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EPIDEMIOLOGY AND PATHO-PHYSIOLOGICAL STUDIES IN *Trypanosoma evansi* INFECTED CAMELS AND BUFFALOES IN PAKISTAN

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This study describes some epidemiological and patho-physiological aspects of *Trypanosoma evansi* (causative agent of "surra") infection in camels (n=255) and Nili Ravi buffaloes (n=233). Clinical ailments like fever, shivering, edema of pads and urticarial swelling were observed in infected animals. Overall prevalence of *T. evansi* infection in buffaloes was 9.01 and 15.12% based on blood smear examination and PCR, respectively, while in camels, the prevalence was 10.58 and 15.29% based on smear examination and PCR, respectively. Results on epidemiological data did not indicate significant association of different factors of hosts with prevalence of *T. evansi*. Hematological parameters including hematocrit, mean corpuscular volume (MCV), leukocyte counts, neutrophils, eosinophils and monocytes increased significantly ($P<0.05$) while erythrocyte counts, hemoglobin (Hb) concentration, mean corpuscular hemoglobin concentrations (MCHC), and lymphocytes decreased significantly in infected buffaloes. In infected camels, leukocyte counts, neutrophils, eosinophils, monocytes, and basophils increased significantly ($P<0.05$) while erythrocyte counts, Hb concentration, hematocrit, and lymphocytes decreased significantly. Serum biochemical parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in infected camels while ALT and ALP, LDH, and Lipid per oxidation product (LPO) in infected buffaloes increased significantly ($P<0.05$). Results showed significantly decreased values of AST, total proteins, magnesium, calcium, and phosphorus in infected buffaloes.

Keywords: *Trypanosoma evansi*; Camel; Buffalo, Pathology; Physiology

INTRODUCTION

Livestock sector plays an important role in the economy of Pakistan, and different dairy animals are known as the mainstay for the livelihood of people of the Southern Punjab. In Pakistan, dairy animals such as cattle, buffaloes, sheep, goats, and camels mainly reared for milk and meat production under tropical and sub-tropical climatic conditions (Ali *et al*., 2016; Ali *et al*., 2017; Hussain *et al*., 2017; Hussain *et al*., 2018; Hussain *et al*., 2020). However, bacterial, viral and different parasitic infections have been one of the major threats to dairy animals and different livestock operation systems (Batool *et al*., 2019; Zafar *et al*., 2019; Chemweno *et al*., 2019; El-Shanawany *et al*., 2019; Hussain *et al*., 2019a; Li *et al*., 2020). Among different parasitic infections, *Trypanosoma evansi* (*T. evansi*) is haemoflagellate parasite and causative agent of "surra" that is an important disease of domestic and wild animals (Truc *et al*., 2007; Chau *et al*., 2016). *Trypanosoma evansi* is mechanically transmitted by biting flies and is a common disease of different wild and domestic animals including cattle, dogs, buffaloes, horses, and deer (Yadav *et al*., 2011; Desquesnes *et al*., 2013; Hussain *et al*., 2016; Hussain *et al*., 2018; Mahmoud *et al*., 2020). *T. evansi* most commonly causes trypanosomiasis in animals but is not known to be zoonotic. It causes enormous concern for the livestock farmers not only by lowering the milk or meat production but also by increasing the management cost in terms of management practices and

