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Prevalence of trypanosomiasis in horses in Chennai by blood smear and PCR study

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Abstract
The aim of the present study was to explore the prevalence of trypanosomiasis in horses. A total of 55 whole blood samples along with blood smears were collected from clinically suspected horses exhibiting signs of anorexia, lethargy, weight loss, and fever, presented at Madras Veterinary College Teaching Hospital, Madras Veterinary College, Chennai during the period from September- 2021 to December- 2021. Blood smears were examined for the presence of organism. Molecular diagnosis was carried out using polymerase chain reaction amplifying repeatative sequence probe pMu Tec 6.428 in genomic DNA of *Trypanosoma evansi.* Blood smear examination revealed 9.1 (5/55) per cent of horses were positive for *Trypanosma evansi* whereas PCR detected 12.7 (7/55) per cent positivity. This results suggested that the PCR technique may be effectively used to detect trypanosomiasis in horses.

Keywords: Horse, *Trypanosma evansi*, blood smear, PCR

1. Introduction
Trypanosomiasis (Surra) is a deadly and an economically important disease of horses caused by *Trypanosoma evansi*, which is characterized by fever, gradual emaciation, anaemia, subcutaneous edema, neurological symptoms, and death. Griffith Evans found *T. evansi*, the first pathogenic mammalian Trypanosome, in the blood of Indian equines and dromedaries in 1880 (Hoare, 1972, Desquesnes *et al*., 2013) [4, 2]. Several species of hematophagous flies, including Tabanids and Stomoxys, have been implicated in mechanical transmission of infection from host to host. The disease is common across India, especially in low-lying areas. However, it is seasonal, with a higher incidence during the monsoon and post rainy seasons due to the predominance of Tabanus flies (Dodiya *et al*., 2020) [3]. In recent years, molecular diagnosis such as polymerase chain reaction (PCR) have been extensively employed in India for the diagnosis and large scale screening of horse blood samples for trypanosomiasis (Naseema *et al*., 2017) [7]. So, the aim of the present study was to explore the current prevalence of trypanosomiasis in horses in Chennai.

2. Materials and Methods
2.1 Collection of Samples
A total of 55 whole blood samples along with blood smears were collected from clinically suspected horses exhibiting signs of anorexia, lethargy, weight loss and fever, presented at Madras Veterinary College Teaching Hospital, Madras Veterinary College, Chennai during the period from September- 2021 to December- 2021. Among 55 horses, 49 males and 06 females with age groups varying from 2 years to 22 years of different breeds; Thoroughbred (51), Marwari (2), Kathiawari (1) and Rocky mountain (1), were presented. Whole blood (3 ml) sample was collected aseptically with EDTA coated vials from jugular vein for DNA extraction and the samples were stored at -80°C until further processing.

2.2 Blood smear examination
Thin blood smears were prepared at the time of collection of blood. Leishman-Giemsa cocktail staining procedure was carried out as per procedure described by Senthil *et al*. (2015) [9]. Stained smears were examined under light microscopy (Olympus, Japan) for the presence of extracellular organism. The organism was identified morphologically.

2.3 DNA extraction from whole blood

Total genomic DNA was extracted from collected whole blood samples using DNeasy® Blood and Tissue Kit (Qiagen, Netherland) according to manufacturer's protocol. The quality of DNA in the final elutes was estimated using Nano spectrophotometer (NanoDrop™ One, Thermoscientific, USA). Extracted DNA samples were preserved at -80 ºC until further analysis.

2.4 Conventional PCR for the detection of the organisms

Polymerase chain reaction was performed targeting repeatative sequence probe pMu Tec 6.428 in genomic DNA of *Trypanosoma evansi* (Wuyts *et al*., 1994) [11]. The primers used (Table-1) amplifies 227 base pair (bp) product. The PCR was conducted in a T100 thermal cycler (Bio-Rad) with a final reaction volume of 25 µL containing 12.5 µL of 2X Taq DNA Polymerase master mix (red), 1 µL of each primer (10 pmol/µL), 5.5 µL of nuclease free water (NFW), and 5 µL of the template DNA. The cyclical conditions were based on the protocol described by Ravindran *et al*. (2008) [8] (Table-2). The amplified products were visualized by electrophoresis in 2% agarose gel and the same was documented in gel doc mega UV transilluminator (Bio- Rad, USA) with 100 bp reference molecular weight marker.

