INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18F–0365/2018/20–7–1607–1612
DOI: 10.17957/IJAB/15.0679
http://www.fspublishers.org

Full Length Article

Molecular Identification of *Trypanosomes* and Their Effects on Hematological and Biochemical Parameters in Donkeys in Punjab, Pakistan

Asmat Ullah Khan[1], Anas Sarwar Qureshi[1*], Muhammad Farooque Hassan[2], Sarmad Rehan[1] and Adeel Sarfraz[3]
[1]*Department of Anatomy, University of Agriculture, Faisalabad-38040, Pakistan*
[2]*Department of Veterinary Pathology, Shaheed Benazir Bhutto University of Veterinary and Animal Science, (SBBUVAS), Skrand, Pakistan*
[3]*University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Bahawalpur-63000, Pakistan*
[*]For correspondence: anas-sarwar@uaf.edu.pk

Abstract

The study was designed to investigate the molecular identification and prevalence and of trypanosomiasis and its effects on hematological and biochemical parameters in donkeys. Blood samples were collected from 657 donkeys of three districts: Gujranwala, Gujrat, and Mandi Bahaudin in Punjab province of Pakistan. Prevalence of *Trypanosoma* was observed by microscopy of Giemsa's stained blood smear; whereas, serum and hematological parameters were determined by serum biochemistry and hematologic analyzer, respectively. Multiplex PCR was used to differentiate the species of *Trypanosoma* in diseased animals. Out of 657 donkeys screened 58 (8.83%) were detected positive for trypanosomiasis by microscopic examination. Gujranwala was found to have highest prevalence (11.58%) followed by Gujrat (8.23%) and Mandi Bahaudin (5.39%), respectively. Hemoglobin, red blood cells count, packed cell volume and mean corpuscular volume were significantly ($P<0.05$) lower in infected animals; whereas, total leucocyte count and lymphocyte count were significantly ($P<0.05$) higher in infected animals. Total protein, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase were significantly ($P<0.05$) increased in the infected animals. Macrocyte, microcyte, acanthocyte, dacrocyte and bizarre shaped red blood cells were observed in infected animals. Multiplex PCR showed that *Trypanosoma evansi* was the most prevalent species. © 2018 Friends Science Publishers

Keywords: Molecular identification; *Trypanosoma*; Prevalence; Hematological values; Serum values

Introduction

Livestock accounted for 58.3% of the agriculture sector and 11.4% of the overall GDP during 2016–2017. Among various livestock species, working donkeys are the source of income generation for poor communities, being kept for transportation of people, goods, and many agricultural purposes (Simenew *et al*., 2011). Donkey population is estimated to be 5.2 million (Anonymous, 2016) in Pakistan with a growth rate of 2.95 per annum in Pakistan (Hasnain and Usmani, 2006). A number of parasitic, bacterial and viral diseases affect the donkey's health and sometimes cause mortality in these animals. *Trypanosoma (T.)* is the etiological agent of trypanosomiasis in donkeys. Not only it causes disease in donkeys, but also it affects human population in the world particularly in Africa and Latin America. A recent study has revealed that *Trypanosoma* can infect all the domesticated animals (Fatihu *et al*., 2009).

Trypanosomiasis (Surra) is a chronic infection in the equine. The main species of trypanosomes that cause trypanosomiasis in animals are *T. congolense*, *T. vivax* and *T. evansi* (Abenga *et al*., 2002). They are transmitted biologically, but can also be transmitted through the mechanical means. The genus *Glossina* is the main vector for biological transmission (Clausen *et al*., 2003). In this disease, animals show intermittent fever, anorexia, severe weight loss, petechial hemorrhages on the third eyelid, anemia, and edema under abdomen, scrotum and limb area. Few animals in later stages show nervous sign and death (Ngaira *et al*., 2002). Working donkeys are the neglected animals and no research was conducted on molecular identification of *Trypanosoma* species of donkeys in Pakistan. Comparative effect of trypanosomiasis on hematology and serum values of infected as well as healthy donkeys has not been also observed. Therefore, in continuation with the existing knowledge, the present study was planned to jot down:

To cite this paper: Khan, A.U., A.S. Qureshi, M.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: 1607–1612

a. Microscopic prevalence of trypanosomiasis in donkeys
b. Hematological examination of trypanosoma infected and non-infected animals
c. Serological analysis of trypanosome infected and non-infected animals
d. Molecular identification of different *Trypanosoma* species

Materials and Methods

Study Area

Gujranwala, Gujrat and Mandi Bahaudin are three districts of Punjab, Pakistan. Gujranwala is located between 32.16 latitude and 74.19 longitude while Gujrat is located between 32.57 latitude and 74.08 longitude; whereas, Mandi Bahaudin is located 32.58 latitude and 73.48 longitude.

