RESEARCH ARTICLE

Prevalence of Haemoprotozoan Infection in Equines of Maharashtra

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Abstract

The prevalence of haemoprotozoan infection in equines was studied by screening 121 clinically suspected blood samples by PCR and Giemsa stained method for the presence of *Theileria equi, Babesia caballi* and *Trypanosoma evansi* from the horses admitted at Veterinary Clinical Complex of the college from Shirwal, Panchgani, Mahabaleshwar, and Baramati. Out of 121 horses, 12 (9.92%) were positive for *Theileria equi* by PCR and 6 (4.95%) by microscopic examination and all horses were negative for *B. caballi* and *T. evansi*. The blood smear examination technique was less sensitive (4.95%) when compared to PCR for the detection of *T. equi* infection. The highest area-wise prevalence of *T. equi* infection was observed in Panchgani (12.90%) followed by VCC Shirwal (10.71%), Mahabaleshwar (8.82%), and Baramati (7.14%). The prevalence of *T. equi* was recorded highest in the Marwari breed (15.79%) and horses of age group 2-18 years (12.5%). The highest prevalence of *T. equi* in horses was recorded in the month of May (33.33%). These findings can help make a comprehensive control and preventive strategy of equine piroplasmosis in Maharashtra.

Key words: *Babesia caballi*, Horse, Prevalence, *Theileria equi, Trypanosoma evansi*.

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Introduction

Haemoprotozoan infections represent a significant health concern in equines worldwide (Tirosh-Levy *et al.*, 2020). These infections are primarily caused by various protozoan parasites that invade the bloodstream of horses, ponies, and donkeys, leading to debilitating symptoms and potential fatalities if left untreated. Transmission typically occurs through the bite of infected ticks or flies, which serve as vectors for these pathogens. Additionally, vertical transmission from infected mares to their offspring can perpetuate the spread of these parasites within equine populations (Onyiche *et al.*, 2019). In tropical and subtropical areas, parasitic infections are the primary cause of equine diseases that result in large economic losses (Velusamy *et al.*, 2014). Piroplasmosis-infected equines exhibit various symptoms such as fever, haemoglobinuria, pale mucous membranes, jaundice, petechial haemorrhages on the nictitating membrane, peripheral edema, and in some cases, mortality (Onyiche *et al.*, 2019).

Piroplasmosis and Trypanosomosis can be diagnosed using a variety of methods including Giemsa stained blood smears, ELISA and PCR which has been demonstrated to be a sensitive, specific and useful diagnostic tool (Rampersad *et al.*, 2003). PCR using the VSG gene is sensitive for confirming *T. evansi* (Sengupta *et. al.*, 2010; Jadhav *et al.*, 2019). Multiplex PCR using the 18S rRNA gene is sensitive for detecting *T. equi* and *B. caballi* (Aziz *et al.*, 2019). The present study was aimed to investigate the prevalence of haemoprotozoan infection in equines in Mahabaleshwar, Panchgani, Baramati and horses presented to the Veterinary Clinical Complex of Veterinary College, Shirwal, Maharashtra.

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Materials and Methods

Sample Collection and Serological Examination

A total of 121 horses were screened for theileriosis, babesiosis and trypanosomosis. Samples were collected from the different areas of western Maharashtra including Mahabaleshwar (Boat riding place, 34), Panchgani (31), Baramati (28) and horses presented to the Veterinary Clinical

Complex (28) of the KNP College of Veterinary Science, Shirwal (Satara, Maharashtra), India. Laboratory work was carried out at the Department of Veterinary Epidemiology and Preventive Medicine of the college. The duration of the present study was April 2022 to March 2023.

Blood was withdrawn aseptically from the jugular vein and collected in a sterile clot activator vacutainer and a tube containing an anticoagulant EDTA. Serum was separated after centrifugation for 25 min at 1735 g and kept immediately at -20°C until further processing.

Blood Smear Examination

Blood smears from each horse were prepared on a glass slide at the time of blood collection and fixed with methanol. Blood smears were stained using Giemsa (Himedia Laboratories, India) and processed further for microscopic examination for any evidence of haemoparasites. Parasitaemia in the Giemsa-stained blood smears was 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) (Laha *et al*., 2004).

Molecular Detection

To confirm *Theileria equi, Babesia caballi* and *Trypanosoma evansi,* all 121 suspected samples were subjected to PCR. All the chemicals and reagents required for PCR were supplied by HiMedia. Pvt. Ltd. A standardized protocol to extract DNA as mentioned by manufacturers (Himedia kit) was adopted. All 121 samples were screened for both *Theileria equi* and/or *Babesia caballi* by using oligonucleotide primers sequences Bec-UF1 forward and Bec-UR reverse ("Catch all" *Babesia* spp. and *Theileria* spp.) of product length 876 bp for *Babesia* spp. and 913 bp for *Theileria* spp. Then for species-specific identification a common forward primer sequence Bec-UF2 was used and with that for identification of *B. caballi* reverse primer Cab-R and for *Theileria equi* reverse primer Equi-R were used for the product 540 bp and 392 bp, respectively (Table 1).