treatment. Many species of the parasite have been identified but *Trypanosoma evansi* is the most common and endemic in several tropical and sub-tropical areas of the world (Hussain *et al*., 2018). The disease has a debilitating nature in dairy animals and its propagation is mainly favored and directly linked with climatic conditions of the regions and the presence of vectors in the area. The high environmental temperature and relative humidity favors the proliferation of its vectors and thus increases chances of occurrence of the disease. Another epidemiological factor is its transmission in the reservoir hosts that include cattle and buffaloes. Among dairy animals, the buffaloes are considered as the most susceptible to the disease as compared to other domestic livestock. *Trypanosomiasis* has a substantial negative impact on dairy animals and causes high mortality and reproductive losses as well. Clinically, the disease is represented by anemia, decreased milk production, reduced working efficiency, immunosuppression, and abortions (Desquesnes *et al*., 2013). There is a marked reduction in total erythrocytes count (TEC), packed cell volume and hemoglobin concentration in infected animals (Hilali *et al*., 2006). Different investigations have proved that not only the *Trypanosoma* affects the blood cells, but it also has harmful effects in certain body tissues. Moreover, the parasite brings about certain changes in the chemical profile of blood which induce oxidative stress to the buffaloes (Pandey *et al*., 2015; Hussain *et al*., 2018). Regardless of being somewhat expensive and technical, PCR is usually useful in diagnosis of the disease. Parasite detection using PCR technique has many advantages over serology and microscopy techniques with respect to the specificity, sensitivity and rapidity. The molecular basis of disease diagnosis in the field has helped to improve the detection rate of surra in different herds (Sumbria *et al*., 2015, Sumbria *et al*., 2017).

Despite different previous studies conducted in tropical and sub-tropical areas of Pakistan on cattle, buffaloes, camels and equines, no information is available from pastoral areas about the presences of animal trypanosomiasis due to non-tsetse transmitted trypanosome infection. Hence, the present study was carried out to investigate the strain based non-tsetse transmitted trypanosome infection and its pathophysiological impacts in infected buffaloes and camels.

MATERIALS AND METHODS

Animals: This study was carried out at two districts (Lodhran and Bahawalpur) of Punjab province. A total of 255 camels reared at Cholistan desert conditions, district Bahawalpur and a total of 233 buffaloes of mixed breeds kept at different villages of tehsil Lodhran of district Lodhran and having different age and sex were randomly included in this study during the month of October and November 2018 for screening of *Trypanosoma* infection. The data regarding age of animals was obtained from the owners or attendants. The animals with history of pyrexia, lacrimation and having different other physiological abnormalities (body condition, pulse, and respiration rate) were screened for the presence of *Trypanosoma* infection.

Blood Sampling: For parasitological investigation, blood was obtained from marginal ear veins and jugular vein of each animal (camel and buffalo) according to instructions and the guidelines of International Animal Ethics and Welfare Committee in glass test tubes with and without anticoagulant (EDTA; 1mg/ml) (Hussain *et al*., 2016). Equal number of blood samples (20) from healthy and infected animals of both species was collected for different blood and serum biochemical analysis (Hussain *et al*., 2018).

Parasitological Investigation: Thin blood smears for each animal were prepared using microscopic glass slides for microscopic identification of flagellated Trypanosomes. All the smears were made from fresh blood (Hussain *et al*., 2018). All the microscopic blood smears were dried, fixed with absolute ethanol and immediately stained with Giemsa stain. For different morphological alterations in red blood cells and the presence of *Trypanosoma*, all the blood smears were carefully observed under light microscope (Hussain *et al*., 2016). The collected blood samples were also subjected to PCR for the confirmation of different strains of parasite. The DNA was extracted from the blood by phenol-chloroform method and PCR was performed by using specific primers (TBR1 forward, 5'GAATATTAAACAATGCGCAG'3 and TBR2 reverse, 5'CCATTTATTAGCTTTGTTGC'3) according to the procedure as described earlier (Desquesnes *et al*., 2001).

Hematological Studies: Blood samples collected without anticoagulant were subjected to different hematological parameters including total erythrocyte counts, hemoglobin concentration, total leukocyte and differential leukocyte counts, hematocrit along with mean corpuscular hemoglobin quantity were determined (Hussain *et al*., 2016).

Blood and Serum Biochemistry: All the serum samples were investigated for determination of serum proteins (serum total proteins and albumin quantity), different enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), lactate dehydrogenase (LDH), mineral contents and a lipid peroxidation product (malondialdehyde concentration) according to the previously used protocol (Hussain *et al*., 2019b).

