Journal of Animal & Plant Sciences, 32(3): 2022, Page: 663-669
ISSN (print): 1018-7081; ISSN (online): 2309-8694
http://doi.org/10.36899/JAPS.2022.3.0467

EFFICACY OF QUINAPYRAMINE SULPHATE, ISOMETAMEDIUM CHLORIDE AND DIMINAZENE ACETURATE FOR TREATMENT OF SURRA

M. Amjad[1], M. H. Saleem[*1], M. Z. Iqbal[1], A. Hassan[6], A. Jabbar[1], M. Ashraf[2], M. Qasim[3], A. Ullah[1], M. M. Tolba[4], H. A. Nasser[5], S. Naaz[7] and I. Ahmad[8]

[1]Department of Veterinary Medicine, [2]Department of Theriogenology, [6]Department of Veterinary Surgery and Pet Sciences, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore, Pakistan;
[3]Department of Economics, Finance, and Statistics Jonkoping University, Sweden
[4]Biomedical Informatics and Biotechnology Group, Informatics and Systems Department, Division of Engineering Research, National Research Centre, Cairo, Egypt;
[5]Microbiology and Public Health Department. Faculty of Pharmacy, Helipolis, University, Egypt.
[7]Livestock and Dairy Development Department Punjab Pakistan
[8]Department of Veterinary Clinical Sciences, University of Poonch Rawalakot Azad Kashmir-Pakistan
Corresponding Author email: dr_mhs@uvas.edu.pk

ABSTRACT

Trypanosomiasis (Surra) is a parasitic and zoonotic disease caused by *Trypanosoama evansi*, transmitted by insect vectors *Tabanus* and *Stomoxys* mechanically. The aim of the present study was to determine the therapeutic efficacy of various trypanosidal drugs against trypanosomiasis in Thoroughbred horses. Horses having clinical signs of trypanosomiasis were diagnosed through blood smear through a microscope were selected for this study. The infected horses were divided into three experimental groups for therapeutic trials. Animals in group A were treated with a single dose of quinapyramine sulphate @ 3000mg/ml per 50 /kg body weight; group B was treated with a single dose of isometamedium chloride Hydrochloride@ 0.5 mg/2.5 ml of 1% solution per 50/kg body weight; group C was treated with a single dose of diminazene aceturate@ 2360 mg/15 ml per 100/kg. Results revealed that significant ($P<0.0001$) decline in the values of erythrocyte counts (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), platelets (PLT) and a significant ($P<0.0001$) increase in white blood cells (WBC), granulocytes, and monocytes in infected horses as compared to healthy ones. Therapeutic trials indicated that quinapyramine sulphate that showed 100% efficacy at 21th days had significantly higher than isometamedium chloride and diminazene aceturate (95.83 and 75% efficacy, respectively). The hematological parameters of recovered horses were significantly restored to normal values on day 21 after treatment. It is concluded that quinapyramine sulphate is the drug of choice against trypanosomiasis in Thoroughbred horses.

Keywords: Trypanosomiasis, Thoroughbred horses, Trypanosidal drugs, hematological effect, therapeutic efficacy.

Published first online October 20. 2021 Published final May 30. 2022

INTRODUCTION

Trypanosomiasis is a parasitic and zoonotic disease caused by *Trypanosoma evansi* that is commonly known as Surra in horses and camel, discovered by the German Veterinary officer Evans in 1880 (Juyal, P.D 2011; Echeverria *et al.* 2019). This parasite can also infect sheep, goats, cattle, buffalo, dogs, and wild animals (Herrera *et al.* 2004; Fernández *et al.* 2009; Desquesnes *et al.* 2013). This disease is widely present in Africa (Fikru *et al.* 2015), America (Wells, 1984), Asia (Tuntasuvan *et al.* 2003), Europe (Tamarit *et al.* 2010) and have been first reported in camels in Pakistan (Tehseen *et al.* 2015).

