



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2019; 7(6): 401-404

© 2019 JEZS

Received: 21-09-2019

Accepted: 23-10-2019

KG Jadhav

Department of Veterinary
Epidemiology and Preventive
Medicine KNP College of
Veterinary Science Shirwal,
Maharashtra, India

MD Meshram

Department of Veterinary
Epidemiology and Preventive
Medicine KNP College of
Veterinary Science Shirwal,
Maharashtra, India

KP Khillare

Department of Veterinary
Epidemiology and Preventive
Medicine KNP College of
Veterinary Science Shirwal,
Maharashtra, India

RS Ghadge

Department of Veterinary
Epidemiology and Preventive
Medicine KNP College of
Veterinary Science Shirwal,
Maharashtra, India

BP Kamdi

Department of Veterinary
Epidemiology and Preventive
Medicine KNP College of
Veterinary Science Shirwal,
Maharashtra, India

KS Bait

Department of Veterinary
Epidemiology and Preventive
Medicine KNP College of
Veterinary Science Shirwal,
Maharashtra, India

Corresponding Author:**KG Jadhav**

Department of Veterinary
Epidemiology and Preventive
Medicine KNP College of
Veterinary Science Shirwal,
Maharashtra, India

Prevalence of trypanosomosis in horses of Western Maharashtra

KG Jadhav, MD Meshram, KP Khillare, RS Ghadge, BP Kamdi and KS Bait

Abstract

A total sum of 136 blood samples of horses were collected from various location and organized farms of Nasik, Baramati, Pachgani, Mahabaleshwar and Maharashtra Police Academy, Nasik. All the samples were screened by microscopic examinations for prevalence of *Trypanosoma* spp. and further confirmed by PCR. Out of 116 samples 12 samples (10.34%) were found to be positive by microscopic examinations and 4 samples were further confirmed as *Trypanosoma evansi* by PCR. For PCR assay primers targeting Variable Surface Glucoprotein (VSG) gene having amplicon size of 400 base pair were used. The prevalence of Trypanosomosis was recorded highest in age group 2-18 years (10.71%) while, lower prevalence was recorded in the age group 0-12 months age (5.26%). For 1-2 years age group, and 18 years age group, the prevalence was recorded as 8.57% and 7.69% respectively. The incidence of Trypanosomosis was recorded highest in Marwari breed (14.28%), followed by Non-descript (9.37%) Spiti (7.69%), Kathiyawadi (6.89%) and Thorough breeds (3.70%).

Keywords: trypanosomosis in horses and variable surface glucoprotein

Introduction

Trypanosomosis is a disease affects all the animals and cause chronic veneral disease in horses. The disease is widely distributed in Asia, Africa and South America, mainly affecting the horses, buffaloes, cattle, camels and wild ruminants. Trypanosomosis is an arthropod borne blood protozoan disease commonly known as Surra (Hoare, 1964), caused by *Trypanosoma evansi*. Flies, including Tabanids and Stomoxys are responsible for transferring infection from host to host, acting as mechanical vectors and characterized by fever, progressive emaciation, anaemia, subcutaneous oedema, nervous signs and death. Among the wild life, it also affects tiger, deer, foxes, jackals, hyenas, also mongoose^[1]. The disease is endemic in nature and occurs in low-lying areas. However, this disease is a seasonal, during rainy and post rainy seasons the incidence is higher due to preponderance of Tabanus flies. 'Surra' in India is generally considered as a disease prevalent mostly in animals of Northern India and prevalence of the disease in equines of Northern India have been reported earlier^[2-4]. The current "gold standard" method for haemoprotozoan diagnosis in many laboratories is microscopic examination of Giemsa's-stained thick and thin blood films. But it requires an expert with experience and well-developed pattern recognition skills to provide an accurate diagnosis despite the development of new diagnostic technologies, microscopic examination remains the method commonly used to diagnose haemoprotozoan.

PCR is more sensitive than conventional parasitological techniques^[5-7]. PCR method appear to be a promising molecular diagnostic technique are also effective for diagnosing haemoprotozoan in equine blood samples^[8]. This study aimed to investigate the prevalence of Trypanosomosis among horses, from three district of western Maharashtra by examining the clinical symptoms of disease horses and detecting the parasite by microscopic examination and further confirmed by PCR.