Statistical analysis: Data collected in the present study were analyzed by using suitable statistical tools. The prevalence was determined by chi-square test. Odd ratio and 95% C.I. were also determined. Data on blood biochemical parameters were subjected to Student's t-test, using SPSS. The significant difference was considered at $P<0.05$.

RESULTS

The overall prevalence of 9.01% of *Trypanosomiasis* infection based on microscopic smear examination, and 15.12% with PCR in buffaloes while 10.58% based on microscopic smear examination and 15.29% (Figure 1) on the basis of PCR in camel was recorded (Table 1). The PCR test gave 2.28 times higher positive results, which is significant than blood smear-based test results in buffalo. While in camel, the PCR gave 52% higher positive results than smear test results and the chi-square difference was non-significant. The results of Chi-square test did not reveal significant differences of blood-based smear tests between male and female of both camel and buffaloes (Smear positive or PCR positive). Similarly, the difference of blood based positive test results between age groups was also non-significant in both camel and buffaloes (Smear positive or PCR positive).

Table 1. Frequency of clinical signs observed in buffaloes (43) and camels (39) infected with *trypanosoma evansi*

Clinical sign	No.	Percentage
Buffalo		
Anorexia/inappetence	39	90.6
Pyrexia	39	90.6
Depression/lethargy	33	76.6
Pale mucous membrane	35	81.3
Dehydration	33	76.6
Lymphadenomegaly	11	25.5
Petechiae/ecchymosis	23	53.4
Diarrhea/constipation	17	39.5
Icterus	39	90.6
Corneal opacity/ocular signs	31	72.0
Ataxia/nervous signs	17	39.5
Emaciation	31	72.0
Respiratory signs/dyspnoea	33	76.6
Congested mucous membrane	29	67.4
Ascites/peripheral edema	23	53.4
Camel		
Anorexia/inappetence	29	74.3
Pyrexia	33	84.6
Depression/lethargy	31	79.4
Pale mucous membrane	37	94.8
Dehydration	35	89.7
Lymphadenomegaly	09	23.1
Petechiae/ecchymosis	27	69.2
Diarrhea/constipation	23	58.9
Icterus	29	74.3
Corneal opacity/ocular signs	31	79.4
Ataxia/nervous signs	15	38.4
Emaciation	29	74.3
Respiratory signs/dyspnoea	27	69.2
Congested mucous membrane	31	79.4
Ascites/peripheral edema	29	74.3

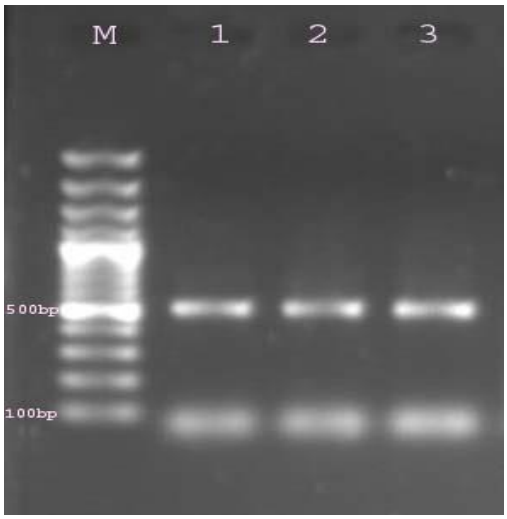

Figure 1. PCR products and electrophoresis (1.5% agarose, stained with ethidium bromide) of TBR gene (535bp) specific for DNA amplification of *Trypanosoma evansi*: Lanes (1-3) positive reactions. M: DNA marker.

The PCR based findings on *Trypanosomiasis* in camels and buffaloes indicated that this test was more sensitive to detect *Trypanosomiasis* in buffaloes than camel. The occurrence of the disease was found 25% more as compared to the smear method.