The transmission of the disease occurs through biological insect vectors that is Stomoxys and Tabanus species (Muieed *et al.* 2010) as well as via vampire bat (*Desmodus rotundus*) in South America (Desquesnes *et al.* 2013). Several clinical signs of Trypanosomiasis have reported in horses, i.e., intermittent fever, severe conjunctivitis, anemia, anorexia, weakness, petechial hemorrhages on third eyelid, loss of body weight, cutaneous eruption, edema of reproductive organ, and also cause nervous signs like ataxia, hind quarter paralysis, hyperexcitability when it crosses blood-brain barrier (Elshafie *et al.* 2018). The hemoparasite, including *Trypanosoma evansi* causes economic losses up to 7,486,000 US$ in the form of mortalities, reduced fertility, reduction in draught capacity, treatment cost, and extra maintenance cost of infected animals (Mukhtar *et al.* 2017). Therefore, proper diagnosis, treatment, and control of this disease are necessary.

The tentative diagnosis of Trypanosomiasis under field conditions is based on clinical signs. The clinical signs are not sufficient for diagnosing the disease that is usually confused with other chronic diseases, particularly helminthiasis and malnutrition; therefore, laboratory diagnosis is necessary for proper treatment (Kumar *et al.* 2013). In acute infection, this parasite can be

demonstrated in fresh blood through thick and thin smear examination, but in chronic infection, it can be detected in blood smear collected from lymphoid tissue due to low level of parasitemia (Halder *et al.* 2019).

The control of this disease depends upon proper management, diagnosis and treatment. Moreover, there is a limitation in the diagnosis and control of disease in this region due to many problems like lack of knowledge, improper management and development of drug resistance. In this study hematological effect and efficacy of quinapyramine sulphate (QS), isometamedium chloride (IC) and diminazene aceturate (DA) have been evaluated against trypanosomiasis in horses**.** Therefore, we hypothesized that different trypanosomal drug improve the health status of horses with similar efficacy against trypanosomaisis and improve hematological parameters. The aim of this study was (a) to evaluate the efficacy of different trypanosomal drug, (b) health status of infected animals and (c) determine the changes in the hematological parameters in horses.

MATERIALS AND METHODS

Study area: The current study was carried out in Tehsil Pattoki (2 stables of Village Wha Adan and 1 stable of Changa Manga) district Kasur, Punjab-Pakistan. During this study, the samples from the horses were collected at a stable and referred to Veterinary Teaching Hospital Ravi Campus UVAS. The study design and all procedures in the study were approved under the guidelines of ethical committee of the University of Veterinary and Animal Sciences, Lahore, Pakistan.

Inclusion Criteria of Animals and smears examination: The clinically infected horses having age 5-10 years were included in the study. The age of individual animals was documented on the basis of information provided from the owner. The horses showed various clinical signs of high fever, petechial hemorrhages on the third eyelid, anemia, weakness, conjunctivitis, and nervous signs, some extant like ataxia. Preparation of blood smear slides was done from blood collected from jugular vein for parasitological examination. A drop of 4-5µl blood on the slides and thin smear was prepared. Smears were air dried, fixed with methanol and stained with Giemsa for parasitological examination. The small drop of cedarwood oil was placed at the end of the blood smear. It was then examined by a microscope using a 100X oil immersion objective lens to detect parasite extracellularly, as previously reported by (Durrani *et al.* 2017). The parasite (*T. evansi*) was diagnosed based on its morphological feature (Tamarit *et al.* 2010). When parasites were confirmed in the smear, these animals were selected for study rather than other animals with only clinical signs and no confirmation of parasites in the smear. After the selection of experimental animals, Blood samples were collected for analysis of hematological parameters.

Sample collection and hematological examination: The blood samples were collected from the jugular vein and stored in the EDTA vacutainer until hematological analysis. Blood samples were transported to clinical medicine laboratory, UVAS-Lahore for hematological analysis. Hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), granulocytes, monocytes and platelets were done by hematology analyzer (Durrani *et al.* 2017).

Therapeutic Trials: The total infected Thoroughbred horses (n=72) based upon inclusion criteria were randomly divided into three groups. Each group contains (n=24) horses. The drug was injected after the proper weight of the animal by weight scale.

Group A: Animals in group A were treated with a single dose of quinapyramine sulphate (Interquin®-Netherlands) @ 3000mg/ml per 50 /kg body weight (intramuscular) IM.

