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RESEARCH ARTICLE

Prevalence of Trypanosomiasis, Risk Factors Analysis and Hematological Alterations in Equines of Gujranwala, Pakistan

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ABSTRACT

Trypanosomiasis is an infectious disease of equines characterized by significant economic impacts due to reduced work efficiency, anemia, and mortality. The current study was devised to assess the prevalence, risk factor association, and hematological alterations in the district of Gujranwala, Punjab, Pakistan, in light of the importance of equines in our country as well as the significant losses imposed by trypanosomiasis. For this purpose, 300 equine blood samples (horses=100; mules=100; donkeys=100) were taken by ear-tip puncture and jugular venipuncture aseptically, and examined under a microscope using the Giemsa staining method. Various associated risk factors were also evaluated by using the chi-square test to find out the association with the occurrence of the disease. Moreover, the impact of trypanosomiasis on different blood parameters (Hb, ESR, TEC, TLC, DLC, and PCV) was also assessed in 30 Trypanosomiasis-affected equines. The results depicted that the overall prevalence of Trypanosomiasis in equines was estimated as 13.67%, with individual prevalence of 16%, 13%, and 12% in horses, mules, and donkeys, respectively. The risk factor analysis showed that the prevalence of trypanosomiasis infection in Pakistani equine was significantly ($P<0.05$) correlated with tick infestation, prior tick infestation history, and tick control status. The significant reduction in Hb, TEC, PCV, and lymphocytes was noted. The ESR value showed an upward trend with no notable changes in TLC, monocytes, basophils, neutrophils, and eosinophils. These findings implicate the burden of Trypanosomiasis among equines, which can be mitigated by an effective therapeutic strategy.

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INTRODUCTION

Equine trypanosomiasis is a significant yet neglected hemoprotozoan disease that affects horses, mules, and donkeys worldwide. Trypanosoma is the genus of unicellular flagellate protozoa that causes trypanosomiasis (Shoraba *et al.*, 2024) and is presented as a haemolymphatic and neuropathological disease resulting in three clinical forms: surra, dourine, and nagana (Raftery *et al.*, 2025). The disease is primarily caused by *T. evansi*, the causative agent of Surra, although other species like *T. equiperdum*, *T. brucei*, and *T. vivex* also affect equines in certain regions of the world, such as Asia, the Middle East, and Africa (Büscher *et al.*, 2019). Trypanosomiasis is primarily transmitted mechanically by biting flies, such as *Tabanus* and *Stomoxys* spp., and cyclically by tsetse flies in endemic areas of Africa (Rodrigues *et al.*, 2005). However, species of *Tabanus* tend to be the most significant vectors, with only the females being hematophagous (Marques *et al.*, 2025).

Equines in the endemic regions are essential for rural economies and have a growing role in supporting the livelihoods of around 600 million people globally (Raftery *et al.,* 2025) but trypanosomiasis drastically affects their performance (Stringer *et al.,* 2015) The infection causes a significant workload reduction by diminishing performance in transportation and agriculture, resulting in income losses to the community (Vourchakbé *et al.,* 2020). The disease is clinically characterized by intermittent fever, progressive weight loss, petechiation on serosal membranes, anemia, edema (genitalia or limbs), and generalized weakness (Halder *et al.,* 2019). The chronic manifestation of the disease leads to immunosuppression, which exacerbates secondary bacterial infections, along with ataxia, recumbency, and a lowered fertility rate (Giordani *et al.,* 2016). Mortality rate increases under stressful conditions or in animals with poor nutrition (Rodrigues *et al.,* 2009).

One of the quintessential features of trypanosomiasis is its marked effect on hematological parameters. Equine trypanosomiasis significantly disrupts hematological parameters by inducing anemia through hemolysis and immune-mediated RBC destruction (Sudan, 2023). These changes result in decreased RBC count, Hemoglobin (Hb) levels, and hematocrit (HCT), increased MCV, MCH, and MCHC, along with changes in total leukocytic count and platelet numbers, which are mostly reported (Parashar *et al.,* 2018; Shoraba *et al.,* 2024; Ullah *et al.,* 2025). These alterations not only aid in disease diagnosis but also correlate with the progression and severity of disease (Jaiswal *et al.,* 2015).

