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Original article

Molecular detection and therapeutic study of *Trypanosoma brucei evansi* from naturally infected horses in Punjab, Pakistan

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Abstract

Trypanosomiasis is one of the severe pathogenic infections, caused by several Trypanosoma species, affecting both animals and humans, causing substantial economic losses and severe illness. The objective of this study was to determine the molecular diagnosis and the risk factors associated with trypanosomiasis in District Jhang, Punjab, Pakistan. For this purpose, blood samples were randomly collected from 200 horses. A predesigned questionnaire was used to collect data on risk factors before the sample collection. The microscopy examination through Giemsa staining, formol gel test and PCR techniques were used to find the prevalence. The prevalence was recorded as 22.5% with microscopy examination, 21% through formol gel test and 15.5% with PCR based results. Analysis of risk factors associated with *Trypanosoma brucei evansi* occurrence was carried out using Chi-square test. It showed the prevalence of *Trypanosoma brucei evansi* was significantly ($p<0.05$) associated with sex, age, rearing purpose and body condition whereas non-significantly ($p>0.05$) with insects control practices. This study supports the idea that PCR is a sensitive, robust and more reliable technique to diagnose trypanosomiasis. It was concluded that *Trypanosoma brucei evansi* is widely prevalent in Jhang (Pakistan), highlighting a dire need to develop control strategies and education programmes to control this disease in developing countries.

Key words: equines, prevalence, *Trypanosome brucei evansi*, therapeutic trial, horses

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Introduction

Horses are grown in Pakistan for numerous purposes, including racing, farming, transportation, companionship, exhibition and breeding (Tehseen et al. 2017). According to economic survey of Pakistan in 2017, there were 4.9 million working equines in Pakistan. Trypanosomiasis (Surra) caused by hemoprotozon parasite *Trypanosoma brucei evansi,* is one of the leading veterinary concerns in the world due to its drastic effects on the health, performance and production of the affected animals. The ailment has detrimental effects on animal health, resulting in immense economic losses (Kandeel and Al-Taher 2021).

Trypanosoma brucei evansi is a hemoflagellate that is spread mechanically by several species of blood sucking flies (mainly *Tabanids* and *Stomoxys*), but in Latin America, the vampire bat (*Desmodus rotundus*) is a vector and reservoir host of *Trypanosoma brucei equinum* causing disease, 'Mal de Caderas' (Stephen 1986). The dogs and cats after getting infected with biting flies serve as the source of transmission for the Trypanosomes in the animal settings with a potential risk to humans (Cardinal et al. 2008). The protozoon is also found in the blood, body fluids and in the in tissues of certain organs (Kennedy 2013). *Trypanosoma brucei evansi* was first to be described by Griffith Evans in the blood samples of equines and dromedaries camels in 1880 (Aregawi et al. 2019). The word "surra" comes from the Indian language and indicates "rotten" which describe the condition of the animals after chronic disease development (Desquesnes et al. 2013).

Trypanosome brucei evansi infects a wide variety of animal hosts, but equids, camels and bovines are most adversely affected animals. It has been diagnosed and depicted in Africa, Asia, South America, and the Middle East (Berlin et al. 2012). The disease was recorded in different regions of Pakistan (Aslam et al. 2010). In livestock, several species of trypanosomes are present, namely *T, brucei, T. vivax, T, evansi, T, congolense* (Baticados et al. 2012).

Clinically, the disease is characterized by emaciation, reduced fertility, neurological signs and immunosuppression combined with anemia and ultimately death in both domestic and wild vertebrates (Tehseen et al. 2015). The symptoms usually appear in horses after 15-20 days of the incubation period associated with fever, emaciation, anemia, fatigue and laziness depending on disease intensity (Sobia et al. 2018). Nervous signs may also develop during the terminal phase of the infection in horses infected with *Trypanosoma brucei evansi* (Ereqat et al. 2020). Horses and camels are highly prone to *trypanosomiasis* and may die within a few weeks or in a month (Yadav et al. 2014).