Group B: Animals in group B were treated with a single dose of isometamedium chloride Hydrochloride (Veridium^{TM}-France) @ 0.5 mg/2.5 ml of 1% solution per 50/kg body weight slow (intravenous) IV.

Group C: Animals in the group were treated with a single dose of diminazene aceturate (Veriban®-France) @ 2360 mg/15 ml per 100/kg body weight IM.

The efficacy of the drugs was measured based upon the disappearance of clinical signs, clearance of *Trypanosome evansi* from blood smear and improvement in hematological parameter on day 7, 10, 14 and 21 after treatments. The efficacy of the drug was determined as follows. After the treatment, all the animals were kept under intense observation to note the reoccurrence of clinical symptoms of Surra or any other side effect.

Percentage efficacy Trypanocidal Drug = (No of animals recovered after treatment × 100)/ (Total animals in the group) (Durrani *et al.* 2017).

Statistical analysis: The data regarding the effect of trypanosomiasis on blood parameters were analyzed by using (ANOVA) and Tukey post hoc applied after ($P<0.05$). The data regarding the efficacy of drugs were analyzed by one way ANOVA. All statistical analyses were done on the statistical software SPSS version. 20. The blood parameters data was presented mean±SEM and efficacy was presented as %.

RESULTS

The spindle shape intercellular *Trypanosome evansi* was identified under the microscope in clinically infected thoroughbred horses before treatments, as shown in Figure 1. Hematological biomarkers revealed that RBCs, hemoglobin, packed cell volume and platelets were significantly (P<0.0001) decreased value in diseased horses as compared to healthy. However, mean values of WBCs, lymphocytes, monocytes and granulocytes were significantly (P<0.0001) increased in diseased horses than healthy, which clearly showed infection (Table 1).

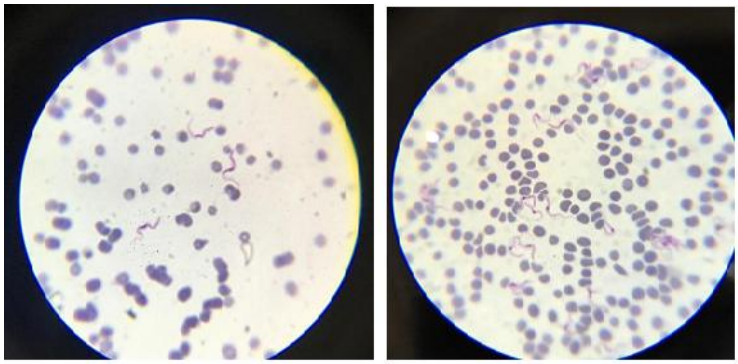

Figure 1. *Trypanosoma evansi* under the microscope in clinically infected Thoroughbred horses

Table 1: Comparison of healthy and disease horses blood parameter.

Blood parameter	Healthy	Disease	p-value
Red Blood cell	7.4275±.97	4.9250±.63	0.000
Hemoglobin	12.26±.79	9.6225±1.50	0.001
Pack cell volume	35.96±2.68	27.35±3.55	0.000
White Blood cell	7.96±1.87	13.78±.93	0.000
Platelets	133.12±15.5	87.25 ± 7.55	0.000
Lymphocytes	2.81±1.19	5.63±.64	0.000
Monocytes	0.72±.14	1.075±.23	0.003
Granulocytes	4.68 ±1.16	8.69±.78	0.000

The post-treatment observation on days 3-21 showed significant restoration in hematological parameters in all treatment groups, meanwhile at day 21 no *Trypanosome evansi* was presented under microscope. The RBCs, Hb, PCV and platelets of both treatments' groups (QS, IC and DA) were significantly increasing from 3 to 21 days after treatment; moreover, there values were at normal level at 21 days after treatments. The results of WBCs, granulocytes and monocytes were significantly decreasing from 3 to 21 days of treatments in QS, IC and DA, which showed decreasing of infection in both groups, as shown in table 2-4.

Table 2: Change in hematological parameters after treatment with quinapyramine sulphate in horses.