Sample Collection

The study population consisted of working donkeys of all age groups that were selected randomly from three districts of Punjab including Gujranwala, Gujrat and Mandi Bahaudin. Blood samples (8 mL) from 657 donkeys were collected from jugular vein aseptically through sterile syringe following the guideline of international animal ethics and welfare committee. About 4 mL blood was transferred into tubes containing EDTA (ethylene diamine tetraacetate) for hematological study while the rest was put in EDTA-free clot activator tubes for serum separation.

Microscopic Examination

A drop of blood was placed on a clean glass slide and a coverslip placed on it, allowing the blood to spread as a thin layer of cells. This was then examined under microscope to observe motile trypanosomes. For morphological examination, thin smear then drawn out with another slide and air dried. Fixation of the slide was carried out with methanol for 2-3 min and 5% Giemsa stain was used for staining purpose followed by rinsing with distilled water (pH 7.2). The slide was then air dried and examined under a high power 100X microscope by using immersion oil.

Hematological and Serum Biochemical Examination

Sysmex hematologic analyzer was used to examine the hematological profile of trypanosome infected and non-infected healthy animals. Healthy animals were free from blood parasites with body condition score ≥2. Screening panel included total erythrocyte count (TEC), total leucocyte count (TLC), differential leucocyte count (DLC), packed cell volume (PCV), hemoglobin concentration (Hb), erythrocyte sedimentation rate (ESR), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The serum biochemical profile was analyzed for total proteins (TP), albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP) by commercially available kits in the RX Monza of Randox RX Series.

PCR Amplification

Multiplex PCR assay was performed for molecular detection of *Trypanosoma* species in donkeys. DNA was extracted from the blood following Ghatak *et al.* (2013) and amplification was carried out in thermal cycler (2720 Applied Biosystem®). Samples were loaded in thermal cycler containing *Trypanosoma* species specific primers master mix solution and DNA templates then the conditions of thermal cycler were adjusted according to optimization levels.

Primers

The species-specific primer sequences used are shown in Table 1.

PCR amplification solution: Total 80 μL amplification solution was prepared which contained 25 μL water, 45 μL master mix, 4 μL extracted DNA and 1 μL of each primer.

Thermal Cycler Conditions

The PCR tubes having PCR mixture were placed into thermocycler. Initial denaturation was done at 94^{o}C, followed by 39 cycles of denaturation, annealing, and extension, a final extension was carried out at 72^{o}C and the amplified sample was stored at 4^{o}C. The duration and different levels of temperature used are given in Table 2. The extension was carried out at 72^{o}C for 10 min. PCR products were held at 4^{o}C until separated by electrophoresis on a 1% agarose gel and visualized under a UV Trans-illuminator.

Statistical Analysis

Data were analyzed using a randomized design. F-test was used to compare data from positive and negative samples at 5% level of significance (α=0.05). The results were considered significant if $P < 0.05$ level. Chi-square test was used to see if there exists any significant difference between different treatment groups.

Results

Prevalence of Trypanosomes

Prevalence of trypanosomiasis was 8.83% in donkeys (n=657), detected positive under a microscope. Gujranwala had the highest prevalence 11.58% of trypanosomiasis (Table 3); whereas, Mandi Bahaudin had the least prevalence 5.39% but they were non-significant.

Table 1: Species-specific primer sequence

Trypanosomavivax (Cox, 2007)	TVW1: 5'-CTGAGTGCTCCATGTGCCAC-3' TVW2:5'CCACCAgAACACCAACCTGA-3'	150 bp
Trypanosoma congolense (Almeida *et al*., 1998)	GOL 5'-GAGAACGGGCACTTTGCGATTTTC-3' GOL5'-GACAAACAAATCCCGCACAACCAT-3' 314 bp	
Trypanosoma evansi (Birhanu *et al*., 2015)	RoTat-F:5'-GCGGGGTGTTTAAAGCAATA-3 RoTat-R:5'ATTAGTGCTGCGTGTGTTCG-3	205 bp