Trypanosomiasis infections resulting from *T. evansi* or *T. equiperdum* are fatal if left untreated. Various trypanocidal compounds, such as diminazene aceturate, isometamidium chloride, quinapyramine sulphate, etc., are widely used for the prevention and control of Trypanosomiasis (Jaiswal *et al.,* 2015; Raftery *et al.,* 2018). Despite the availability of these drugs, treatment failures and emerging drug resistance have been frequently documented in equines (Delespaux *et al.,* 2010; Gibson, 2005). Field-based treatment trials are thus necessary for the evaluation of the therapeutic efficacy of trypanocidal drugs, monitoring the restoration of hematological parameters, and therefore guiding the effective disease management strategy (Davkharbayar *et al.,* 2020; Raftery *et al.,* 2025).

Given the growing spread of equine trypanosomiasis, its marked hematological changes, and issues regarding treatment efficacy, extensive studies are needed to evaluate prevalence, delineate hematological changes, and assess therapeutic responses among affected equines. The present study bridges this gap by investigating the distribution of trypanosomiasis in equines, analyzing its impact on various blood parameters, among naturally infected equines in the district of Gujranwala.

MATERIALS AND METHODS

Study area and Study design: This study was conducted in various tehsils of Gujranwala district (32.1476° N, 74.1914° E), Pakistan (Fig. 1). The present study project was designed to find out the distribution of trypanosomiasis in equines, its effect on various hematological parameters, along with treatment trials on affected equines, and risk factor analysis. Samples were collected from suspected equines and processed at the Medicine Research Laboratory, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan.

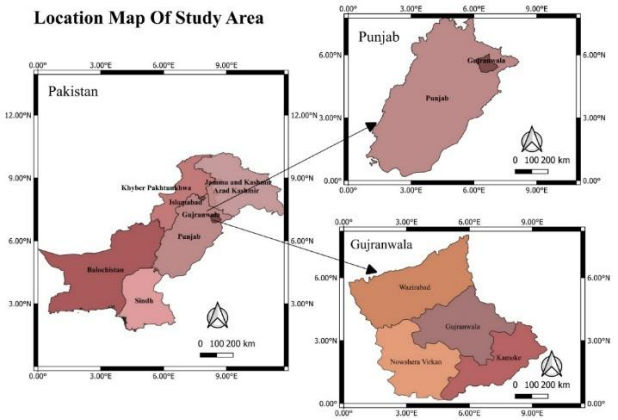

Fig. 1: QGIS map of the study area

Animal ethics: All study procedures were conducted in compliance with the IACUC.

Study population and sampling criteria: A total of 300 blood samples from equines (n=100 horses; n=100 mules; n=100 donkeys) suspected of trypanosomiasis were collected from various private and public animal hospitals of Gujranwala, Pakistan, from July 2025 to September 2025. Blood sampling was carried out irrespective of breed, age, sex, and other parameters (Sabir *et al.,* 2018). The samples were taken from equines exhibiting any of the following clinical signs: petechial hemorrhages on the third eyelid, intermittent fever, anemia, loss of weight, pale mucous membrane, and recumbence (Singh *et al.,* 2021). The data related to all suspected equines were collected in a pre-tested questionnaire.

In addition to sampling, a detailed questionnaire containing animal-related and management-related information was filled out on the spot to check the association of various risk factors like age, breed, and housing with the parasite (Hussain *et al.,* 2016).

Sampling procedure: Approximately 10 mL of blood samples were collected from the equine ear vein and jugular vein using 10 mL sterile disposable syringes, in accordance with aseptic protocols (Zahoor *et al.,* 2022). The blood samples were immediately transferred to clearly marked and labeled

EDTA (Ethylene Diamine Tetraacetate) coated vacutainers BD® for hematological analysis (Khan *et al.*, 2018). Following blood sample collection, the samples were transported under cold chain maintenance to the Medicine Research Laboratory, Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore, Pakistan.

Additionally, a predesigned questionnaire was prepared and filled with the necessary information concerning related risk factors associated with the trypanosomiases in equines in Punjab, Pakistan. The chi-square test was used, and the information was later analyzed to check the association of different risk factors with trypanosomiasis in equines.