	Quinapyramine sulphate					
Blood Parameter	**0**	**3**	**7**	**10**	**14**	**21**
RBC	4.85±1.9	5.84±0.33*	6.60±0.14*	6.90±0.48*	7.17±0.32*	7.70±0.52*
HB	8.41±1.03	9.47±0.24*	10.87±0.48*	11.83±0.70*	12.3±0.35*	12.83±0.39*
PCV	27.41±1.9	30.37±2.73*	32.06±2.29*	33.88±2.16*	33.97±1.51*	36.45±3.04*
WBC	13.22±0.3	10.37±1.89*	9.73±1.61*	8.60±1.47*	7.26±0.78*	7.23±1.09*
Granulocytes	7.96±0.68	6.06±1.20*	5.78±0.76*	4.82±0.82*	4.05±0.68*	4.12±0.87*
Monocytes	1.53±0.23	0.97±0.07*	0.77±0.12*	0.60±0.16*	0.61±0.15*	0.48±0.11*
Lymphocytes	2.28±0.62	2.37±0.47	2.4±0.32	2.38±0.54	2.47±0.24	2.48±0.51
Platelets	89.25±7.32	100.25±8.79*	110.87±8.9*	123.62±7.53*	122.75±12.4*	126.25±11*

Data are expressed in mean±S.D, drugs of treatment: D1 (Quinapyramine) Days of treatment 0, 3, 7, 10, 14 and 21. Asterisk shows the level of significance (P<0.05). Reference (Normal values of RBCs, HB and PCV, WBC, Granulocytes, Lymphocytes, Platelets) from (Pritchard *et al.* 2009).

Table 3: Change in hematological parameters of after treatment with isometamedium chloride in horses.

	Isometamedium chloride					
Blood Parameter	**0**	**3**	**7**	**10**	**14**	**21**
RBC	4.65±0.28	5.61±1.61*	6.26±0.44*	6.59±0.10*	6.83±1.91*	7.01±0.18*
HB	7.82±0.33	8.35±0.85*	9.94±0.58*	11.21±0.62*	11.94±0.70*	12.41±0.39*
PCV	25.64±1.04	28.58±1.20*	30.80±1.35*	32.35±1.21*	33.25±1.11*	34.88±0.66*
WBC	12.58±0.36	11.10±1.06*	10.14±1.22*	9.34±1.37*	8.50±0.99*	7.78±0.80*
Granulocytes	8.08±0.65	6.65±0.22*	4.88±0.61*	4.45±0.80*	4.37±0.31*	3.97±0.39*
Monocytes	1.56±0.25	0.88±0.06*	0.62±0.09*	0.65±0.11*	0.60±0.10*	0.55±0.07*
Lymphocytes	2.2±0.45	2.32±0.34	2.35±0.51	2.5±0.86	2.7±0.33	2.72±0.22
Platelets	85.85±8.99	96.14±4.38*	113.28±8.09*	118.85±2.60*	121.14±7.01*	124.5±5.87*

Data are expressed in mean±S.D, drugs of treatment: D2 (Isometamedium) Days of treatment 0, 3, 7, 10, 14 and 21. Asterisk shows the level of significance (P<0.05). Reference (Normal values of RBCs, HB and PCV,WBC, Granulocytes, Lymphocytes, Platelets) from (Pritchard *et al.* 2009).

The therapeutic efficacy of QS was significantly higher (P<0.005) than IC and DA. Moreover, overall efficacy of QS, IC and DA at 21th day post treatment was (100%, 95.83 %, and 75%; P<0.005) after treatment, as shown in table 5.

Table 4: Efficacy of different drugs against trypanosomiasis in horses at various days.

Various drugs	**Efficacy of various days**				
	Total animals	**Day 3**	**Day 7**	**Day 14**	**Day 21**
Quinapyramine sulphate*	24	22 (91%)	24 (100%)	24 (100%)	24 (100%)
Isometamedium chloride*	24	19 (79%)	20 (83%)	21 (87%)	21 (87%)
Diminazene aceturate	24	8 (33%)	14 (58%)	15 (63%)	15 (63%)

(P-value=0.001, *highly significant).