Materials and methods

Study Area

The present study on "Prevalence of Trypanosomosis in horses" was undertaken for the period of six months (January 2019 to June 2019) and sample were collected from different areas of Mahabaleshwar, Panchgani, Baramati and Nasik. Laboratory work was carried out at Department of Veterinary Epidemiology and Preventive Medicine, DIS Pune, Nasik

Polyclinic, Central Instrumentation Facility of Krantishin Nana Patil College of Veterinary Science, Shirwal, District-Satara 412801. In this study, total 136 horses were examined and 116 sample were screened for Trypanosomosis.

Sample Collection

For the present study, a total of 136 blood samples were

collected from jugular vein in a tube containing an anticoagulant, Ethylene diamine tetra acetic acid (EDTA). These blood samples were kept on ice and were processed on the day of collection. The blood samples with EDTA were used for the parasitological diagnosis. DNA was isolated from blood on same day.

Table 1: Number of horses and samples collected from horses

Sr. No	Area	No. of Horses	No of samples	No. of Blood Smear
1.	Teaching Veterinary Clinical Complex, Shirwal	25	25	20
2.	Maharashtra Police Academy, Nasik	17	17	17
3.	Nasik Polyclinic	30	30	25
4.	Panchgani (Table Land)	27	27	22
5.	Mahabaleshwar (Boat riding Place)	20	20	15
6.	Baramati Region	17	17	17
	Total	136	136	116

Blood smear examination

Total 116 animals were screened for protozoan infection by making thin blood smear. The smears prepared were dried in air, stained with Giemsa stain (30-60 seconds) (by Hi Media Pvt Ltd), and washed with tap water. These well stained blood smears were examined under the high power and oil immersion (x 100) microscope. Trypanosome parasitaemia in the Giemsa stained blood smears were denoted as '+' for 1 to 4 number of parasites/field (X1000); '++' for 5 to 9 number of parasites / field (X1000) and '+++ for more than 9 parasites/field (X1000) [9].

Staining Protocol: Giemsa's staining

1. Treat the dried blood film with methanol for 3-5 minutes.
2. Immerse the slide in the staining fluid containing 30 drops (0.67 ml) of Giemsa's Stain (So11by HiMedia Pvt Ltd) in 30 ml distilled water and stain it for 30-40 minutes.
3. Wash with distilled water allowing the preparation to differentiate for 1 to 3 minutes.
4. Dry the film in air and examine.

Molecular Detection

Polymerase Chain Reaction (PCR)

To confirm the *T. evansi* 4 representative positive samples on Giemsa stain were subjected for Polymerase Chain Reaction (PCR). All the chemicals and reagents required for PCR were supplied by Hi Media. Pvt. Ltd

DNA extraction protocol from blood samples

Standardized protocols to extract DNA as mention by

Table 4: Steps and conditions of thermal cycling for VSG gene primer used in PCR

	Initial Denaturation	Final Denaturation	Annealing	Initial Extension	Final Extension
Temp	94 °C	94 °C	54 °C	72 °C	72 °C
Time	3 min	1 min	1 min	1 min	10 min
	35 cycles				

Electrophoresis of PCR products

After completion of PCR, the amplified products were analyzed and confirmed by agarose gel electrophoresis.

Results

1. Blood Smear Examinations

Total 116 thin stained (Giemsa) blood smears were examined under oil immersion microscopy. Twelve (10.34%) blood smears were found positive by blood smear examination for

manufactures (Himedia kit) was adopted.

Table 2: PCR primer used for detection of Variable Surface Glycoprotein (VSG) gene

Gene	nucleotide sequence	Product length
VSG	Forward primer DITRYF 5_- CGA CCA GCC AGA ACG AGC AGA ACG AGC AGA AT-3_and reverse primer DITRYR 5_- CTT GTC GAT CGA GTT GAC GGT-3_	400.bp (10)

Table 3: Composition of PCR mixture used for detection of VSG gene

Sr no	Components	Concentration (µl)
1.	PCR master mix	12.5
2.	DNA template	5
3.	Primer mix	2
4.	Nuclease free water (NFW)	5.5
5.	Total reaction volume	25

Reference Primers and Positive Control.

Reference primers and Positive DNA sample (positive control) was procured from Himedia.

PCR protocol

The cycling programme performed in a thermocycler is described in table was followed as per Sengupta *et al.*, (2010) with slight modifications [10].