Blood smear microscopy is the easiest and most frequently used screening test to diagnose *trypanosomiasis* for which a thin blood smear slide prepared is stained with Giemsa stain and observed under a light microscope at 100X magnification. *Trypanosoma brucei evansi* is monomorphic, slender-shaped, with an elongating membrane and well-developed free flagellum present outside the cell surface (Muieed et al. 2010). Morphologically, *Trypanosoma brucei evansi* is a hemoparasite measuring about 14-29 *u*m in length and 1.5-3.45 *u*m in width and contains nucleus (Hassan-Kadle et al. 2019). However, the rate of parasitemia is usually lower and fluctuates notably in the chronic phase, so the involvement of trypanosome can go unreported. Therefore, a more robust and more precise technique needs to be used. In recent years, DNA-based technologies including PCR have been used for the detection of trypanosomiasis (Simwango et al. 2017). Other serological diagnostic tests such as card agglutination test and enzyme-linked immunosorbent assay are also used for detection of *Trypanosoma brucei evansi* infection (Jiang et al. 2018).

The PCR is a very tactful and precise approach for diagnosing surra and has been frequently used in certain countries (Baticados et al. 2012). In addition, the actual sensitivity and specificity of such techniques depend directly on the blood volume examined and the examiner's skills and experience (Elshafie et al. 2013). This method has also been used for large-scale analysis of trypanosomes samples (Simwango et al. 2017). Keeping in view the severity of the disease and economic losses caused by it, the present study was designed to find its prevalence, associated risk factors and therapeutic trial of different drugs.

Materials and Methods

The Ethics Committee of the CVAS, Jhang (Sub Campus University of Veterinary & Animal Sciences, Lahore), initially approved the present thesis (No. CVAS/11516 dated 07-01-2020) and then the final approval of the material was accorded by the Directorate of Advanced Studies (DAS/537 dated 15-06-2020) of the University. Verbal and written consents were obtained from each owner/attendant before collecting blood samples and delivering the treatments to their animals.

A total of 200 blood samples were randomly collected from different breeds of horses from district Jhang Punjab, Pakistan. About 5 ml of the blood sample was collected from the jugular vein of the animals by using 10 ml disposable syringe following aseptic protocol and immediately transferred to properly labelled purple-capped vacutainer (BD Vacutainer® spray-

Table 1. The overall prevalence of *Trypanosoma brucei evansi* in horses based on different tests in District Jhang, Pakistan.

Horses	No. of horses tested	Positive	Prevalence
Microscopy	200	45	22.5%
Formol gel test	200	42	21.0%
PCR based prevalence	200	31	15.5%

-coated K_2EDTA) tubes. Afterward, the sample was transported under the cold chain to Medicine Laboratory (College of Veterinary and Animal Sciences Jhang) for analysis. The blood samples in EDTA tubes were used for the parasitological examination and extraction of DNA for PCR amplification.

Blood smear preparation and Giemsa staining was done according to our standard lab procedures. Briefly, a drop of blood sample was placed on a clean and dry (grease-free) glass slide near an end. Another dry slide with a plane edge was placed near the blood drop on the first glass slide at a 45º angle and spread it uniformly. After drying of the slides were soaked in absolute methyl alcohol for 3-5 minutes and stained with 10% diluted Giemsa stain and observed under oil emersion lens (100X magnification) in the laboratory.

The formol gel test was also used to detect trypanosomal infection as previously performed by (Aslam et al. 2010). Briefly, serum samples were transferred to smaller tubes and two drops of 37 percent formaldehyde solution were added. The analysis was found to be positive if the sample coagulated and become white spontaneously and negative if the serum remained unchanged or coagulated after 30 minutes.