Table 2: Thermal cycler conditions

Solution/Primer	Process	Temperature	Time
Primers	Initial denaturation	94°C	4 min
GOL Primer 314 bp	Denaturation	94°C	30 sec
	Annealing	50°C	40 sec
	Extension	72°C	30 sec
TVW Primer 150 bp	Denaturation	94°C	1 min
	Annealing	60°C	40 sec
	Extension	72°C	30 sec
RoTat Primer 205 bp	Denaturation	94°C	1 min
	Annealing	59°C	1 min
	Extension	72°C	1 min
	Final extension	72°C	15 min
	Infinite hold	4°C	∞

Table 3: Prevalence of trypanosomiasis in three districts

District	Animals screened	Infected	Prevalence (%)	Chi-square value	P-value
Gujranwala	259	30	11.58%	5.000	0.172
Gujrat	231	19	8.23%		
Mandi Bahaudin	167	9	5.39%		
Total	657	58	8.83%		

Significant if *P<0.05*

Prevalence of Trypanosomiasis among Sex and Age Groups

Trypanosomiasis was more prevalent in males 9.8% than females 7.88% (Table 4). In this study, males and females under one year of age had the least prevalence (male 6.32% and female 6.67%); whereas, the highest prevalence was observed in the animals with age more than 6 years (male 12.33% and female 9.76%). Pregnant dry 13.3%, pregnant lactating 16.6% is not significantly higher than non-pregnant dry and lactating.

Morphology of RBC

In this study, trypanosomes were observed in Giemsa stained slides under the microscope. Red blood cells of different shapes were seen which included microcyte (Fig. 1), macrocyte (Fig. 2), acanthocyte (Fig. 3), dacrocyte (Fig. 4) and bizarre shaped cells (Fig. 5).

Serum and Hematological Values of Infected and Healthy Animals

Values of various hematological and serum parameters were compared between infected and non-infected animals.

Table 4: Distribution of experimental animals according to sex and age (Chi Square analysis)

	Animals screened	Infected	Prevalence	Chi-square value	P-value
Male	327	32	9.8%	0.742	0.389
Female	330	26	7.88		
Male					
Less than one year	95	6	6.32%	2.340	0.505
1 to 3 year	76	7	9.21%		
3--6 year	83	10	12.0%		
More than 6 year	73	9	12.33%		
Female					
Less than one year	90	6	6.67%	0.896	0.826
1 to 3 year	78	5	6.41		
3 to 6 year	80	7	8.75%		
More than 6 year	82	8	9.76%		
Pregnant or dry					
Pregnant dry	15	2	13.3%	2.255	0.521
Pregnant lactating	18	3	16.6%		
Non-pregnant dry	26	1	3.8%		
Non-pregnant lactating	23	2	8.69%		

Significant if *P<0.05*

Table 5: Mean ±SEM values of hematological and serum biochemical parameters of infected and healthy donkeys

Parameters	Infected donkeys	Healthy donkeys	P-Value
Hemoglobin g/dL	6.34 ±1.34*	10.7± 0.933	0.0001
RBC million/cm^3	4.29 ± 0.57*	6.28± 0.49	0.0001
Packed cell volume %	19.26± 2.94*	33.70± 2.33	0.0001
MCV μm^3	45.26±7.83*	54.12±4.89	0.0001
MCH pg	14.87± 2.95*	16.59± 2.96	0.0028
MCHC g/dL	34.1± 8.3	31.9±8.30	0.0973
Total leucocyte 10^3/cm^3	15.26± 2.35*	11.63± 1.50	0.0001
Lymphocyte %	63.45±4.37*	40.9± 2.87	0.0001
Neutrophil %	28.40±3.81*	51.3± 3.02	0.0001
Monocyte %	4.22±1.72	4.57±1.15	0.2139
Eosinophil %	4.00±1.24*	3.10± 0.79	0.0014
Alkaline phosphatase U/L	396.82+78.83*	337.22±44.51	0.0004
Total protein g/dL	7.88±1.33*	6.77±1.24	0.0058
Albumin g/dL	2.71±0.47*	3.22±0.77	0.0045
Aspartate aminotransferase U/L	387.00± 42.72*	242.8 ±42.18	0.0001
Alanine aminotransferase U/L	24.82± 6.00*	19.01±7.06	0.0010

Significant if *P<0.05*

Hemoglobin, red blood cells count, packed cell volume (PCV) and neutrophil count were significantly decreased in infected animals as compared to healthy animals (Table 5); whereas, total leucocyte count and lymphocyte count increased significantly. All the parameters including total proteins alkaline phosphatase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly higher in the infected animals as compared to healthy animals while, albumin was significantly decreased in infected animals as compared to in healthy animals.