Trypanosomosis. The high parasitemia (+++) was recorded in one horse whereas moderate (++) parasitaemia was observed in two horses and low (+) parasitemia was observed in nine horses (Table No 5). During the study, it was observed that the increased parasitemia was related to high fever and other parameter like increase in heart rate, respiration rate, pulse rate, gut motility. (fig. 1 and table 5)

Table 5: Parasitaemia of infected horses

Sr. No	No. of Infected Horses	Body condition	Parasitemia
1	1	emaciated	+++ (high)
2	2	poor	++ (moderate)
3	9	dull	+ (low)

- a. Overall prevalence:** Present investigation revealed *Trypanosoma* Spp. in 12 (10.34%) samples out of 116 samples.
- b. Area wise prevalence of Trypanosomosis in Horses:** Total 136 samples were collected for the present study, out of which 17 samples were from Baramati, 30 from Nasik, 27 from Panchgani, 17 from Maharashtra Police Academy, Nasik, 25 from TVCC KNP College and 30 from Nasik Polyclinic (Table 4.1, Fig 4.1). Highest number of positive samples were from Panchgani (11.11%), followed by Mahabaleshwar (10.00%), Nasik (6.38%), Baramati (5.88%) and TVCC (4%). The presence of Trypanosomosis is therefore confirmed in the present study.
- c. Age wise prevalence of trypanosomosis in horses:** Influence of Trypanosomosis on age was also taken into consideration in the present study. It was observed that highest prevalence was recorded in the age group 2-18 years (10.71%) while, low prevalence was recorded in the age group 0-12 months age (5.26%). In 1-2 years age group and more than 18 years age group, prevalence was recorded as 8.57% and 7.69% respectively.
- d. Breed wise prevalence of Trypanosomosis horses:** The present data was analyzed for the breed wise prevalence. Blood samples comprised mainly of Kathiawadi, Marwari, Thorough breeds, ND and Spiti breeds of horses. Highest breed wise prevalence was observed in Marwari breed (14.28%), followed by Non-descript (9.37%) Spiti (7.69%), Kathiawadi (6.89%) and Thorough breeds (3.70%)
- e. Polymerase Chain Reaction (PCR):** Representative positive blood samples by Giemsa staining were subjected for isolation of DNA by standardized protocol mentioned by manufacturer (Himedia kit). DNA samples were checked qualitatively by 1.2 agarose gel electrophoresis under trans-illuminator and then subjected to PCR. Four blood samples were subjected to PCR following the standardized protocol for confirmation of *Trypanosoma* spp. All samples revealed 400 bp amplicons after agarose gelelectrophoresis indicating *T. evansi* positivity. Similarly, the reference (positive control) sample also produced approximately 400 bp amplicon (fig. 2)

Discussion

The results of the overall prevalence was similar with Deepak Sumbria *et al.*, (2014) studied the prevalence of Trypanosomosis in central and western Punjab and found 6.8% and 13.63% prevalence respectively^[11], Mavadiya *et al.*, (2010) reported the prevalence of 28.21% of Trypanosomosis which was higher compared to the present study^[12].

The results of Area wise prevalence was accordance with Silva *et al.*, (1995) who reported an outbreak of Trypanosomosis due to *Trypanosoma evansi* in horses of the Pantanal Mato-grossens region of Brazil^[13]. Kumar *et al.*, (1994) diagnosed *Trypanosoma evansi* infection during an outbreak in horses, mules and donkeys in a village Mathura,

India, with a morbidity rate of 100% and mortality rate of 66.6% in horses and 33.3% in donkeys^[14]. Higher prevalence from the samples received from Panchgani in the present study may be due to higher incidence of vectors present in this area.

The results of Age wise prevalence of trypanosomosis was agreement with Kirit Chavda (2012) who studied the 68 horses showing the sign of surra in horses and found higher incidence in adult group of horse (80.95%) compared to aged group of horses (19.05%)^[15]. Furthermore Mavadiya *et al.*, (2010) also studied a total 39 horses and observed higher incidence in adult group of horses (57.14%) followed by aged group of horses (14.28%)^[12]. Findings of both these studies were in concordance with the findings of present study.

The results of the breed wise prevalence was similar with Mavadiya *et al.*, (2010) investigated a total 39 horses for *Trypanosoma evansi* based on blood smear examination, and found higher prevalence in Kathiawadi breed (38.86%) followed by in Non-descript breed (33.33%) and then Marwadi breed (22.22%)^[12].

A finding of present study was in the accordance with findings obtained by Sengupta *et al.*, (2010)^[10]. They could detect the *T. evansi* on 3rd day of post infection in experimentally infected animals. This is because, VSG gene is most reliable for the detection of *T. evansias* it is expressed in early, middle and late stage of infection as well as in carrier phase of disease (Robinson *et al.*, 1999)^[16].