Blood smear formation/ diagnostic technique: Thin blood smears were prepared for morphological examination by the method suggested by (Pradesh, 2014). A drop of blood (3-5 µl) was taken on one end of a clean, dry glass slide. Another plane-edge clean glass slide was placed near the blood drop at a 45° angle, and the drop was spread uniformly. The slides were air-dried and fixed in absolute methyl alcohol as well as stained with 10% Giemsa stain. A drop of cedar wood oil was dropped onto the smear, and slides were analyzed through an oil immersion lens under a total magnification of 100X (Tehseen *et al*., 2015; Hussain *et al*., 2016; Khan *et al.,* 2018; Sabir *et al*., 2018; Zahoor *et al*., 2022). The presence of *Trypanosomes* was identified by their morphological characteristics. The prevalence of animals affected with trypanosomiasis was calculated by using the formula.

$$\textbf{Prevalence}\ (\%) = \frac{\text{No. of positive samples}}{\text{Total no. of samples}} \times 100$$

Hematological studies: About 5 ml of blood samples from 36 trypanosomiasis-positive equines (horses=12; donkeys=12; mules=12) were placed into separate tubes having anticoagulant coated with EDTA@ 1 mg/ml of blood to analyze various key hematological parameters such as hemoglobin (Hb) values, total erythrocytes count (TEC), erythrocytes sedimentation rate (ESR), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), Mean cell hemoglobin (MCH), packed cell volume (PCV), total leukocytes count (TLC), differential leukocytes count (DLC), and platelets through hematological analyzer (Parreira *et al.*, 2016; Agina *et al*., 2021; Shoraba *et al.*, 2024; Yamazaki *et al*., 2022).

Statistical analysis: The prevalence of trypanosomiasis was calculated by dividing the number of positive samples by the total number of samples collected, while the data on risk factors were analyzed using Pearson's chi-square test. The results of hematological parameters were analyzed using Student's t-test. The significance of results was checked at 5% level of significance using SPSS (Version 20).

RESULTS

Overall prevalence of trypanosomiasis in equines: This study revealed that, out of 300 equines (horses=100, mules=100, and donkeys=100) suspected of trypanosomiasis from various tehsils of the district Gujranwala, 41 (13.67%) tested positive for trypanosomiasis by direct smear microscopy. The highest prevalence for trypanosomiasis was detected in horses, 16%, followed by mules, 13%, and then donkeys, 12% (Table 1).

Table 1: Prevalence of equine trypanosomiasis in different animals

No. of animals examined based on Microscopy	Positive	Negative	Prevalence (%)
Horses (n=100)	16	84	16
Mules (n=100)	13	87	13
Donkeys (n=100)	12	88	12
Total (n=300)	41	259	13.67

Risk factors associated with equine trypanosomiasis: The findings of various factors associated with trypanosomiasis revealed that trypanosomiasis was more common in tick-infested animals (19%) than in tick-free animals (4.9%), according to the risk factors assessment.

Trypanosomiasis infection was also more common in animals that had previously had tick
infestation or any tick-borne illness. Trypanosomiasis in equines was shown to be substantially correlated ($P<0.05$) with both tick infestation and prior tick infestation history.

Age, sex, and the introduction of new animals were risk factors that were not shown to be statistically significant ($P>0.05$) linked to the incidence. The details are provided in Table 2.

Analysis of hematological parameters of trypanosomiasis-positive equines: Values of various hematological parameters were analyzed across horses, mules, and donkeys, exhibiting marked hematological alterations when compared with healthy equines. Hemoglobin concentration (Hb), packed cell volume (PCV), and Total erythrocyte count (TEC) exhibited a significant reduction in affected horses, mules, and donkeys, indicating a marked trend towards anemia associated with trypanosomiasis. This trend was accompanied by markedly elevated erythrocyte sedimentation rate (ESR) across all equine species, indicating inflammatory activity.