Table 1: Primer Sequence

Primer	Sequence (5'-3')	Product size	Reference
Forward (21 mer)	TGCAGACGACCTCACGCTACT	227 bp	Wuyts *et al*., 1994
Reverse (22 mer)	CTCCTAGAAGCTTCGGTGTCCT		

Table 2: PCR cyclical conditions

Initial Denaturation	90 ºC	7 min	
Denaturation	90 ºC	30 sec	30 cycles
Annealing	60 ºC	30 sec	
Extension	72 ºC	30 sec	
Final extension	72 ºC	7 min	

3. Results and Discussion

In the present investigation, out of 55 blood smears stained by Leishman- Giemsa cocktail staining, 9.1 (5/55) per cent were found positive for *Trypanosma evansi* (Fig-1, Table-3), which is concordant with findings of Abo-Shehada *et al*. (1999) [1], who reported 9.6 percent of prevalence in horses in Jordan by blood smear examination. Out of 55 whole blood samples, 12.7 (7/55) per cent were showed specific amplification as a distinct 227 bp band on agarose gel (Fig-2, Table-3), which is in close accordance with Naseema *et al*. (2017) [7], who reported 13.75 percent prevalence of trypanosomiasis by PCR in horses in an organized stud farm in Chennai, however they could not detect any trypanosomes by wet film examinations. The benefit of blood smear examination is that, no additional special equipment is required except a light microscope, making it a low-cost and technically straight forward method of identifying parasites, particularly in *T. evansi* endemic nations (Killick-Kendrick, 1968) [5]. When the level of parasitaemia remains low and fluctuates, parasite detection techniques like blood smear examination are not always reliable (Laha and Sasmal, 2009) [6]. In the present study, PCR is more sensitive than blood film examination for the diagnosis of trypanosomiasis in horses and can be used for the large scale screening so that diagnosis is possible at the earliest (Shyam *et al*., 2013) [10].

Table 3: Prevalence of *T. evansi* in horses by blood smear and molecular diagnosis

No. of Animals	Blood smear examination positive	Prevalence (%)	PCR positive	Prevalence (%)
55	5	9.1	7	12.7

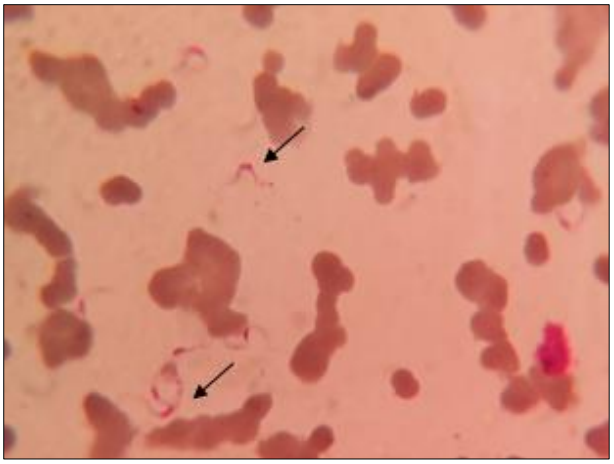

Fig 1: *T. evansi* (arrow mark) in blood smear (Leishman-Giemsa cocktail stain) at 100x magnification

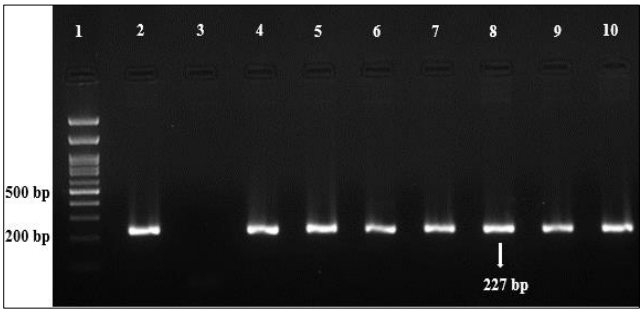

Fig 2: Molecular diagnosis of *T. evansi* by PCR, Lane 1 - 100 bp DNA ladder, Lane 2- Positive control, Lane 3- Negative control, Lane 4 to 10- test positive samples

4. Conclusion

The present investigation to unveil the current prevalence of trypanosomiasis in horses in Chennai detected 9.1 per cent positive by blood smear examination and 12.7 per cent positive by PCR. When compared to blood smear examination, PCR was found to be more sensitive in detecting *T. evansi* in infected animal blood. As a result, PCR may be utilised for large-scale screening of animals for trypanosomiais, allowing for early diagnosis.

5. Acknowledgement

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