Conclusion

The overall prevalence of equine Trypanosomosis observed was 10.34% in horses by microscopic examinations. The prevalence Trypanosomosis was recorded highest in the age group of 2-18 years (10.71%) while, lower incidence was recorded in the age group of 0-12 months age (5.26%). Trypanosomosis prevalence was highest in Marwari breed (14.28%), followed by Non-descript (9.37%) Spiti (7.69%), Kathiawadi (6.89%) and thorough breeds (3.70%). Variable Surface Glycoprotein (VSG) gene based PCR method was found sensitive and used for *T. evansi* confirmation.

References

- Radostits OM, Gay CC, Hinchcliff KW, Constable PT. Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 10th Ed., W.B. Saunders Company, London, UK, Trypanosomiasis in animals, 2009, 1537-1538.
- Chaudhri SS, Yadav CL, Gupta RP, Ruprah NS. Parasitic Infection of equines in Haryana. Indian journal of Animal Sciences. 1985; (55):766-769.
- Singh V, Chaudhari SS, Kumar S, Chhabra MB. Polyclonal antibody-based antigen-detection immunoassay for diagnosis of *Trypanosoma evansi* in buffaloes and horses. Journal of Veterinary Parasitology. 1995; (56):261-267.
- Soodan JS, Singh KB, Juyal PD, Khakhaira SS. Incidence of *Trypanosoma evansi* in equines in Punjab state. Journal of Veterinary Parasitology. 1995; (9):133-134.
- Wuyts N, Chokesajawatee N, Sarataphan N, Panyim S. PCR amplification of crude blood on microscope slides in the diagnosis of *Trypanosoma evansi* infection in dairy cattle. Annales de la Societe Belge de Medecine Tropicale. 1994; (75):229-237.

6. Desquesnes M. Evaluation of a simple PCR technique for the diagnosis of Trypanosoma vivax infection in the serum of cattle in comparison to parasitological techniques and antigen-enzyme-linked immunosorbent assay. *Acta Tropica*. 1997; (65):139-148.
7. Masake RA, Majiwa PAO, Mollo SK, Makau JM, Njuguna JT, Maina M *et al.*, Sensitive and specific detection of Trypanosoma vivax using the polymerase chain reaction. *Experimental Parasitology*. 1997; (85):193-205.
8. Gibson DG, Young L, Chuang RY, Venter JC, Hutchison CA. 3rd, Smith HO, Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nature Methods*. 2009; (5):343-5
9. Laha R, Bera AK, Panja P, Sikdar A. Clinical and haematobiochemical studies in natural trypanosomiasis of equines. *Indian Journal of Animal Sciences*. 2004; (5):125-127.
10. Sengupta PP, Balumanhendiran M, Suryanarayana VVS, Raghavendra AG, Shome BR, Gajendra MR *et al.* PCR-based diagnosis of surra- targeting VSG gene in small laboratory rodents and buffalo. *Veterinary Parasitology*. 2010; (171):22-31.
11. Sumbria D, Singla LD, Amrita Sharma, Aman Dev Moudgil, Equine MS. trypanosomiasis in central and western Punjab: Prevalence, haemato-biochemical response and associated risk factors. *Acta Tropica*, 2014; 138:44-50.
12. Mavadiya SV, Raval SK, Mehta SA. Epidemiological aspects of Trypanosoma evansi infection in horses. *Indian Journal of Field Veterinarians*. 2010; 6(1):51-53.
13. Silva RAMS, Arosemenab NAE, Herreras HM, Sahib CA, Ferreira MSJ. Outbreak of trypanosomiasis due to Trypanosoma evansi in horses of Pantanal Mato-grossense, Brazil. *Journal of Veterinary Parasitology*. 1995; 60(1-2):167-171.
14. Kumar A, Saxena SC, Sharma SD, Joshi BP. Epidemiology and therapeutic studies of a field outbreak of equine trypanosomiasis. *Indian Veterinary Journal* 1994; (7):74-76.
15. Kirit Chavda. Clinical studies on surra in horses. 2012, 36-37.
16. Robinson NP, Burman, Melville SE, Barry JD. Predominance of duplicate VSG gene conversion in antigenic variation in African trypanosomiasis. *Molecular and Cellular Biology*. 1999; (19):5839-5846.