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DETECTION OF *TRYPANOSOMA EVANSI* IN WHOLE BLOOD OF DOMESTIC ANIMALS BY DNA AMPLIFICATION METHOD

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ABSTRACT

Trypanosoma evansi, **a blood protozoan parasite causing 'surra' in domestic animals is widely prevalent in different parts and is of significant economic importance in livestock production. Parasitaemia is often intermittent and is not always possible to demonstrate the parasites in blood and hence diagnosis of trypanosomosis is often difficult. The present investigation has been carried out with the aim of detecting *T. evansi* in cattle, buffaloes and equines in the state of Haryana by parasitological (WBF), and DNA-detecting (TE-PCR) tests. Out of 205 field blood samples tested, only 2% were positive for *T. evansi* by WBF while PCR using synthetic oligonulceotide primers (21 mer sense and 22 mer antisense) targeted to a repetitive nuclear DNA sequence of *T. evansi* detected 60.49% samples positive. Study revealed TE-PCR to be highly sensitive to the conventional parasitological method and could be used for early of diagnosis of trypanosomosis in domestic animals.**

Key words*:* Diagnosis, TE-PCR, Trypanosoma evansi, WBF.

Trypanosoma evansi, a unicellular haemoflagellate causes surra in domestic animals which has the economic significance due to its high morbidity, mortality and chronic debilitating effects. It is the most widely geographically distributed pathogenic trypanosome in Africa, South and Central America and Asia (Luckins, 1998; Pathak and Khanna, 1995; Wernery and Kaaden, 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). The disease has been reported from the states of Haryana, Punjab, Uttar Pradesh, and Jammu and Kashmir in northern India; Rajasthan, Gujarat, and Maharashtra towards west, Andra Pradesh, Karnataka, Tamil Nadu towards south, and Bihar and West Bengal in eastern India. Surra may occur in acute, sub-acute, chronic and inapparent forms. Acute and sub-acute forms of the disease are usually fatal. In buffaloes, cattle and camels, the disease is usually chronic, though acute cases have also been reported. Under field conditions the disease can be confused with any other chronic wasting disease, notably helminthosis and malnutrition.

Though the disease has been studied for past many decades, the definite diagnosis still suffers from low sensitivity and specificity, and as a result, the epidemiology of the disease is far from completely understood. The parasitological examinations frequently fail to detect patent infections because parasitaemia is scanty in peripheral blood in the chronic forms (Killick-Kendrick, 1968). Antibody detection based serological diagnosis has limitations of differentiation between current and past infections, persistent titres and occurrence of false positive results. So PCR is relied for diagnosis of trypanosomosis which was found to be highly sensitive and specific in the present study.

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TABLE 1. Detection of Trypanosoma evansi in domestic animals by TE-PCR.

Animal species	Number of blood samples examined	TE-PCR					
		Number Positive			Percent Positive		
		Strong	Weak	Total	Strong	Weak	Total
Cattle	88	5	53	58	5.68	60.23	65.91
Buffalo	46	5	30	35	10.87	65.22	76.09
Equine	71	0	31	31	-	43.66	43.66
Total	**205**	**10**	**114**	**124**	**4.48**	**55.61**	**60.49**

TE-PCR: T. evansi specific Polymerase Chain Reaction.

Collection of blood: Two hundred and five blood samples from naturally infected, suspected and healthy cattle (n=88), buffaloes (n=46), and equines (n=71) from different places of Haryana state were collected in heparinised vials. The blood samples were examined by Wet Blood Film (WBF) method (Killick-Kendrik, 1968) and positive sample was inoculated (@0.2 ml) into an albino rat intraperitoneally for bulk harvest of T. evansi for extraction of DNA.

Genomic DNA extraction: Genomic DNA was extracted from T. evansi infected rat blood and field blood samples using phenol: chloroform: isoamyl alcohol (25:24:1) following the method of Sambrook and Russell (2001). A set of primers specific to *T. evansi* repetitive DNA sequence probe pMUTec 6.258 as described by Wuyts *et al.* (1994) were used for amplification by TE-PCR.(Table- 1)

Forward primer (21 mer)

5'-TGCAGACGACCTGACGCTACT-3'