A significant decrease in platelet counts was visible across all equine animals. In contrast, lymphocyte percentages were markedly reduced in affected horses, mules, and donkeys, suggesting lymphopenia as the common feature of disease. Similarly, basophil

Table 2: Association of various risk factors with trypanosomiasis in equines

Study Variable	Category	No. of Equines	Positive (%)	P-value
Sex	Male	143	14 (9.79)	0.704
	Female	157	27 (13.7)	
Age	≤ 4 Year	122	23 (18.85)	0.806
	>4 Year	178	18 (10.11)	
Tick Infestation	Yes	201	30 (14.93)	0.023
	No	99	11 (11.11)	
Previous Tick History	Yes	181	29 (16.02)	<0.001
	No	119	12 (10.08)	
Tick Control Status	Yes	169	20 (11.83)	0.043
	No	131	21 (16.03)	
Introduction of new wild animals	Yes	157	28 (17.83)	0.493
	No	143	13 (09.09)	

P<0.05 indicates significant results

Table 3: Blood parameters averages in trypanosomiasis-affected horses

Parameters	Infected Horses (n=12)	Healthy Horses	P-value
Hemoglobin (g/dl)	06.92±1.59	14.30±4.47	0.0001
ESR (mm/20 min)	66.47±15.53	17.10±4.84	$8.0 *10^{-9}$
TEC (10^{12}/L)	06.98±1.43	14.70±4.47	$4.01*10^{-9}$
TLC (10^{12}/L)	07.96±1.02	08.70±1.05	0.319
PCV (%)	31.66±2.74	39.06±7.87	0.0005
Platelets (10^{12}/µl)	162.50±35.00	322.6±79.26	$3.07*10^{-6}$
Monocytes (%)	01.43±0.41	01.87±0.39	0.354
Lymphocytes (%)	31.43±11.12	45.00±10.91	0.006
Basophils (%)	00.09±0.05	01.10±0.51	$2.2*10^{-6}$
Eosinophil (%)	01.24±0.33	01.67±0.43	0.121
Neutrophils (%)	47.00±10.80	54.60±10.19	0.116

Table 4: Blood parameters averages in trypanosomiasis-affected mules

Parameters	Infected Mules (n=12)	Healthy Mules	P-value
Hemoglobin (g/dl)	06.11±1.40	14.30±4.08	$4.109*10^{-5}$
ESR (mm/20 min)	67.43±16.05	17.60±4.74	$1.55*10^{-8}$
TEC (10^{12}/L)	04.98±1.06	14.50±4.43	$6.91*10^{-6}$
TLC (10^{12}/L)	07.90±0.79	08.65±1.04	0.129
PCV (%)	28.80±3.05	40.00±7.96	0.001
Platelets (10^{12}/µl)	146±28.48	324±79.38	$2.04*10^{-6}$
Monocytes (%)	01.50±0.39	01.90±0.39	0.283
Lymphocytes (%)	26.00±8.3	44.45±10.82	0.0007
Basophils (%)	00.37±0.42	01.09±0.51	0.001
Eosinophils (%)	01.42±0.33	01.70±0.43	0.069
Neutrophils (%)	49.10±10.07	54.40±9.95	0.176

Table 5: Blood parameters averages in trypanosomiasis-affected donkeys

Parameters	Infected Donkeys (n=12)	Healthy Donkeys	P-value
Hemoglobin (g/dl)	06.57±1.09	14.30±4.72	$5.069*10^{-5}$
ESR (mm/20min)	68.40±10.3	17.00±4.9	$3.44*10^{-11}$
TEC (10^{12}/L)	04.25±0.85	14.72±4.47	$3.27*10^{-6}$
PCV (%)	28.30±3.47	39.16±8.15	0.001
TLC (10^{12}/L)	05.22±0.79	08.69±1.03	0.110
Eosinophils (%)	00.51±0.46	01.73±0.41	0.036
Lymphocytes (%)	26.00±9.76	44.95±10.6	0.003
Neutrophils (%)	50.48±11.5	54.80±10.2	0.247
Platelets (10^{12}/µl)	145.2±32.06	323.4±78.53	$1.92*10^{-6}$
Monocytes (%)	01.30±0.46	01.88±0.4	0.179
Basophils (%)	01.50±0.65	01.10±0.51	0.039

percentages were significantly reduced in diseased horses and mules, while basophil percentages were significantly higher in diseased donkeys. Notably, eosinophils indicated no marked changes in horses and mules, while a significant reduction was observed in donkeys. Similarly, neutrophil and monocyte percentages exhibited no significant variations among all affected and healthy equine species. All the details of these parameters are shown in Tables 3, 4, and 5.