DNA was isolated from whole blood using commercially available (Thermo Scientific Gene JET Whole Blood Genomic DNA Purification Mini Kit #KO781). DNA quality was checked by electrophoresis on an agarose gel. The primers ESAG 6/7 forward ACA TTC CAG CAG GAG TTG GAG and ESAG 6/7 reverse CAC GTG AAT CCT CAA TTT TGT (manufactured by Gene Link™) were used to amplify an approximately 450 bp region of the 18S rRNA gene. PCR was performed in total 25ul of volume of reaction mixture having 12ul Master Mix (VizPure™ PCR 2X Master), 2ul forward and reverse primers each, 4ul of DNA and 5ul of nuclease free water. PCR amplification was performed in thermal cycler (Applied Biosystems® Veriti® 96-Well Thermal Cycler). The cycling condition for *Trypanosome evansi* was at 94°C for 5 minutes, followed by 40 cycles at 93°C for 30 sec, 60°C for 30 sec, and 72°C for 1 minute. The final extension step was performed at 72°C for 10 min. Amplified DNAs were examined by agarose gel electrophoresis (1.3%). A 100 bp ladder was used to check the size of amplified DNA (Thermo scientific®).

A total 18 positive horses were randomly divided into three groups A, B, and C for therapy to evaluate the comparative efficacy of drugs. The horses of group A were treated with Tryban® (Quinapyramine sulphate) @ 5mg/kg body weight intramuscularly (UM Enterprises). The horses of group B were treated with Diminazene® (Diminazen diaceturate) @ 3.5-7mg/kg intramuscularly for 3 days (Star laboratories Pvt. Ltd) whereas Neem leaves powder (*Azadirachta indica*) @ 60 g daily PO was used to treat group C against trypanosomiasis. After elapsing of 7 days post-treatment, blood samples were taken again from the treated animals to recheck the status of animal.

The following formula was employed to calculate the efficacy of used drugs.

Therapeutic efficacy % = No. of recovered animals / No. of total animals × 100

The data thus obtained were analyzed using Pearson Chi-square test for the parameters pertaining to the prevalence of the protozoan while the therapeutic efficacy of the selected drugs was determined by using the SPSS software version 21.

Results

Out of 200 blood samples of horses screened for *Trypanosoma brucei evansi* infection, 45 (22.5%) were detected positive through Giemsa stain, 42 (21.0%) through formol gel test and 31 (15.5%) based on PCR as shown in Table 1. The microscopy was not precisely able to distinct *Trypanosoma evansi* spp. in the blood sample. It may be due to visual confusion in the identification of trypanosome species. Positive samples detected through PCR on electrophoresis gel are shown below in Fig. 1.

Analysis of risk factors associated with *Trypanosoma brucei evansi* occurrence was carried out using Chi-square statistics (Table 2). It showed the prevalence of *Trypanosoma brucei evansi* was significantly associated with the various risk factors. The prevalence was significantly higher (p=0.011) in males as compared with the females. The prevalence of the disease was higher in animals above 4 years of age than in the younger animals. There was a significant difference in prevalence among age groups (p=0.032). The prevalence of disease was significantly (p=0.027) higher