DISCUSSION

Many studies were reported regarding prevalence of trypanosoamsis in camel, but in my knowledge no specific study on horses was reported. In the current study, *Trypanosoama evansi* was diagnosed based on morphological features as described by (Baba 2011). In the current study, thin blood smear examination was done for diagnosis of extracellular spindle shape *T.evansi.* These morphological findings coincide with the result of (Muieed *et al.* 2010; Durani *et al.* 2017). By Giemsa-stained thin blood smear examination, lower and poor detection of *T. evansi* as compared to other serological and molecular diagnostic tests. Therefore, by microscopic examination no better infection burden has observed (Singh *et al.* 2019). However, by detection of *T. evansi* specific antibodies, may give a better estimation of the infection burden as has been shown in previous studies (Ahmad *et al.* 2005; Yusuf *et al.* 2013; Singh *et al.* 2012). The previous studies showed that overall estimate prevalence of 19.4%, with low sensitivity of CATT/*T. evansi* and the PCR tests (43% - 53%) but better than the sensitivity of Giemsa-stained thin blood smear examination (Da Silva *et al.* 2011; Elshafie *et al.* 2018).

In the current study, various clinical signs were observed in infected thoroughbred horses, including intermittent high fever (104 ºF), pale mucus membrane, anemia, weakness, petechial hemorrhages on the third eyelid, conjunctivitis, enlarge lymph node, and incoordination. Similar clinical signs were observed in camel and donkeys (Hussain *et al.* 2016; Durani *et al.* 2017; Elshafie *et al.* 2018; Oparah *et al*. 2017). The high rise of body temperature is due to toxins liberated by the trypanosome parasite in the blood and change the set point of body temperature in the hypothalamus due to the release of the pyrogenic stimuli in trypanosomiasis infection (Hörchner *et al*. 1920).

The treatment trails of various trypanocidal drugs showed that quinapyramine sulfate was more effective against trypanosomiasis infection in horses. The therapeutic efficacy of quinapyramine sulphate was 100% @ 1mg/ kg against trypanosomiasis in horses. Hematological analysis on days 3, 7, 10, 14 and 21 show significant restorations of blood parameters after treatment in recovered horses; our finding is in agreement with the studies (Ahmad *et al.* 2005; Singh *et al.* 2012; Yusuf *et al.* 2013). The isometamedium chloride has therapeutic efficacy 87.5 % against trypanosomiasis in horses, and significantly, the restoration blood parameter occurs on days 3, 4, 7 10, 14 and 21 after treatment. The results coincide with the finding of (Gutierrez *et al.* 2010). In previous reports, isometamedium chloride has good therapeutic efficacy against trypanosomiasis in field conditions (Durrani *et al.* 2017). The difference in results

may be due to species difference and difference in severity of infection. Diminazene aceturate has 62.5 % therapeutic efficacy against trypanosomiasis that is lower than quinapyramine and isometamedium. The results coincide with the finding of (Da Silva *et al.* 2011). Similarly, the lower efficacy of diminazene aceturate had been reported and relapse of infection occurs in mule and horses after treatment (Tuntasuvan *et al.* 2003). The lower efficacy of diminazene aceturate against trypanosomiasis is due to resistance development (Zhang *et al.* 1991). The resistance of *Trypanosoma evansi* to Diminazene aceturate has been reported recently in India (Sivajothi1 and Sudhakara, 2016). While in contrast, it reported that the combination of diminazene aceturate and vitamin E showed high efficacy in equines against trypanosomiasis (Singh R *et al.* 2019). The high efficacy of diminazene aceturate may be due to vitamin E, which enhance the immunity of equines.