Prevalence of Trypanosomiasis through PCR

Trypanosoma evansi, *T. congolenese* and *T. vivax* can be transferred mechanically or biologically (Abenga *et al*., 2002) so there might be the presence of different trypanosome species in the naturally infected animals.

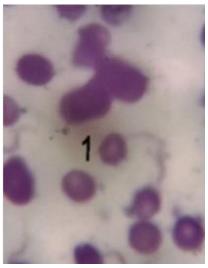

Fig. 1: Microcyte

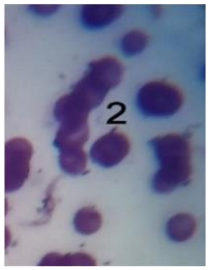

Fig. 2: Macrocytic

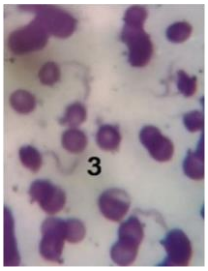

Fig. 3: Acanthocyte

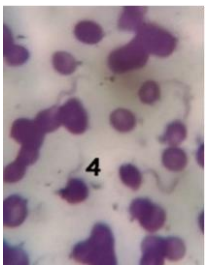

Fig. 4: Dacrocyte (Tear droplet cells)

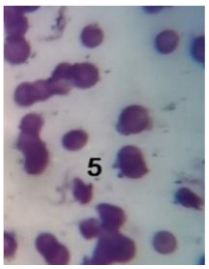

Fig. 5: Bizarre shape (Giemsa Stained- 1000X)

To identify the prevalence of each *Trypanosoma* species in trypanosomiasis infected animals, three species-specific primers were used for *T. vivax, T. congolense* and *T. evansi. T. aevansi* was targeted (Fig. 6) by RoTat at 205 base pair; whereas, *T. vivax* and *T. congolense* were not observed in these infected donkeys (Table 6). Our study showed that *T. evansi* was only and the most prevalent specie in the working donkeys of Pakistan. It also indicated that only *T. evansi* was mechanically transmitted in Pakistan; whereas, *T. congolense* and *T. vivax* were neither transmitted mechanically nor biologically.

Table 6: Prevalence of trypanosomiasis using PC

Sample	*T. evansi*	*T. congolense*	*T. vivax*
Infected samples	58	0	0
Prevalence	100%	0	0

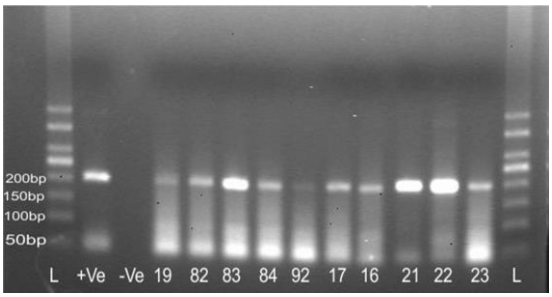

Fig. 6: PCR product from amplification of DNA taken from the blood of donkeys. The primers used are specific for *T. evansi, T. vivax,* and *T. congolense*. Band at 205 bp indicated *T. evansi*. L is the molecular marker, +ve and –ve are positive and negative control; 19, 82, 83, 84, 92, 17, 16, 21, 22, 23 are amplified DNA of infected samples

Discussion

This study was conducted to investigate the molecular identification and prevalence of trypanosomiasis and its effects on hematological and biochemical parameters in donkeys. Blood samples were collected from 657 donkeys of three districts: Gujranwala, Gujrat, and Mandi Bahaudin in Punjab province. Blood screening indicated 8.83% trypanosomiasis infection in donkeys; whereas, Multiplex PCR showed that only *T. evansi* is prevalent in Pakistan. This study will help in developing strategies to curb the trypanosomiasis.