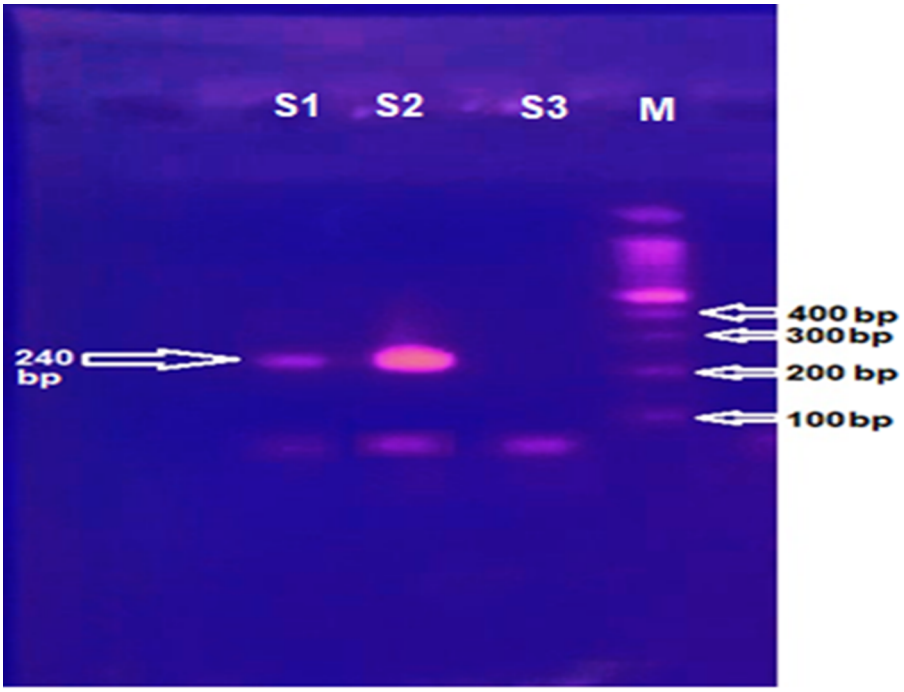

Fig. 1. PCR products from DNA of *T. evansi* in the tested samples. Lane marked as M represents marker (ladder), S1 and S2 are positive samples at 240bp.

Table 2. PCR based prevalence of *Trypanosoma brucei evansi* and its associated risk factors in horses of Jhang district, Pakistan.

Variables	Level	No. of horses tested	Positive	Prevalence %	*P*-value
Sex	Male	120	23	19.16	0.011*
	Female	80	8	10.0	
Age	>4 years	100	19	19.0	0.032*
	1-4 years	70	9	12.85	
	< 1 year	30	3	10.0	
Purpose	Transportation	140	27	19.28	0.027*
	Breeding	60	4	6.66	
Body Condition	Good	107	5	4.67	0.012*
	Emaciated	93	26	27.95	
Insects control	No	143	24	16.78	0.25
	Yes	57	7	18.28	

* Values indicate statistically significant difference ($p<0.05$) associated with various risk factors

in working animals as compared with the breeding animals. The disease was highly associated with emaciation (p=0.012) as compared with good body condition. However, the insects control practices did not represent a significant role (p=0.25).

Therapeutic efficacy of all compounds was assessed based on disappearance or amelioration of clinical signs, absence of *Trypanosoma brucei evansi* parasite in blood examination and PCR on day 7 post-treatment. The results of therapeutic trial were depicted in Table 2. In group A, all the six animals were treated with Tryban® (*Quinapyramine sulphate*) @ 5mg/kg on alternative days along with supportive therapies and recovered successfully. The efficacy of Tryban® (*Quinapyramine sulphate*) was 100%. The group B treated with *Diminazine aceturate* along with supportive treatment, four animals recovered. The efficacy of *Diminazine aceturate* was 66.6%. In group C treated with Neem leaves powder (*Azadirachta indica*) @ 60 g daily PO along with same supportive therapy, three animals recovered without any side effects. The efficacy of Neem leaves powder (*Azadirachta indica*) was 50% (Table 3). The drug *Quinapyramine sulphate* and Neem leaves powder (*Azadirachta indica*) was very effective in removing the Trypanosomes from blood with less side effects.

Table 3. Drug trials and their efficacy percentage.

Group	Therapeutic agents, their composition, Dosage and route of administration	No. of horses treated	No. of horses recovered	Drug efficacy
A	Tryban® (Quinapyramine methyl sulphate) @ 5mg/kg body weight SC (UM Enterprises)	6	6	100%
B	Diminazene® (diminazene diaceturate) @ 3.5-7mg/kg IM for 3 days	6	4	66.6%
C	Neem leaves powder (Azadirachta Indica) @ 60g daily PO for 7 days	6	3	50%

Discussion

In the present study the prevalence of trypanosomiasis by microscopy in horses was 22.5%. Several studies in Pakistan reported the prevalence of *Trypanosoma brucei evansi* in different species. The prevalence of trypanosomiasis was 13.7% in Sindh, Pakistan (Shah et al. 2004). The difference in rate of infection of trypanosomiasis in Sindh, Pakistan may be due to difference in environmental conditions and climate changes as compared with Punjab. Similarly, 2% prevalence was reported in horses and donkeys by thin smear blood microscopy from Gujranwala, Pakistan (Aslam et al. 2010).