The hematological studies of infected horses showed a significant decrease in hemoglobin (HB), pack cell volume (PCV), and total erythrocytes count (TEC) that results in anemia. The anemic condition had been reported in trypanosomiasis infection (Stijlemans *et al.* 2007). The researcher suggested various factors that include the release of sialidase enzyme by the parasite, immunologic mechanisms, depression of erythrogenesis, and hemolytic factors such as free fatty acid and hemolysis are concerned for anemia development in trypanosomiasis infection (Adamu *et al.* 2008). The trypanosomes release the sialidase enzymes that adhere to sialic acid on the surface of erythrocytes, exposing the residues of Galactosyl. The macrophages recognized this residue by d-galactose specific lectins on macrophages leading to erythrophagocytosis resulting in a decrease in RBC count and anemia (Sallau *et al.* 2008). The parasite survives in the extracellular fluid of the host (Mijares *et al.* 2010) causes increased lipid peroxidization, calcium-ATPase activity, increased osmotic fragility of RBC and oxidative damage of erythrocytes is due to reduction in reduced glutathione. The affected erythrocytes are removed by the spleen through the mononuclear phagocytic system that results in a decrease in pack cell volume (PCV) (Stijlemans *et al.* 2007). While furthermore, IFN-A, TNF-α, and IL-1 produced as the result of immune responses also lead to decrease PCV value in infected animals (El-Bahr and El-Deeb, 2016). The results of this research coincide with the finding of (Khan *et al.* 2018) they also reported the decrease in red blood cell (RBC), pack cell volume (PCV) and hemoglobin in trypanosomiasis infection, the infected horses also showed leukocytosis that includes monocytosis, granlocytosis (eosinophilia, neutrophilia). These results of our studies coincide with the study of (Hussain *et al.* 2016). The leukocytosis in trypanosomiasis infection in cattle, sheep, and goats. The increase in leukocytes count is due to a rise in neutrophil (neutrophilia), monocytes (monocytosis) and eosinophil (eosinophilia) that occur as a result of the immunological response of the host influenced by the surface glycoprotein of *trypanosoma* species (Oparah *et al.* 2017). The infected horses also showed thrombocytopenia that occurs due to increased splenic sequestration of platelets, and disseminated intravascular coagulation reaction that causes destruction of platelets reported in trypanosomiasis infection (da Silva *et al.* 2011; Kipper *et al.* 2011).

Finally in conclusion, trypanosomiasis is present in the stable horses of Pakistan and causes various clinical signs and hematological changes. Quinapyramine sulfate has significantly more effective against trypanosomiasis in horses and significant restoration of blood parameters in recovered horses.

REFERENCES

Adamu, S., N.D.G. Ibrahim, A.J. Nok and K.A.N. Esievo (2008). Sialyltransferase activity probably counteracts that of sialidase as one of the possible mechanisms of natural recovery or stabilization of erythrocyte mass in trypanosome-infected animals - A perspective. African. J. Biotechnol., 7(25): 4992–5001.

Ahmad, S., A.A. Nasir and A.H. Awan (2005). Therapeutic Drug Trial in Albino Mice Against Trypanosomiasis. Pakistan Vet. J., 25(1): 49–50.

Baba, M.S.B. and B.A.H. Zainal-Abidin (2011). The effects of *nerolidol, allicin* and berenil on the morphology of *Trypanosoma evansi* in mice a comparative study using light and electron microscopic approaches. Malaysian Appl Biol. 40(1): 25–32.

Da Silva, A.S., H.A. Garcia Perez, M.M. Costa, R.T. França, D. De Gasperi, R.A. Zanette, J.A. Amado, S.T. Lopes, M.M. Teixeira and S.G. Monteiro (2011). Horses naturally infected by Trypanosoma vivax in southern Brazil. Parasitol. Res., 108(1): 23–30.

Desquesnes, M., A. Dargantes, D.H. Lai, Z.R. Lun, P. Holzmuller and S. Jittapalapong (2013). *Trypanosoma evansi* and surra. A review and perspectives on transmission, epidemiology and control, impact, and zoonotic aspects. Biomed. Res. Int., 2013:321237.

Durrani, A.Z., Z. Bashir, K. Mehmood, M. Avais, H. Akbar, W. Ahmad and M. Azeem (2017). Use of physiological biomarkers in diagnosis along with field trials of different trypanisidal drugs in camels of Cholistan desert. Microb. Pathog., 108: 1–5.

Echeverria, J.T., R.L. Soares, B.A. Crepaldi, G.G. de Oliveira, P.M.P. da Silva, R.C. Pupin, T.B. Martins, H.P.K. Cleveland, C.A.D.N. Ramos and F.D.A. Borges (2019). Clinical and therapeutic aspects of an outbreak of canine trypanosomiasis. Rev. Bras. Parasitol. Vet., 28(2): 320–4.