Prevalence of trypanosomiasis was observed to be 8.83% in above mentioned three districts. No district wise data was available to compare these findings. Previously, Abbasi *et al.* (2014) reported 8.46% trypanosomiasis in donkeys. Hussain *et al.* (2016) reported 6.71% trypanosomiasis in donkeys. However, Hassan *et al.* (2006) reported 3.3% trypanosomiasis prevalence among equines in Punjab. This variation indicates the possibility of low trypanosomiasis pressure in other districts of Punjab than the ones in the current study.

There was no significant difference in the prevalence of trypanosomiasis with sex, age and reproductive status of animals. However, a non-significant higher prevalence of trypanosomiasis was observed in male and adult working donkeys than females and young ones. Reason for higher incidence of disease in male animals than females could be due to the fact that male animals travel from one place to another to provide transportation services more than females. Thus, males have a higher probability of acquiring an infection. Frequent travelling can also compromise their immune response to infection due to the stress of fatigue (Kassa *et al.*, 2011). Hussain *et al.* (2016) reported no significant difference in trypanosomiasis among the sex and age groups of donkeys in Pakistan. Our result showed agreement with the Bogale *et al.* (2012) who also observed a non-significant higher prevalence in male animal than females. Mekibib *et al.* (2010) observed that sex and age had no influence on prevalence of trypanosomiasis.

Red blood cells of different shapes such as macrocyte, microcyte, acanthocyte, dacrocyte and bizarre shaped cells were seen in infected donkeys. No literature was available to compare effects of trypanosomes on red blood cell's morphology for three districts of Punjab. However, Silva *et al.* (1995) reported the morphological alteration of red blood cells by *Trypanosoma* species in dogs and horse. Acanthocyte, microcyte, macrocyte, dacrocyte, microspherocyte, anisocytosis and bizarre shaped red blood cells appeared in the dogs and horse infected with natural trypanosomiasis. Morphological alteration in the red blood cells might be due to liver abnormalities since; this organ plays a major role in removing blood parasites (Alsaffar *et*

al., 2007). Hussain *et al*. (2016) has reported macrocyte, microcyte, elliptical cells and tear droplet cell in camel infected with natural trypanosomiasis.

Our study showed significant alteration in hematological and serum parameters in the trypanosomiasis infected donkeys. Cadioli *et al*. (2006) in donkeys, Hilali *et al*. (2006) in buffalo calves, Chamond *et al*. (2010) in mice, Ohaeri and Eluwa (2011) in sheep and Padmaja (2012) in camels reported variation in the hematological parameters due to trypanosomiasis in infected animals. In trypanosomiasis, the main pathological changes in blood are the reduction in hemoglobin and hematocrit values. Lashing movement of Flagella, hemodilution (Reddy *et al*., 2016), hemolysis of RBCs (Rossi *et al*., 2017), erythrophagocytosis (Ohaeri and Eluwa, 2011) and metabolites released from trypanosomes (Naessens, 2006) are the main causes that change hematological parameters in infected animals. Sialidase enzyme released from trypanosomes breaks sialic acid on cell membrane of erythrocytes and make the erythrocyte more susceptible for phagocytosis that in turn produce anemia in the infected animals (Ohaeri and Eluwa, 2011). Padmaja (2012) and Hussain *et al*. (2016) reported a significant reduction in RBCs, hemoglobin, and hematocrit in trypanosomiasis infected animals which were in complete agreement with our findings.

Leucocytosis with lymphocytosis was found in positive animals in our study which showed complete agreement with Sivajothi *et al*. (2015) who reported significant increase in total leucocyte count in the infected rabbit. Ohaeri and Eluwa (2011) reported an increase in leucocyte in infected sheep, goat, and cattle wherein lymphocytosis was observed in all these cases. In contrast to all these, Padmaja (2012), Chaudhary and Iqbal (2000) reported decreased lymphocyte count in infected camels. Reason behind this decreased lymphocyte count could be the immune suppression. Secondly, these reports could have been about initial stages of infection where neutrophil increased and lymphocyte decreased.