The prevalence of Trypanosoma *spp*. has been reported by several countries. The 56.25% prevalence of *Trypanosoma brucei evansi* in camels was reported in Iraq (Al-Amery et al. 2017) and 17.61% from Iran (Mirshekar et al. 2017) and 0.96% from Egypt (Henidy et al. 2019), 15.25% prevalence in buffaloes from India (Gangwar et al. 2019), 9.1% in horses and donkeys from Gambia (Pinchbeck et al. 2008) and 34.4 percent in camel samples 6.8 percent of donkey and 7.7% of dog samples were found positive by thin blood smear microscopy from India (Mandovera et al. 2008). Our study findings are consistent with these studies that *Trypanosoma brucei evansi* is widely prevalent in these countries, like in Pakistan.

Formol-gel test is a non-specific biochemical test that has been used to detect the chronic infection. However, researchers have attempted to report the prevalence of *Trypanosoma* spp. through this test. Sobia et al. (2018) revealed 51.1% prevalence *Trypanosoma* species through formol gel test from cattle, goat, sheep, donkeys and camels from Pakistan. Similarly, 24% equines were found to be positive through the formol gel test from Lahore, Pakistan (Tehseen et al. 2015, Aslam et al. 2010). In the present study, we found 21% prevalence of trypnosoma from district Jhang.

In the current study, it has been found that PCR-based techniques are more sensitive and precise for the identification than microscopy and other techniques. In addition, this test was also used in the large scale for analysis of trypanosomes samples (Desquesnes et al. 2013). The results of the current research were also accordance to the findings of Alanazi et al. (2018), who found that PCR was more specific for the detection of tryponosomal antigens. In another study (Henidy et al. 2019), it was shown that the use of PCR in the diagnosis of trypanosomiasis is more empathetic and precise, particularly in cases with low infection rates, as well as potentially valuable in epidemiological studies. Similarly, Aslam et al. (2010) also found that PCR test sensitivity for the detection of trypanosomes in horses and donkeys blood samples was higher than Formol Gel test. In the present study, PCR based prevalence of *Trypanosoma brucei evansi* is 15.50%. Some of the samples that were positive in microscopy and formol gel test, were negative when analyzed with PCR. These findings are consistent with (Baticados et al. 2012) who also found that PCR is more precise and sensitive technique than other tests.

In the present study the prevalence of *trypanosomiasis* is higher in older animals than the younger animals. Our research is in accordance with the study of Elhaig et al. (2018) who also indicated the greater prevalence in old horses as compared with adult animal. This may be attributed to extreme stress by using them to transport goods from one location to another or due to poor management. In contrast to our study Tehseen et al. (2017) found the non-significant disparity between age groups, indicating that all age groups are equally affected by *trypanosomiasis* (surra). In the present study, sex-wise prevalence is higher in male horses than female horses that is in accordance with the findings of Nurcahyo et al. (2019) who also found a higher prevalence in males as compared with female. Our findings are inconsistent with Hasan et al. (2006) who found both sex are equally affected. The lower prevalence in breeding horses at farm as compared to transport horses may be related to effective management, good nutrition and accessibility of veterinarians. Horses with poor body condition and used for transportation purpose at brick-kilns showed higher prevalence for *Trypanosoma brucei evansi* that may be due to

stress, work overload, poor nutrition, and increased burden of other infections. Our results are consistent with findings of Hasan et al. (2006), who found a higher prevalence in horses that were emaciated and used for draft purposes.