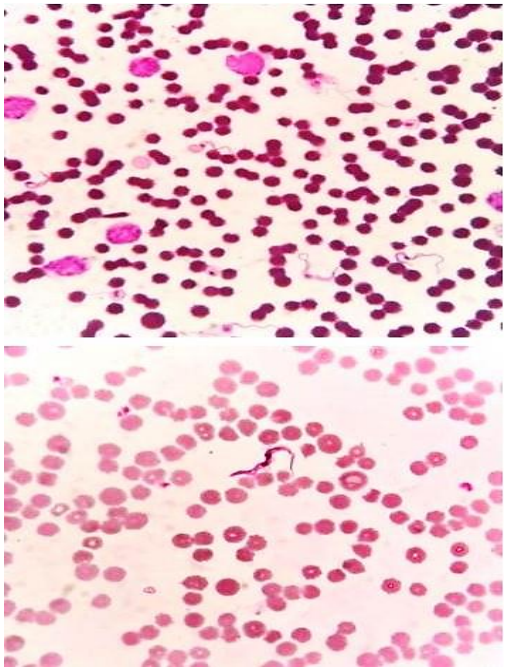

Figure 2. Blood smear of *Trypanosoma evansi* positive buffalo showing presence of parasite and hypochromatic red blood cells (Giemsa Staining-1000X).

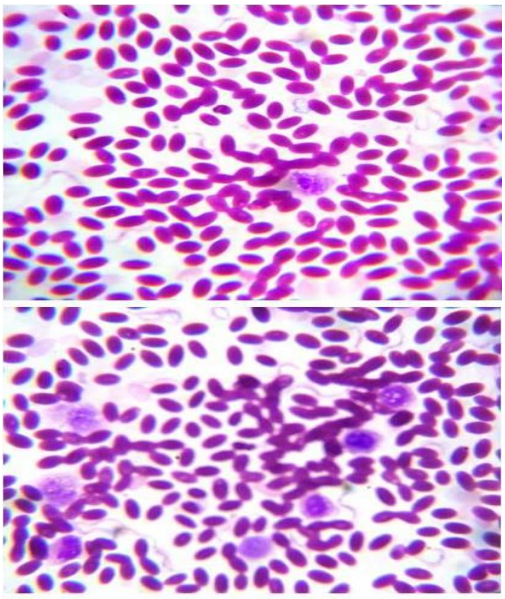

Figure 3. Blood smear of *Trypanosoma evansi* positive camels showing poikilocytosis, anisocytosis and presence of parasite and hypochromatic red blood cells (Giemsa Staining- 1000X).

Various physiological abnormalities or clinical abnormalities of the buffaloes and camels recorded included anorexia, pyrexia, lacrimation, edematous legs, hyperemic eyes, and frequent urination. In addition, in camels brisket edema was observed in few cases (03). Furthermore, many infected animals were emaciated, depressed and lethargic. Microscopic observation of blood smear revealed morphologically various strains of parasite on the basis of flagellum in buffaloes (Fig. 2) and camels (Fig. 3).

The results of hematological parameters showed that erythrocytes counts and erythrocyte indices including Hb concentration and MCHC were significantly reduced (P<0.05) in *T. evansi* infected animals (buffaloes and camels) in comparison to non-infected animals (Table 3). The hematocrit values were higher (P<0.05) in buffaloes but were lower in camel. The leukocyte counts, neutrophils, monocytes and eosinophils increased significantly (P<0.05) both in infected camels and buffaloes, while the lymphocyte counts decreased significantly in infected animals of both the species. Results revealed non-significant difference in basophils counts in *T. evansi* infected buffaloes in comparison to non-infected (Table 3) while in infected camels it increased significantly. Results on microscopic observation of blood smear of camels indicated presence of morphologically different red blood cells (Fig. 2). The frequency of microcytes, macrocytes, red blood cells with nuclear remnants along with polychromasia and hypochromasia were increased in trypanosome infected camels. Results on serum biochemistry showed that the quantity of total serum proteins in camels and buffaloes were significantly reduced (P<0.05) when compared to non-infected animals.

The results on various serum enzymes showed that the quantity of ALT and ALP were significantly (P<0.05) higher in infected animals. The AST was lower (P<0.05) and higher in infected buffaloes and camels, respectively. The LDH was higher in buffaloes. The values of oxidation product (malondialdehyde) were increased significantly (P<0.03) in infected buffaloes (Table 3). Results showed that different serum macro-minerals including magnesium, calcium, and phosphorus decreased significantly in infected buffaloes (Table 3).