Our study reported a significant higher value of serum total proteins in the infected animals which might be due to released immunoglobulin from the defense system in response to disease (Taylor and Authié, 2004; Baral *et al*., 2007). Increase in serum protein could also be due to the release of tissue specific enzyme and disruption of the cell membrane in the infected animals. Several studies reported significant increase in total protein in the trypanosomiasis infected animals e.g., Orhue *et al*. (2005) reported this in rabbit, Gutierrez *et al*. (2005) in camel and Hilali *et al*. (2006) in buffalo calves, Hussain *et al*. (2016) in donkeys and Oparah *et al*. (2017) in Nigerian donkeys. Decreased albumin in our study was also reported by Orhue *et al*. (2005), Ohaeri and Eluwa (2011). The reasons of hypoalbuminemia were hepatic injuries due to centrilobular degeneration and hypoxia in trypanosomiasis (Hussain *et al*., 2016). Decreased albumin level maintains osmolality that causes compensation in hyerglobulinemia (Ahmadi-Hamedani *et al*., 2014). Edema in the dependent part of the body in trypanosomiasis might be due to decreased albumin level (Enwezor and Sacky, 2005).

In our study, all the serum enzyme including alkaline phosphatase ALP, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly higher in the infected animals. Takeet and Fagbemi (2009), El-Baky and Salem (2011) showed complete agreement with our study who reported a significant rise inalkaline phosphatase in infected animals. Oparah *et al*. (2017) reported significant rise in ALT, AST and ALP in Nigerian donkeys infected with trypanosomiasis hepatic necrosis in naturally infected camel with trypanosomiasis might be the reason of increased ALP, AST and ALT (El-Baky and Salem, 2011). A significant increase in ALP, AST, and ALT was also reported in infected rabbit by Takeet and Fagbemi, (2009). Taiwo *et al*. (2003), Sivajothi *et al*. (2015), Hussain *et al*. (2016) also reported significant rise AST and ALT in infected animals. Tissue damage (necrosis) and inflammatory changes in liver, kidney, heart, and muscle cause a significant increase in these enzymes. Host immune system lysed trypanosomes in different stages of infection which might also cause an increase in ALT and AST (Taiwo *et al*., 2003; Takeet and Fagbemi, 2009).

Conclusion

To the best of our knowledge, this is the first ever report on prevalence of trypanosmiasis in donkeys in Pakistan using species-specific primers. Since, *Trypansoma evansi* was the only species detected by PCR, therefore, more emphasis should be laid on *T. evansi* infection, control and treatment in future in Pakistan. Hematological parameters were altered significantly in infected animals that ultimately affected the donkey health and productivity of animals. Precautionary measures should be taken to prevent flies (vectors) from biting the animals. Epidemiologists and policy makers are suggested to devise species and area specific mitigation measures against trypanosomiasis infection in working equines. PCR based (species specific) investigation of trypanosomiasis in other equine and livestock species are also strongly recommended.

Acknowledgements

The authors acknowledge Faculty of Veterinary Science UAF, The Islamia University of Bahawalpur, and Center of Excellence in Vaccinology & Biotechnology of University of Balochistan Quetta for allowing access to laboratory facilities and cooperation during our work.

References

Abbasi, I.H.R., H.A. Sahito, M.I. Sanjrani, F. Abbasi, M.A. Memon, D.R. Menghwar, N.A. Kaka, M.N. Shah and M. Memon, 2014. A disease complex pathogen "*Trypanosoma congolense*" transmitted by tsetse fly in donkeys. *Herald J. Agric. Food Sci. Res.*, 3: 44–48

Abenga, J.N., F.N.C. Ewenzo, F.AG. Lawani, C.S.J. Ezebuiro and K.M. David, 2002. Prevalence of trypanosomiasis in trade cattle at slaughter in Kaduna State, *Niger. J. Parasitol.*, 23: 107–110

Ahmadi-Hamedani, M., K. Ghazvinian and M.M. Darvishi, 2014. Hematological and serum biochemical aspects associated with a camel (*Camelus dromedarius*) naturally infected by *Trypanosoma evansi* with severe parasitemia in Semnan, Iran. *Asian Pac. J. Trop. Biomed.*, 4: 743–745

Almeida, P.J.L.P., M. Ndaoa, B. Goossensb and S. Osaerb, 1998. PCR primer evaluation for the detection of trypanosome DNA in naturally infected goats.*Vet. Parasitol.*, 80: 111–116.