After confirmation of infection through PCR, all animals were treated with *Diaminazine aceturate, Quinapyramine sulphate*, and Neem leaves powder (*Azadirachta indica*). Quinapyramine sulphate had an outstanding response as treatment. Four out of 6 animals were cured after the first dose and two animals were cured after the second dose. The efficacy of the drug was considered to be 100%. Our results are inconsistent with the study of Hota et al. (2019) who used *Quinapyramine sulphate* in India, for the treatment of *T. evansi* and Matovu et al. (2020) who used it for the treatment of "*surra"* in horses and camels and obtained highest efficacy. *Diminazene diaceturate* was also found to have a good effect on the disease and showed to have 66.6% efficacy, but the drug showed adverse reaction as reported earlier (Raftery et al. 2018). Neem leaves powder (*Azadirachta indica*) was found to be 50% effective in curing of animals with less toxic effects. These results are similar with the study reported by Omoja et al. (2011). The findings of Mahboob et al. (2008) are also consisted with our study. They used *Azadairchta indica* dried leaves at the same dose in horses infected with strongylosis and cured the animal. The efficacy of 10% neem water extract against nematodes in sheep was observed by Amin et al. (2011) and significant declines in EPG count were observed. The anti-trypanosomal activity of *Azadirachta indica* may be due to the presence of secondary metabolites in the plant capable of producing radicals that act against the metabolism of the parasite (Enyanwu et al. 2018). The pathological effect of trypanosoma is mediated by release of cytokines and nitric oxides. Active Neem (*Azadirachta indica*) compounds such as phenol act to neutralise poisonous chemicals, thereby break the life cycle of the organism. Ajabe et al. (2018) also suggested that the failure of the extract to completely kill the parasite may be attributed to the low levels of active compounds in the aqueous extract.

Conclusion

Trypanosomiasis is highly prevalent in Jhang in subacute and chronic form. The PCR test is more sensitive technique for identification trypanosomal infection in horses compared to Formol Gel test and Thin Blood Smear microscopy. Quinapyramine sulphate can be used effectively to treat the infected animals. Neem leaves powder has proved to possess anti trypanosomal potentials and can be used in case of unavailability of a drug, especially in ruler areas as a natural remedy. This study suggested the higher rural authorities and Government officials to make a policy for the control of this disease.

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References

Ajabe JS, Borikar ST, Digraskar SU, Siddiqui MF, Narladkar BW, Kumbhar NS **(2018)** Therapeutic efficacy of *Azadirachta indica* (Neem) leaves powder against helminthosis in donkeys. Pharm Innov J 7: 370-372.

Al-Amery AM, Faraj AA, Majeed SM **(2017)** Detection of Haemoprotozoa in camels in Al-Najaf province, Iraq. Int J Adv Biol Res 7: 238-241.

Alanazi AD, Puschendorf R, Salim B, Alyousif MS, Alanazi IO, Al-Shehri HR **(2018)** Molecular detection of equine trypanosomiasis in the Riyadh Province of Saudi Arabia. J Vet Diagn Invest 30: 942-945.

Amin MR, Mostofa M, Islam MN, Asgar MA **(2010)** Effects of neem, betel leaf, devil's tree, jute and turmeric against gastrointestinal nematodes in sheep. J Bangladesh Agril Univ 8: 259-263.

Aregawi WG, Agga GE, Abdi RD, Buscher P **(2019)** Systematic review and meta-analysis on the global distribution, host range, and prevalence of *Trypanosoma evansi*. Parasit Vectors 12: 67.

Aslam A, Chaudhary ZI, ur Rehman H, Ashraf K, Ahmad N, Yaqub T, Maqbool A, Shakoori AR **(2010)** Comparative evaluation of parasitological, serological and DNA amplification methods for diagnosis of natural trypanosomal infection in equines. Pakistan J Zool 42: 371-376.