Table 2. PCR and Giemsa smear examination based frequency of *Trypanosoma evansi* in camels and buffaloes.

Species/ sex/age	No.	Positive		95%C.I.	Odd Ratio/ P value
		N	%		
Giemsa smear test					
Buffalo					
Sex					
Male	69	07	10.14	4.55 - 19.04	**1.21** [reciprocal =
Female	164	14	8.53	4.94 - 13.59	0.83]
Age groups					
< 1	47	3	6.38	1.65 - 16.39	Mantel-Haenszel
2-3	73	9	12.32	6.18 - 21.42	chi-sq. P = 0.983
4-5	67	5	7.46	2.79 - 15.76	
> 5	46	4	8.69	2.82 - 19.66	
Polymerase chain reaction					
Sex					
Male	69	15	21.73	13.20 - 32.61	**1.35** [reciprocal =
Female	164	28	17.07	11.89 - 23.41	0.74]
Age groups					
< 1	47	7	14.89	6.75 - 27.25	Mantel-Haenszel
2-3	73	15	20.54	12.44 - 30.95	chi-sq. P = 0.827
4-5	67	13	19.40	11.24 - 30.16	
> 5	46	8	17.39	8.42 - 30.36	
Giemsa smear examination					
Camels					
Sex					
Male	62	08	12.90	6.18 - 23.03	**1.36** [reciprocal =
Female	193	19	9.84	6.21 - 14.68	0.74]
Age Groups					
< 1	53	5	9.43	3.54 - 19.68	Mantel-Haenszel
2-4	69	7	10.14	4.55 - 19.04	chi-sq. P = 0.692
5-6	81	9	11.11	5.56 - 19.41	
>6	52	6	11.53	4.81 - 22.46	
Polymerase chain reaction					
Sex					
Male	62	09	14.51	7.32 - 24.97	0.92 [reciprocal =
Female	193	30	15.45	10.94 - 21.18	**1.08**]
Age groups					
< 1	53	6	11.32	4.72- 22.06	Mantel-Haenszel
2-4	69	11	15.94	8.68 - 26.01	chi-sq. P = 0.487
5-6	81	13	16.04	9.23 - 25.26	
>6	52	9	17.30	8.79 - 29.39	

Table 3. Blood profile of *Trypanosoma evansi* positive and healthy buffaloes and camels

Parameters/Species	Healthy (20)	Infected (20)	p-value
Buffalo			
Erythrocyte counts (10^6/µL)	7.69±0.26	6.11±0.31*	0.001
Hemoglobin quantity (g/dL)	12.9±0.7	10.1±0.2*	0.001
Hematocrit (%)	34.65±1.75	44.52±1.45**	0.001
Mean corpuscular volume (fL)	45.8±2.8	67.5±9.2**	0.001
Mean corpuscular hemoglobin concentration (g/dL)	37.3±2.2	22.6±0.8*	0.001
Leukocyte counts (10^3/µL)	9.21±0.38	12.27±0.53**	0.001
Neutrophil (%)	34.3±1.1	50.1±5.3**	0.001
Lymphocyte (%)	49.6±1.6	41.2±3.5*	0.001
Eosinophil (%)	2.45±0.22	3.84±0.25**	0.001
Monocyte (%)	4.15±0.33	5.05±0.76**	0.001
Basophil (%)	0.57±0.05	0.64±0.09	0.094
Camels			
Erythrocyte counts (10^6/µL)	7.12±0.45	4.75±0.24*	0.001
Hemoglobin quantity (g/dL)	12.05± 0.69	9.14± 0.43*	0.001
Hematocrit (%)	40.48±2.2	28.6±2.1*	0.001
Mean corpuscular volume (fL)	57.1±3.9	60.6±5.7	0.295
Mean corpuscular hemoglobin concentration (g/dL)	29.3±2.1	31.6±3.2	0.309
Leukocyte counts (10^3/µL)	12.2± 1.2	17.5±1.5**	0.001
Neutrophil (%)	32.2± 1.3	46.6±2.4**	0.001
Lymphocyte (%)	49.5±4.6	40.5±2.6*	0.001
Eosinophil (%)	2.26±0.17	3.84±0.14**	0.001
Monocyte (%)	2.24±0.35	3.92±0.44**	0.001
Basophil (%)	0.73±0.18	1.48±0.06**	0.001