Alsaffar, A.M.H., A.F. Hasoon and A.H. Farag, 2007. Blood picture and hepatic changes in rabbits experimentally infected with *Trypanosoma evansi* Iraqi strain. *Med. J. Babylon*, 4: 358–364

Anonymous, 2016. *Pakistan Economic Survey*, 24: 45

Baral, T.N., P.D. Baetselier, F. Brombacher and S. Magez, 2007. Control of *Trypanosoma evansi* infection is IgM mediated and does not require a type I inflammatory response. *J. Infect. Dis.*, 195: 1513–1520

Birhanu, H., R. Fikru, M. Said, W. Kidane, T. Gebrehiwot, A. Hagos and P. Büscher, 2015. Epidemiology of *Trypanosoma evansi* and *Trypanosoma vivax* in domestic animals from selected districts of Tigray and Afar regions, Northern Ethiopia. *Parasites Vectors*, 8: 212

Bogale, B., F. Kelemework and M. Chanie, 2012. Trypanomosis in Camel (*Camelusromedaries*) in DeloMena District. *Acta Parasitol. Glob.*, 3: 12–15

Cadioli, F.A., L.C. Marques, R.Z. Machado, A.C. Alessi, L.P.T.C. Aquino and P.A. Barnabé, 2006. Experimental *Trypanosoma evansi* infection in donkeys: hematological, biochemical and histopathological changes. *Arq. Bras. Med. Vet. Zootec*., 58: 749–756

Chamond, N., A. Cosson, M.C. Blom-Potar,G. Jouvion, S. D'Archivio, M. Medina, S.D. Bergere, M. Huerre, S. Goyard and P. Minoporio, 2010. *Trypanosoma vivax* infections: pushing ahead with mouse models for the study of Nagana. I. Parasitological, hematological and pathological parameters. *PLoS Negl. Trop. Dis.*, 4: 792

Chaudhary, Z. and J. Iqbal, 2000. Incidence, biochemical and haematological alterations induced by natural trypanosomosis in racing dromedary camels. *Acta Trop*., 77: 209–213

Clausen, P.H., S. Chuluun, R. Sodnomdarjaa, M. Greiner, K. Noeckler and C. Staak, 2003. A field study to estimate the prevalence of *Trypanosoma equiperdum* in Mongolian horses. *Vet. Parasitol*., 115: 9–18

Cox, A.P., 2007. *Epidemiological Analysis of Host Populations with Widespread Sub-Patent Infections*: African Trypanosomiasis

El-Baky, A.A.A. and S.I. Salem, 2011. Clinico pathological and Cytological Studies on Naturally Infected Camels and Experimentally Infected Rats with *Trypanosoma evansi*. *World Appl. Sci. J*., 14: 42–50

Enwezor, F.N.C. and A.K.B. Sackey, 2005. Camel trypanosomosis-a review. *Vet. Arhiv*, 75: 439–452

Fatihu, M.Y., S. Adam, N.D.G. Ibrahim, L.O. Eduvie and K.A.N. Esievo, 2009. The effect of experimental *Trypanosoma vivax* infection on the thyroid gland in Zebu bulls. *Vet. Arhiv*, 79: 429–437

Ghatak, S., R.B. Muthukumaran and S.K. Nachimuthu, 2013. A simple method of genomic DNA extraction from human samples for PCR-RFLP analysis. *J. Biomol. Tech.: JBT*, 24: 224

Gutierrez, C., J.A. Corbera, M.C. Juste, F. Doreste and I. Morales, 2005. An outbreak of abortions and high neonatal mortality associated with *Trypanosoma evansi* infection in dromedary camels in the Canary Islands. *Vet. Parasitol*., 130: 163–168

Hasnain, H.U. and R.H. Usmani, 2006. *Livestock of Pakistan*, pp: 8–9. Livestock Foundation

Hassan, M.U., G. Muhammad, C. Gutierrez, Z. Iqbal, A. Shakoor and A. Jabbar, 2006. Prevalence of *Trypanosoma evansi* infection in equines and camels in the Punjab region, Pakistan. *Ann. NY Acad. Sci*., 1081: 322–324

Hilali, M.A., A. Abdel-Gawad, A. Nassar and Abdel-Wahab, 2006. Hematological and biochemical changes in water buffalo calves (*Bubalus bubalis*) infected with *Trypanosoma evansi*. *Vet. Parasitol*., 139: 237–243

Hussain, M., Z. Saeed, M. Gulsher, R.S. Shaikh, M. Ali, M. Akhtar and F. Iqbal, 2016. Molecular Detection and Seasonal Prevalence of *Trypanosoma brucei* and its Effect on Hemato biochemical Parameters in Donkeys from Dera Ghazi Khan District in Southern Punjab, Pakistan. *Pak. J. Zool*., 48: 1781–1786