Table. 1: PCR primer used for detection of 18S ribosomal RNA

Nucleotide sequence	Product length
Bec-UF1 (5'-GTTGATCCTGCCAGTAGTCA-3') Bec-UR (5'-CGGTATCTGATCGTCTTCGA-3')	876 bp & 913 bp
Bec-UF2 (5'-TCGAAGACGATCAGATACCGTCG-3') Cab-R (5'-CTCGTTCATGATTTAGAATTGCT-3')	540 bp
Bec-UF2 (5'-TCGAAGACGATCAGATACCGTCG-3') Equi-R (5'-TGCCTTAAACTTCCTTGCGAT-3')	392 bp

PCR primer used was DITRY F (5'-CGACCAGCCAGAACGAGCAGAAT-3') and DITRY R (5'-CTTGTCGATCGAGTTGACGGT-3') for the detection of the Variable Surface Glycoprotein (VSG) gene, which is specific for the detection of *Trypanosoma evansi* that gives the product of 400 bp indicative of a positive sample.

PCR Protocol

The PCR amplification reaction for 18S ribosomal RNA was carried out in a programmable thermocycler, which included initial denaturation at 96°C for 10 min, followed by 35 cycles each of denaturation at 96°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 10 min. The PCR procedure was run using the appropriate PCR protocol as per Aziz *et al.* (2019). The cycling protocol performed in a thermocycler for VSG gene was as per Sengupta *et al.* (2010) with slight modifications that included initial denaturation at 94°C for 3 min instead of 10 min, and other steps as above. After completion of PCR, the amplified products were analyzed and confirmed by 2% agarose gel electrophoresis.

Results and Discussion

Blood Smear Examination

A total of 121 thin blood smears were examined under oil immersion microscopy for the presence of *T. equi, B. caballi* and *T. evansi*. Out of 121 total samples, six (4.95%) blood smears were positive for *T. equi* (Table 2) and no blood smear was found to be positive for *B. caballi* and *T. evansi*. High parasitaemia (+++) was recorded in one horse, whereas moderate (++) parasitaemia was observed in 3 horses and low (+) parasitemia was observed in 2 horses. It was observed during the study that high parasitaemia was related to high fever, tachycardia and respiration rate. Rampersad *et al.* (2003) reported prevalence of *T. equi* as 9.5% by Giemsa-stained blood smear examination technique. Bahrami *et al.* (2014) also reported a 4.76% prevalence rate of *T. equi* by blood smear examination close to the findings of the present study.

Polymerase Chain Reaction (PCR)

No amplification was observed in DNA extracted from the samples suspected for *Babesia caballi* and *Trypanosoma evansi*. Only fragments of size 913 bp were generated from template DNA representing Theileria infection as described by Aziz *et al.* (2019). Then, this product was subjected to species-specific identification of *Theileria equi* using a unique primer set as described by Aziz *et al.* (2019), which generated fragments of size 392 bp. Out of 121 samples, 12 (9.92%) were positive for *Theileria equi* by PCR which also included samples positive by blood smear examination (Table 2). In this study, Giemsa-stained blood smears were less sensitive than PCR for detecting low levels of parasitemia, subclinical or carrier status. These findings were in agreement with Kumar *et al.* (2020).

Table. 2: Comparison of blood smear examination and PCR for *T. equi*

Parameters	Total no. of cases	Total no. of positive cases	Prevalence rate
Blood smear examination (*T. equi*)	121	06	4.95%
PCR (*T. equi*)	121	12	9.92%

Malhothra *et al.* (1978) found a prevalence rate of 50.1% in North-West India using capillary tube agglutination, which is quite higher as compared to the overall prevalence rate of 9.92% in our study. Nadal *et al.* (2022) also reported the overall prevalence of *T. equi* as 24.5% and the prevalence of *B. caballi* as 2.4% in Europe. Similarly, Sumbria *et al.* (2016) reported a 4.17% prevalence of *T. equi* in Punjab based on the blood smear examinations, which corroborates with the present findings. Karatepe *et al.* (2009) and Alanazi *et al.* (2012) reported prevalence rates closer to the present study as 12.8%, and 10.4% by using IFAT from Turkey and the Central Province of Saudi Arabia, respectively.

Area-wise Prevalence

Out of 28 samples screened from VCC Shirwal, 31 from Panchgani, 34 from Mahabaleshwar, and 28 from Baramati, the highest number of positive samples were from Panchgani (12.90%, 4/31), followed by VCC (10.71%, 3/28), Mahabaleshwar (8.82%, 3/34), and Baramati (7.14%, 2/28). The presence of Theileriosis was therefore confirmed in the present study.