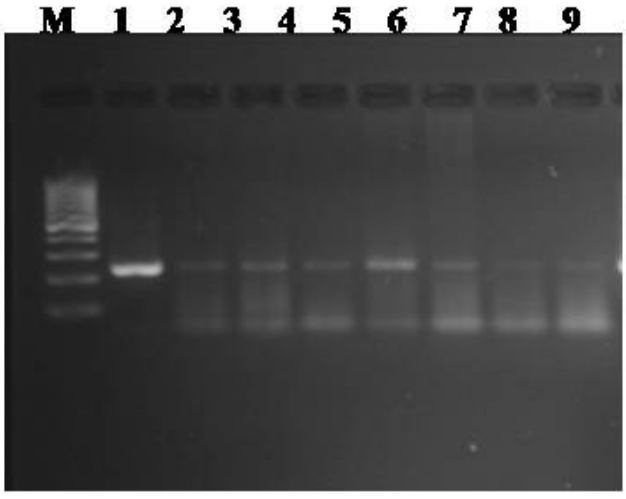
Reverse primer (22 mer)

5'-CTCCTAGAAGCTTCGGTGTCCT-3'

Polymerase chain reaction: TE-PCR was carried out in 50 l reaction mixtures containing 10x PCR buffer with KCl, 1.5mM $MgCl_2$, 200 M each dNTP, primers each at 20 pM and 2U of thermostable Taq DNA polymerase. The cycles included an initial step at 95°C for 4 minutes followed by 29 cycles of denaturing at 95°C for 1 minute, primer annealing at 60°C for 1 minute and primer extension at 72°C for 1 minute. This is followed by last cycle of denaturing at 95°C for 1 minute, annealing at 60°C for 1 minute and. extension at 72°C for 10 minutes and hold at 4°C for indefinite time. Amplification products were resolved in 2% agarose stained with ethidium bromide (5mg/ml) and visualized by UV transillumination and photographs were taken.

Out of 205 blood samples examined by WBF, only four (1.95%) were positive for *Trypanosoma evansi*. The low number of positive samples by WBF can be due to the inherent low sensitivity of the test.

FIG.1. T. evansi - specific PCR using DNA extracted from field blood samples



[Lane M: 100 bp DNA ladder; Lane 1: positive control, Lane 2 - Lane 9 field samples]

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.*, 1995a; Baghel *et al.*, 1996; Singh *et al.*, 2004; Rayulu *et al.*, 2007) and in other countries (Masake and Nantulya, 1991; Trial *et al.*, 1991; Olaho-Mukani *et al.*, 1993; Davison *et al.*, 2000; Ngaira *et al.*, 2003). Wet film examination for the parasite in the infected animals is often the only test used in the field even today, but it is probably the least sensitive test missing 50-80% of the positive cases. Another reason why WBF was low might be probably due to the treatment of animals for trypanosomosis on symptomatological basis which is a quite common practice in the field in India. It was difficult to extract history of treatment of the substantial number of cases, if not all from which the samples had been taken.

TE-PCR reaction was initially optimized using genomic DNA extracted from *T. evansi* infected rat blood which gave a specific band of 227 bp and further the test was applied on field blood samples (Fig.I). Out of two hundred and five samples, 124 (60.49%) were positive by TE-PCR which included 58 cattle (65.91%), 35 buffaloes (76.09%), and 31 equines (43.66%) (Table I). However, most of these samples showed weak band of 227 bp by TE-PCR which indicated that most samples either contained low number of parasites or there were some inhibitors coming in the DNA during its extraction from blood samples. This could be the probable reason for the two WBF-positive samples showing weak positive by TE-PCR. In the present study, TE-PCR positive signal was obtained with template DNA content of 12 trypanosomes extracted from whole blood sample. The limit of detection was higher than that obtained in other studies. Wuyts *et al.* (1994) could achieve limit of detecting 0.5 pg of parasite DNA or one parasite in 10ml of blood sample. Omanwar *et al.* (1999a), however, using the same primers could detect five organisms in 10 l crude blood samples. PCR could detect much larger number of positive samples than WBF. Several investigators have reached similar conclusions using PCR, Mugittu *et al.* (2001) detected trypanosome DNA in 27 (43.55%) out of the 62 parasitologically-negative samples. In another study, Omanwar *et al.* (1999b) using the Wuyts' primers in PCR detected 3 (15%) out of 20 parasitologically-negative blood samples from camels in Rajasthan.

TE-PCR was found to be sensitive when compared to WBF to detect *T. evansi* in infected animal blood. Hence PCR could be used for the large scale screening of domestic animals for trypanosomosis so that diagnosis is possible at the earliest. Moreover TE-PCR has an advantage of detecting the active infection because the test depends on the presence of parasitic DNA.

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