Kassa, T., T. Eguale and H. Chaka, 2011. Prevalence of camel trypanosomosis and its vectors in Fentale district, South East Shoa Zone, Ethiopia. *Vet. Arhiv*, 81: 611–621

Mekibib, B., M. Manegerew, A. Tadesse, F. Abuna, B. Megersa, A. Regassa, S. Mekuria and R. Abebe, 2010. Prevalence of haemoparasites and associated risk factors in working donkeys in Adigudem and Kwiha districts of Tigray region, Northern Ethiopia. *J. Anim. Vet. Adv*., 9: 2249–2255

Naessens, J., 2006. Bovine trypanotolerance: a natural ability to prevent severe anaemia and haemophagocytic syndrome. *Int. J. Parasitol*., 36: 521–528

Ngaira, J.M., B. Bett and S.M. Karanja, 2002. Animal-level risk factors for *Trypanosoma evansi* infection in camels in eastern and central parts of Kenya. *Onderstepoort J. Vet. Res.*, 69: 263

Ohaeri, C.C. and M.C. Eluwa, 2011. Abnormal biochemical and haematological indices in trypanosomiasis as a threat to herd production. *Vet. Parasitol*., 177: 199–202

Oparah, Q.N., A.B.K. Sackey, I.A. Lawal and U.S.A. bdullahi, 2017. Haematological Indices in *Trypanosoma Brucei Brucei* (Federe Isolate) Infected Nigerian Donkeys (*Equus Asinus*) Treated with Homidium and Isometamidium Chloride. *Mac. Vet. Rev*., 40: 73–82

Orhue, N.E.J., E.A.C. Nwanze and A. Akafor, 2005. Serum total protein, albumin and globulin levels in *Trypanosoma brucei*-infected rabbits: Effect of orally administered Scopariadulcis. *Afr. J. Biotechnol*., 4: 1152–1155

Padmaja, K., 2012. Haemato-biochemical studies of camels infested with trypanosomiasis. *Vet. World*, 5: 356–358

Reddy, B.S., K.N. Kumari, S. Sivajothi and V.C. Rayulu, 2016. Haemato-biochemical and thyroxin status in *Trypanosoma evansi*. *J. Parasit. Dis*., 40: 491–495

Rossi, S.M.S., A.A. Boada-Sucre, M.T. Simoes, Y. Boher, P. Rodriguez, M. Moreno, M.L.D. Ruiz, M.L. Marquez, H.J. Finol, C. Sanoja and G. Payares, 2017. Adhesion of *Trypanosoma evansi* to Red Blood Cells (RBCs): Implications in the Pathogenesis of Anaemia and Evasion of Immune System. *Diagn. Pathol. Open*., 2: 2

Silva, R.A.M.S., H.M. Herrera, L.B.D.S. Domingos, F.A. Ximenes and A.M.R. Dávila, 1995. Pathogenesis of *Trypanosoma evansi* infection in dogs and horses: hematological and clinical aspects. *Ciên. Rural*, 25: 233–238

Simenew, K., M. Gezahegne, M. Getachew, M. Wondyefraw, L. Alemayehu and I. Eyob, 2011. Reference values of clinically important physiological, hematological and serum biochemical parameters of apparently healthy working equids of Ethiopia. *Glob. Vet*., 7: 1-6

Sivajothi, S., V.C. Rayulu and B.S. Reddy, 2015. Haematological and biochemical changes in experimental *Trypanosoma evansi* infection in rabbits. *J. Parasitic Dis.*, 39: 216–220

Taiwo, V.O., M.O. Olaniyi and A.O. Ogunsanmi, 2003. Comparative plasma biochemical changes and susceptibility of erythrocytes to in vitro peroxidation during experimental *Trypanosoma congolense* and *T. brucei* infections in sheep. *Isr. J. Vet. Med*., 112–117

Takeet, M.I. and B.O. Fagbemi, 2009. Haematological, pathological and plasma biochemical changes in rabbits experimentally infected with *Trypanosoma congolense*. *Sci. World J*., 4: 29–36

Taylor, K. and E.M.L. Authié, 2004. *Pathogenesis of Animal Trypanosomiasis In: CABI Publishing In the Trypanosomiases*, pp: 331–354. Oxfordshire, Willingford, UK

(Received 08 March 2018; Accepted 14 March 2018)