P-value less than 0.05 indicate the significance abnormality in trypanosome infected buffaloes; *Indicate significant decrease, while ** indicate significant increase in values in each row.

Table 4: Serum biochemical profile of *Trypanosoma evansi* positive and healthy buffaloes and camels

Parameters/Species	Healthy (20)	Infected (20)	p-value
Buffalo			
Alanine aminotransferase (U/L)	83.8±2.6	108.49±4.2**	0.001
Aspartate aminotransferase (U/L)	85.5±2.5	25.12±1.5*	0.001
Alkaline phosphatase (U/L)	83.8±2.6	108.4±4.2**	0.001
Lactate dehydrogenase (U/L)	270.1±20.3	339.8±3.9**	0.001
Serum total proteins ((g/dL)	5.68±0.41	4.27±0.38*	0.001
Lipid per oxidation product	1.59±0.22	2.17±0.24**	0.001
Magnesium (mg/dL)	2.29 ± 0.12	1.43 ± 0.11*	< 0.04
Calcium (mg/dL)	9.11 ± 0.02	7.01 ± 0.04*	< 0.02
Phosphorus (mg/dL)	5.13 ± 0.09	3.39 ± 0.17*	< 0.01
Camels			
Alanine aminotransferase (U/L)	15.8±1.43	34.07±7.57**	0.001
Aspartate aminotransferase (U/L)	30.77±2.45	43.47±3.24**	0.001
Alkaline phosphatase (U/L)	95.47±5.65	127.3±4.1**	0.001
Serum total proteins (g/dL)	6.33±0.50	4.75±0.18*	0.001
Albumin (g/dL)	3.55±0.25	2.23±0.11*	0.001

P-value less than 0.05 indicate the significance abnormality in trypanosome infected buffaloes; *Indicate significant decrease, while ** indicate significant increase in values in each row.

DISCUSSION

Dense population of dairy animals particularly buffaloes, cattle and camels exist in southern Punjab. This region accounts for about more than 52% of agriculture-based landscape where 32% of the total population of this province inhabits and mostly associated with dairy animals for their livelihood. The cattle (Cholistani breed) and camels are the main dairy animals reared for routine livelihood of the people of this region. The buffaloes are mainly kept in villages. In

desert conditions (Cholistan) nomadic system exists and there is seasonal based migration of animals due to shortage of feed and water. The camels are known as the best source of livelihood of people of desert areas including Africa, Asia and Middle East (Diall *et al*., 1993; AL-Samawy *et al*., 2019; Gherissi *et al*., 2020). Due to lack of proper husbandry practices in this region, the chances of occurrence and transmission of different infections agents increase in dairy animals. Therefore, continuous disease monitoring, from time to time is important for control strategies and eradications of various parasitic endemic infections (Riaz *et al*., 2109; Hussain *et al*., 2017; Ali *et al*., 2020). Therefore, due to this reason the current research was carried out to investigate the presence of trypanosome parasite in camels and buffaloes.

The different clinical signs observed in camels and buffaloes in this current study, like high temperature, and lacrimation due to *Trypanosoma evansi* have previously been reported in camels (Baticados *et al*., 2011; Padmaja, 2012; Tehseen *et al*., 2015; Hussain *et al*., 2016), and buffaloes (Sivajothi and Reddy 2017; Hussain *et al*., 2018; Nuryady *et al*., 2019). The findings of our study indicated that there were no significant differences in presence of disease in camels and buffaloes on the basis of age and gender. In contrast to these findings, increased prevalence of infection in old (Bogale *et al*., 2012), young (Kassa *et al*., 2011) and male animals (Bogale *et al*., 2012) have previously been reported.

