal among samples declared as negative by examination of Giemsa stained blood smears, MIT and Ab-ELISA. Detection of infection in more numbers of suspected cases by PCR could unveil more positive cases.

REFERENCES


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