Baticados WN, Castro DL, Baticados AM **(2011)** Parasitological and PCR detection of *Trypanosoma evansi* in buffaloes from Luzon, Philippines. Ceylon J Sci (Bio Sci) 40: 141-146.

Berlin D, Nasereddin A, Azmi K, Ereqat S, Abdeen Z, Eyal O, Baneth G **(2012)**. Prevalence of *Trypanosoma evansi* in horses in Israel evaluated by serology and reverse dot blot. Res Vet Sci 93: 1225-1230.

Cardinal MV, Lauricella MA, Ceballos LA, Lanati L, Marcet PL, Levin MJ, Kitron U, Gurtler RE, Schijman AG (2008). Molecular epidemiology of domestic and sylvatic Trypanosoma cruzi infection in rural northwestern Argentina. Int J Parasitol 38: 1533-1543.

Desquesnes M, Holzmuller P, Lai DH, Dargantes A, Lun ZR, Jittaplapong S **(2013)** *Trypanosoma evansi* and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. Biomed Res Inter 2013: 194176.

Elhaig MM, Sallam NH **(2018)** Molecular survey and characterization of *Trypanosoma evansi* in naturally infected camels with suspicion of a Trypanozoon infection

in horses by molecular detection in Egypt. Microb Pathog 123: 201-205.

Elshafie EI, Sani RA, Hassan L, Sharma R, Bashir A, Abubakar IA **(2013)** Seroprevalence and risk factors of *Trypanosoma evansi* infection in horses in Peninsular Malaysia. Res Vet Sci 94: 285-289.

Enyanwu OM, Igwe OH, Emmanuel E **(2018)** Invivo Anti Trypanosomal Activity of Aqueous Extractof *Azadirachta indica* Leaves on *Trypanosoma brucei brucei* Infected Mice. Int J Infect Dis Ther 3:13-17.

Ereqat S, Nasereddin A, Al-Jawabreh A, Al-Jawabreh H, Al-Laham N, Abdeen Z **(2020)** Prevalence of *Trypanosoma evansi* in livestock in Palestine. Parasit Vectors 13: 21.

Gangwar P, Shukla PC, Singh B, Gawai, P **(2019)** Prevalence of bovine trypanosomosis in and around Jabalpur. J Entomol Zool Stud 7: 9-12.

Hasan MU, Muhammad G, Gutierrez C, Iqbal Z, Shakoor A, Jabbar A **(2006)** Prevalence of *Trypanosoma evansi* infection in equines and camels in the Punjab region, Pakistan. Ann NY Acad Sci 1081: 322-324.

Hassan-Kadle AA, Ibrahim AM, Nyingilili HS, Yusuf AA, Vieira TS, Vieira RF **(2019)** Parasitological, serological and molecular survey of camel trypanosomiasis in Somalia. Parasit Vectors 12: 598.

Henidy S, Ashmawy K, Abuekkada S, Elshanat S, Dewir A **(2019)** Field investigation and comparative evaluation of diagnostic tests of *Trypanosoma evansi* in camels in Egypt. Egypt Vet Med Soc Parasitol J 15: 1-10.

Hota A, Maiti SK, Ghosh RC **(2019)** Therapeutic efficacy of quinapyramine compounds in *T. evansi* affected cattle. Pharma Inov J 8: 467-471.

Kandeel M, Al-Taher A **(2021)** Molecular landscapes of deoxyuridine 5′-triphosphatase (dUTPase) as a drug target against camel *Trypanosoma evansi*. Pak Vet J 41: 235-241.

Kennedy PG **(2013)**. Clinical features, diagnosis and treatment of Human African Trypanosomiasis (Sleeping sickness). Lancet Neurol 12: 186-194.

Mahboob K, Khan JA, Khan MS **(2008)** Prevalence, chemotherapy and haematology of strongylosis in horses of district Layyah. J Anim Plant Sci 18: 117-119.