El-Bahr, S.M. and W.M. El-Deeb, (2016). *Trypanosoma evansi* in naturally infected Dromedary Camels: Lipid profile, oxidative stress parameters, acute phase proteins and proinflammatory cytokines. Parasitology. 143(4): 518–22.

Elshafie, E.I., R.A. Sani, R. Sharma and I.A. Abubakar (2018). Clinical and Hematological Profiles of Malaysian Ponies Experimentally Infected with a Field Strain of *Trypanosoma evansi*. Open Parasitol. J., 6(1): 7–16.

Fernández, D., B. González-Baradat, M. Eleizalde, E. González-Marcano, T. Perrone and M. Mendoza (2009). *Trypanosoma evansi*: A comparison of PCR and parasitological diagnostic tests in experimentally infected mice. Experi. Parasito., 121(1): 1-7.

Fikru, R., Y. Andualem, T. Getachew, J. Menten, E. Hasker, B. Merga, B.M. Goddeeris and P. Büscher (2015). Trypanosome infection in dromedary camels in Eastern Ethiopia: Prevalence, relative performance of diagnostic tools and host related risk factors. Vet. Parasitol., 211(3-4): 175–81.

Gutierrez, C., M. Desquesnes, L. Touratier and P. Büscher (2010) *Trypanosoma evansi*: Recent outbreaks in Europe. Elsevier B.V. 174(1-2): 26-9.

Halder, B., S. Dhara and A. Ghosh (2019). Trypanosomiasis in equines: A brief discussion. International J. Vet. Sciences and Anim. Husbandry., 4(6): 40–4.

Herrera, H. M., A.M.R. Dávila, A. Norek, U.G. Abreu, S.S. Souza, P.S. D'Andrea and A.M. Jansen (2004). Enzootiology of *Trypanosoma evansi* in pantanal, Brazil. Vet Parasitol, *125*(3-4), 263-275.

Hörchner, F., A. Schönefeld and B. Wüst (1983). Experimental infection of horses with *Trypanosoma evansi*. I. Parasitological and clinical results. Ann. Soc. Belg. Med. Trop., (1920) 63(2): 127–35.

Hussain, R., A. Khan, R.Z. Abbas, A. Ghaffar, G. Abbas and F. Ali (2016). Clinico-hematological and biochemical studies on naturally infected camels with trypanosomiasis. Pakistan J. Zool., 48(2): 311–6.

Juyal, P. D., (2011). Newer perspectives in the diagnosis and control of trypanosomosis (Surra) in domestic livestock in India. *Tropmed—Internationale Wissenschaftliche Publikationen*, 1-13.

Khan, A.U., A.S. Qureshi, H.F. Hassan, S. Rehan and A. Sarfraz (2018). Molecular identification of Trypanosomes and their effects on hematological and biochemical parameters in donkeys in Punjab, Pakistan. Int. J. Agric. Biol., 20 :1607–12.

Kipper, M., A.S. Da Silva, C.B. Oliveira, I. Andretta, F.C. Paim, C.B. da Silva, R. Leon, K. Corrêa, D.R. Stainki, S.T.A. Lopes and S.G. Monteiro (2011). Relationship between splenic sequestration and thrombocytopenia in *Trypanosoma evansi* infection in rats. Res. Vet. Sci., 91(2): 240–2.

Kumar, R., S. Kumar, S.K. Khurana and S.C. Yadav (2013). Development of an antibody-ELISA for seroprevalence of *Trypanosoma evansi* in equids of North and North-western regions of India. Vet. Parasitol., 196(3-4): 251–7.

Mijares, A., J. Vivas, C. Abad, M. Betancourt, S. Piñero, F. Proverbio, R. Marín and R. Portillo (2010). *Trypanosoma evansi*. Effect of experimental infection on the osmotic fragility, lipid peroxidation and calcium-ATPase activity of rat red blood cells. Exp. Parasitol., 124(3): 301–5.