DISCUSSION

Developing nations like Pakistan are increasingly threatened by zoonotic parasitic diseases like Trypanosomiasis, which is often neglected, leading to huge economic losses as well as spread to humans (Fong, 2017; Sabir Mughal *et al.*, 2025). Equine trypanosomiasis is a major contributor to morbidity and mortality among equines globally (Raftery *et al.*, 2026). The vector population, like *Tabanus* and *Stomoxys spp.*, is intimately linked to the emergence and development of this parasite in equines (Jones *et al.*, 2008). Several studies in Pakistan have reported trypanosomiasis to be endemic in Pakistan, yet there is no significant data about trypanosomiasis in equines in the Gujranwala district of Pakistan.

The current study investigated the prevalence of equine trypanosomiasis, its hematological findings, and the therapeutic efficacy of commonly used trypanocidal drugs among equines in the Gujranwala district. The current study investigated a significant prevalence of trypanosomiasis (13.67%) among equines, which is in line with the results of Shoraba *et al.* (2024) reporting a prevalence of 12.5% in Dakahlia in the summer season and Golombieski *et al.* (2023) stating a prevalence of 14% through PCR among equines in Brazil, respectively. In contrast, a slightly lower (1.8%) as well as a higher (22.5%) prevalence of trypanosomiasis among equines (Dagnachew *et al.*, 2020) through microscopy in Jabitehenan, Egypt and through microscopy in Jhang (Zahoor *et al.*, 2022), respectively. The marked variation in prevalence across regions of the world may be attributable to factors such as geographical distribution, vectors, climate, differences in technique used, housing conditions, and veterinary care.

In terms of species-wise prevalence, this study reported a comparatively higher prevalence of trypanosomiasis in horses (16%), followed by mules (13%) and then donkeys (12%). Similar findings were reported by Sabir *et al*. (2018), who documented a similar trend in horses, mules, and donkeys with the prevalence of 12%, 5.33%, and 3.67%, respectively. The higher prevalence among horses might be associated with species-specific susceptibility to trypanosomiasis or increased exposure to vectors, etc. Alternatively, the findings of Vourchakbé *et al.* (2020) contradict the findings of the current study, which states that trypanosomiasis prevalence is higher in donkeys (39.3%) than in horses (30.5%). The observed variation in the prevalence of trypanosomiasis among different species may be due to factors like variation in species susceptibility, immune response, management, etc.

The hematological findings in this research clearly indicate the substantial effect of trypanosomiasis on the blood profile of affected equines. A marked reduction in hemoglobin concentration, packed cell volume, and total erythrocyte count among horses, mules, and donkeys was seen in this study, which is similar to previous findings (Parashar *et al*., 2018; Shoraba *et al.*, 2024), indicating the presence of anemia, regarded as a defining feature of Trypanosome infection in equines. Similarly, a significant decrease in platelet counts among all equine species was observed in this study, which is consistent with the findings of Elshafie *et al*. (2018). Marked thrombocytopenia can be explained by damage to platelets due to hemorrhages. Whereas no significant variation pattern was observed for white blood cell percentages, but relative lymphopenia was seen among mules, donkeys, and horses, which is in line with the findings of Marques *et al.* (2000). Alternatively, Elshafie *et al*. (2018) reported monocytosis and reduced eosinophil and neutrophil counts, which is in contrast with our findings. The observed lymphopenia among infected animals may be attributed to immunosuppression due to Trypanosome infection. This study underscores the continued presence of trypanosomiasis among equines and its significant hematological alterations in diseased animals in the Gujranwala district, with isometamidium being most effective against trypanosomiasis infection among equines.

Conclusion

This comprehensive study provides valuable insights into the prevalence of Trypanosomiasis among equines of the Gujranwala district, with slightly higher prevalence in horses, followed by mules and donkeys. Trypanosomiasis-affected animals also exhibited hematological changes, indicative of anemia. Various risk factors were found to be significant for disease occurrence.

Conflict of Interest: The authors declare no conflict of interest.

Authors' contribution: SMA conceived the idea and conducted the research.

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