Mandovera V, Shringi A, Bhardwaj B, Bhati A, Pachaury R, Godara A **(2011)** An Outbreak of Trypanosomiasis in Camel at Rajasthan. Ind J Appl Res 4: 566-567.

Matovu E, Mugasa CM, Waiswa P, Kitibwa A, Boobo, Ndung'u JM **(2020)** Haemoparasitic infections in cattle from a *Trypanosoma evansi* rhodesiense sleeping sickness endemic district of eastern Uganda. Trop Med Infect Dis 5: 24.

Mirshekar F, Yakhchali M, Shariati-Sharifi F **(2017)** *Trypanosoma evansi* infection and major risk factors for Iranian one-humped camels (*Camelus dromedarius*). J Parasit Dis 41: 854-858.

Muieed MA, Chaudhry ZI, Shakoori AR **(2010)** Comparative studies on the sensitivity of polymerase chain reaction (PCR) and microscopic examination for the detection of *Trypanosoma evansi* in horses. Turk J Vet Anim Sci 34: 507-512.

Nurcahyo W, Yowi MR, Hartati S, Prastowo J **(2019)** The prevalence of horse trypanosomiasis in Sumba Island, Indonesia and its detection using card agglutination tests. Vet World 12: 646-652.

Omoja VU, Anaga AO, Obidike IR, Ihedioha TE, Umeakuana PU, Mhomga LI, Asuzu IU, Anika SM **(2011)** The effects of combination of methanolic leaf extract of *Azadirachta indica* and diminazene diaceturate in the treatment of experimental *Trypanosoma brucei brucei* infection in rats. Asian Pac J Trop Med 4: 337-341.

Pinchbeck GL, Morrison LJ, Tait A, Langford J, Meehan L, Jallow S, Jallow J, Jallow A, Christley RM **(2008)** Trypanosomosis in The Gambia: prevalence in working horses and donkeys detected by whole genome amplification and PCR, and evidence for interactions between trypanosome species. BMC Vet Res 4: 7.

Raftery AG, Jallow S, Rodgers J, Sutton DG (**2019)** Safety and efficacy of three trypanocides in confirmed field cases of trypanosomiasis in working equines in The Gambia: a prospective, randomised, non-inferiority trial. PLoS Negl Trop Dis 13: e0007175.

Shah SR, Phulan MS, Memon MA, Rind R, Bhatti WM **(2004)** Trypanosomes infection in camels. Pak Vet J 24: 209-210.

Simwango M, Ngonyoka A, Nnko HJ, Salekwa LP, Ole-Neselle M, Kimera SI, Gwakisa PS **(2017)** Molecular prevalence of trypanosome infections in cattle and tsetse flies in the Maasai Steppe, northern Tanzania. Parasit Vectors 10: 507.

Sobia M, Mirza IS, Nuzhat S, Sonia T, Abul H, Hafiz MA, Muhammad D, Muhammad FQ **(2018)** Prevalence and characterization of Trypanosoma species from livestock of Cholistan desert of Pakistan. Trop Biomed 35: 140-148.

Stephen LE **(1986)**. Trypanosomiasis: a Veterinary Perspective. New York, Pergamon Press, p 450.

Tehseen S, Jahan N, Qamar MF, Desquesnes M, Shahzad MI, Deborggraeve S, Buscher P **(2015)** Parasitological, serological and molecular survey of *Trypanosoma evansi* infection in dromedary camels from Cholistan Desert, Pakistan. Parasit Vectors 8: 415

Tehseen S, Jahan N, Desquesnes M, Shahzad MI, Qamar MF **(2017)** Field investigation of *Trypanosoma evansi* and comparative analysis of diagnostic tests in horses from Bahawalpur, Pakistan. Turk J Vet Anim Sci 41: 288-293.

Yadav SC, Kumar R, Manuja A, Goyal L, Gupta AK **(2014)** Early detection of *Trypanosoma evansi* infection and monitoring of antibody levels by ELISA following treatment. J Parasit Dis 38: 124-127