Muieed, M.A., Z.I. Chaudhary and A.R. Shakoori (2010). Comparative studies on the sensitivity of polymerase chain reaction (PCR) and microscopic examination for the detection of *Trypanosoma evansi* in horses. Turkish J. Vet. Anim. Sci., 34(6): 507–12.

Mukhtar, Y., K. Abdu and A.K. Maigari (2017). Efficacy of Anogeissus leiocarpus (DC) as Potential Therapeutic Agent against Trypanosomiasis Diseases: Inter. J. of health and Pharm. R., 3:1–9.

Oparah, Q.N., A.B.K. Sackey, I.A. Lawal and U.S. Abdullahi (2017). Haematological indices in *Trypanosoma brucei brucei* (Federe isolate) infected nigerian donkeys (*equus asinus*) treated with homidium and isometamidium chloride. Maced. Vet. Rev., 40(1): 73–82.

Pritchard, J. C., C.C. Burn, A.R. Barr and H.R.Whay (2009). Haematological and serum biochemical reference values for apparently healthy working horses in Pakistan. Res. Vet. Sci., 87(3): 389-395.

Sallau, A.B., M.A. Ibrahim, A. Salihu and I.A. Yusuf (2008). Bloodstream Form of *Trypanosoma evansi* Contains $ -Galactosidase. Middle-East J. Sci. Res., 3(2): 49–52.

Sivajothi1 S. and B.R. Sudhakara (2016). Polypeptide Profiles of Diminazene Aceturate Resistant *Trypanosoma evansi* Organisms Isolated from a Buffalo. J. Vet. Sci. Med., 4(1): 2–5.

Singh, R., S.K. Gupta and S. Upadhyay, (2012). Chemotherapy and evaluation of drug efficacy in equines infected with *T. evansi* with antrycide prosalt and isometamedium chloride. Vet. Practitioner., 13(2), 139-42.

Singh, R., A. Tripathi, A. Srivastava and A. Singh, A. (2019). Assessment of Clinical Recovery of Equine Trypanosomosis using Antitrypanosomal Drugs and Antioxidant Supportive Therapy. Int. J. Livest. Res., 9(4):

Stijlemans, B., T.N. Baral, M. Guilliams, L. Brys, J. Korf, M. Drennan, J. Van Den Abbeele, P. De Baetselier and S. Magez (2007). A Glycosylphosphatidylinositol-Based Treatment Alleviates Trypanosomiasis-Associated Immunopathology. J. Immunol., 179(6): 4003–14.

Tehseen, S., N. Jahan, M.F. Qamar, M. Desquesnes, M.I. Shahzad, S. Deborggraeve and P. Büscher (2015). Parasitological, serological and molecular survey of *Trypanosoma evansi* infection in dromedary camels from Cholistan Desert, Pakistan. *Parasites & Vectors*, 8(1): 1-11.

Tamarit, A., C. Gutierrez, R. Arroyo, V. Jimenez, G. Zagalá. I. Bosch, J. Sirvent, J. Alberola, I. Alonso and C. Caballero (2010). *Trypanosoma evansi* infection in mainland Spain. Vet. Parasitol., 167(1): 74–6.

Tuntasuvan, D., W. Jarabrum, N. Viseshakul, K. Mohkaew, S. Borisutsuwan, A. Theeraphan and N. Kongkanjana (2003). Chemotherapy of surra in horses and mules with diminazene aceturate. Vet. Parasitol. 110(3-4): 227–33.

Yusuf, O. S., B.S. Oseni, A.O. Olayanju, M.A. Hassan, A. A. Ademosun and R. Y. Akele (2013). Acute and chronic effects of *Trypanosoma brucei brucei* experimental infection on bone marrow and peripheral blood cells in Wistar rats. Scholars J. App. Med. Sci., 1(6): 1040-1036.

Wells, E.A., (1984). Animal trypanosomiasis in South America. Prev. Vet. Med., 2(1): 31–41.

Zhang, Z.Q., C. Giroud and T. Baltz (1991). In vivo and in vitro sensitivity of *Trypanosoma evansi* and *T. equiperdum* to diminazene, suramin, MelCy, quinapyramine and isometamidium. Acta. Trop., 50(2): 101–10.