In the present study, results showed significant changes in hematological parameters due to *Trypanosoma* infection in camels and buffaloes. The erythrocyte counts, lymphocyte, hemoglobin and mean corpuscular hemoglobin concentration were significantly reduced in trypanosome infected animals while total leukocyte count, neutrophils, eosinophils and monocytes were significantly increased in diseased animals. The evaluations of various hematological parameters are considered as the most important and reliable to know the stage and severity of the disease infected animals. Previously it is reported that the blood biochemical parameters are the useful and reliable biomarkers to determine disease conditions including surra (Ohaeri and Eluwa, 2011; Pandey *et al*., 2015; Hussain *et al*., 2018; Mingala *et al*., 2020). In present study, lower blood values including decreased red blood cells, lymphocyte, MCHC and hemoglobin quantity in infected camels and buffaloes are suggestive of anemia. The significantly decreased erythrocyte count in affected cases could also be related to parasitic infection leading to removal of red blood cells by phagocytic actions of mononuclear cells in lymph nodes and spleen. Moreover, decreased number of erythrocytes also leads to lower values of hematocrit, responsible for vascular abnormalities and dysfunctions resulting in anoxic conditions in variety of tissues (Eyob and Matios, 2013; Hussain *et al*., 2018). Similar reports are available in different previous published studies in camels (Mijares *et al*., 2010; Padmaja, 2012; Eyob and Matios, 2013; Hussain *et al*., 2016) and buffaloes (Pandey *et al*., 2015; Hussain *et al*., 2018). The lower counts of lymphocytes observed in the infected animals in this study may be attributed to the immunosuppressive actions of trypanosome infection (Abubakar *et al*., 2005; Ekanem *et al*., 2008). Results of this study showed increase in white blood cell counts, neutrophil counts, PCV and MCV values in both trypanosome infected camels and buffaloes. These occur as a result of innate immune responses as being the first line of defense among the cells to the infection to fight against the infection which are similar to previous published literature (Padmaja, 2012; Hussain *et al*., 2018). In addition, increased population of neutrophils, eosinophils and basophils are suggestive of parasitic infection and has also been recorded previously in Surra (Ahmad *et al*., 2004; Padmaja *et al*., 2012). The increased frequency of red blood cells like microcytes, macrocytes, cells with nuclear remnants, polychromic and hypochromic cells in infected camels could be due to oxidative stress resulting hypoxic conditions in tissues. Similar reports are also available in naturally infected camels (Hussain *et al*., 2016) and experimental rats (El-Baky and Salem, 2011).

The significantly increased levels of biochemical parameters such as serum enzymes and lipid peroxidation products in infected camels and buffaloes might be due to increased generation and release of free radicals leading to induction of oxidative stress, injury to blood vessels and increased permeability of small blood vessels (Akerstedt *et al*., 2011). Moreover, different previous studies have reported that the increased quantity of different serum biochemical parameters including serum enzymes and lipid peroxidation product could be due to hepatic degeneration, oxidative stress, and hypoxic conditions faced by different body tissues (El-Baky and Salem, 2011; Hussain *et al*., 2018). The significantly lower contents of different serum minerals can be related to abnormal physiological changes induced by oxidative stress leading to poor absorption and lower bioavailability in infected animals (Spears and Weiss, 2008; Kirmizigul *et al*., 2016; Hussain *et al*., 2020) It is also speculated that anemia creates anoxic disorders in diseased animals and eventually leads to development of weakness which exhibits the signs of dysfunction in various organs mainly including the liver and results into release of several enzymes particularly the alanine aminotransferase and aspartate aminotransferase (Olaho-Mukani and Mahamat, 2000; Soylu, 2013).

Conclusion: Keeping in view the findings and investigation of this study, it is therefore suggested that continuous screening and detection of *T. evansi* infection is crucial to enhance the productivity of dairy animals.

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