Age-wise Prevalence

The higher prevalence of *T. equi* was recorded in the age group of 2 to 18 years (12.5%), while low prevalence was recorded in the age group of less than one year (6.25%). In the 1-2 years age group and more than 18 years age group, the prevalence was recorded as 9.37% and 8.0% respectively (Table 3). The difference in age-wise prevalence was found to be non-significant in the present study. These findings were similar to Mavadiya *et al.* (2021). Kumbhar *et al.* (2020) however recorded the overall age-wise prevalence of *T. equi* in equines of various localities of Larkana, as 18.5% and 23.80% in below 2 years and above 2 years age groups, respectively. Bharai *et al.* (2020) among the total positive cases also found a high prevalence of 76.92% in adult horses 2-18 years old followed by 15.38% in 1-2 years, 7.69% in foals of less than one year of age, and no case in horses aged above 18 years.

Table. 3: Age-wise prevalence of *Theileria equi* infection in horses of Maharashtra

Age groups	No. of horses	No. positive	Percentage
< 1 year	16	1	6.25
1-2 years	32	3	9.37
2-18 years	48	6	12.50
> 18 years	25	2	8.00

Breed-wise Prevalence

Blood samples comprised mainly of Kathiawadi, Marwari, Thorough breeds, ND, and Spiti breeds of horses. The highest breed-wise prevalence of theileriosis was observed in the Marwari breed (15.79%), followed by non-descript (9.09%), Spiti (8.33%), Kathiawadi (7.14%), and Thoroughbreds (4.76%, Table 4). The prevalence rate recorded in nondescript equines in the present study was comparable with the results of Bharai *et al.* (2020). The breed-wise prevalence was found to be non-significant in the present study, which corroborated with Dodiya *et al*. (2016) and Bharai *et al.* (2020) from Gujarat. However, the age-wise prevalence was found to be statistically non-significant as has been reported by Hussain *et al.* (2014) and Mavadiya *et al.* (2021).

Table. 4: Breed-wise prevalence of *Theileria equi* infection in horses of Maharashtra

Breed	No. of horses	No. positive	Percentage
Marwari	38	6	15.79
Non-descript	22	2	9.09
Spiti	12	1	8.33
Kathiawadi	28	2	7.14
Thoroughbreds	21	1	4.76

Sex-wise Prevalence

Out of 121 horses, the number of males and females was 80 and 41, respectively. In the present study, a higher prevalence of *T. equi* was recorded in males (11.25%, 9/80) as compared to females (7.32%, 3/41). In the present study, male equines showed higher seropositivity than females, possibly due to more male horses being sampled. This observation concurred well with the report of Mavadiya *et al.* (2021).

Month-wise Prevalence

Among the monthly cases screened in horses, the highest prevalence of *T. equi* was recorded in the month of May (33.33%) followed by February (25.0%), June (20.0%), July (16.67%), April (14.29%), March (11.11%), August (9.09%), October (6.67%), and no case was recorded in January, September, November, and December months. On the contrary, Bharai *et al.* (2020) reported higher incidence of piroplasmosis during June (21.95%), followed by August (12.20%), July (9.76%), April, May, September, October, November and December (7.32% each) and least during January, February and March (4.88% each) months with maximum number of positive cases as per blood smear examination during June to October months. Salib *et al.* (2013) found the highest prevalence of piroplasmosis in July (25.81%) and it was more common in October, January, September, and November, may be due to variations in the climate that promote tick population. Ruegg *et al.* (2006) observed piroplasmosis only between mid-May and mid-July and the maximum prevalence was found in mid-June.

Season-wise Prevalence

In the present study based on seasonal pooled data, the highest prevalence of *T. equi* was observed in summer (17.24%) followed by monsoon (10.87%), while the least occurrence was recorded in winter season (4.35%, Table 5). Bharai *et al.* (2020) found the maximum prevalence of piroplasmosis during summer followed by monsoon and the least in the winter season. The higher prevalence of piroplasmosis during summer is generally association with greater population of ticks/vectors in this season.

Table. 5: Season-wise prevalence of *Theileria equi* infection in horses of Maharashtra

Season	No. of horses	No. positive	%
Winter (Nov. to Feb.)	46	2	4.35
Summer (Mar. to May)	29	5	17.24
Monsoon (Jun to Oct.)	46	5	10.87

Conclusion

Out of 121 horses, 9.92% horses had the presence of *Theileria equi* infection, suggesting that the study area (Western Maharashtra) is enzootic for *T. equi*. The prevalence of *T. equi* was highest in the Marwari breed (15.79%), horses of age group 2-18 years (12.5%), in the month of May (33.33%), and in summer season (17.24%). These findings can help make a comprehensive control and preventive strategy.

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