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HEMATOLOGICAL AND BIOCHEMICAL ALTERATIONS ASSOCIATED WITH TOXOPLASMOSIS IN DROMEDARIES (*CAMELUS DROMEDARIUS*) HABITATING IN CHOLISTAN DESERT OF BAHAWALPUR, PUNJAB, PAKISTAN

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ABSTRACT

Toxoplasmosis is an important cause of reproductive failure in animals, resulting in socio economic losses all over the world. The aims of the current study was to investigate the seroprevalance of *Toxoplasma gondii* in *Camelus dromedarius* (Dromedary camels) and also to assess the hematological and biochemical alterations associated with *T. gondii* infection from February, 2015 to December, 2015. Blood samples were aseptically collected and were analyzed for *T. gondii* prevalence by direct agglutination test kits and hematobiochemical parameters by hematology analyzer. A total of 201 camels of different age were examined from 12 flocks in different Cholistan areas of Bahawalpur. Of 201 selected camels only 36 camels found seropositive of toxoplasmosis. The overall seroprevalence of toxoplasmosis was 17.91% in camels and the prevalence was found significantly ($P<0.01$) highest 31.25% in camels age group of ≥ 13 years and lowest 10.52% in age group of 9-12 years. Camels that suffered from toxoplasmosis (n=60) were subjected to hematological and biochemical analysis and matched with clinically healthy camels as control (n=60). The haematological analysis revealed a significant ($P<0.05$) reduction in the total RBC count, Hb concentration, HCT, PVC, MCH, MCHC, LYM, PLT and PDW in the affected camels. In addition, significant ($P<0.05$) increases in WBCs, MCV, RDW, NEUT, MPV, MXD, platelets and P-LCR were observed. The biochemical analysis revealed a highly significant reduction ($P<0.05$) in the sugar and AST level. Significant ($P<0.05$) increases in ALT, total bilirubin, blood urea, uric acid and blood cholesterol levels were detected in affected camels when compared with the controls.
In conclusion, *T. gondii* was more common in older age group of camel; moreover toxoplasmosis greatly affected the hematobiochemical parameters of camel.

Keywords: Toxoplasmosis, Camels, Age, Prevalence, Hematological, Biochemical

INTRODUCTION

Domestic animals play a vital role in the economy of rural populace of Pakistan. It contributes 11.8% to national GDP and provided 56.3% of value addition in agricultural sector during 2014-15. The earnings from livestock products (milk, meat, wool, leather) have exceeded Rs. 801.3 billion rupees in 2014-15 plus providing thousands jobs to rural families (Anonymous, 2014-15). The camel is important specie which plays an important role in the national economy and social life of a large people of arid region in Pakistan. Camels are famous for their ability to survive in hot and dry environment (Fowler, 1996; Farooq *et al*., 2012). Camel has a capability to change the scanty plant resources of the desert into meat, milk and fiber. Moreover 862 million tons camel milk is produced annually, which cost at Rs 4.8 billion. Moreover, 50,000 tons of camel meat is produced yearly, price at Rs. 350 million (Anonymous, 2014-15).

The camel population in Pakistan is about 1.2 million and is the 3rd larger camel-raising country in the world. The highest population of one-humped camels is in Baluchistan (41.22%), followed by Punjab (21.61%), Sindh (30.23%) and NWFP (7%) (Anonymous, 2014-15). Camels suffer from a variety of parasitic infections, including toxoplasmosis, which have economic significance. *Toxoplasma gondii* infect the camels through ingestion or inhalation of sporulated oocysts that are shed by domestic cats in the pasture. *Toxoplasma gondii* is the dangerous parasite on earth planet as it has no geographical boundaries and is found in almost every mammal and bird species in all around the globe (Carruthers, 2002). Frequent prevalence of *T. gondii*, has been reported in numerous areas of the world (Tenter *et al*., 2000). The majority of natural *T. gondii* prevalence in domestic animals is asymptomatic. Clinically it is manifested by abortion and stillbirth in sheep, goats and cattle (Lashari and Tasawar, 2010; Tasawar *et al*., 2011, 2013). This imposes considerable restrain on animal production throughout the tropics and subtropics (Kazakove *et al*., 1982). The most common method of spread in ruminants is ingestion of feed contaminated by wild felids including house cat are its definitive hosts

where oocysts are produced by sexual reproduction (Tenter *et al*., 2000).

Haematological and biochemical alterations can provide precious information about the severity of the infestation and are considered to be good tools for the diagnosis, prognosis and evaluation of the treatment applied. Additionally, the biochemical profile may help in understanding the host–parasite relationship at a molecular level and to describe the disease clearly (Al-Kaysi *et al*., 2010).

The research has been documented in different parts of Pakistan, reported ovine toxoplasmosis (Lashari and Tasawar, 2010) caprine and bovine toxoplasmosis (Tasawar *et al*., 2011, 2013).

Similar data of camels toxoplasmosis in some other countries are existing such as Osman and Al-Busadah (2000) for Saudi Arabian camels and Chaudhari *et al*., 1996 for Abudehbi racing camels, Dessouky (2006) for Egyptian camels and Sazmand *et al*. (2011) for Iranian camels, Gebremedhin *et al*., 2016 for Ethiopian camel, Bartova *et al*., 2017 for Czech Republic camelids.

Moreover over the haematological and biochemical parameters in camel *Toxoplasma* infestation have been conducted on experimentally or naturally infected camelidae species (Atmaca *et al*., 2015; Al-Kaysi *et al*., 2010), but to our knowledge there is no investigation on toxoplasmosis, haematological and biochemical alterations by *T. gondii* in Cholistani camels so far. Keeping in view the importance of camel toxoplasmosis in the current study was conducted to examine the overall prevalence and host parasite relationship with age, some hematological and biochemical parameters in naturally infected camels with *T. gondii*.

MATERIALS AND METHODS

Study area: The current study was carried out in the Cholistan desert of Bahawalpur district (28°.50'62" N latitude and to 71°.57'24" E longitude) which is located in the Punjab province of Pakistan. It lies at an altitude of 461m above sea level and has an annual rainfall ranging from 100 mm-250 mm and average annual temperature ranging from 5-40°C. The common flora of Cholistan desert includes the perennial grasses, annual grasses, sedge, herbs, shrubs and trees. All the camels were female and were grouped by age into four categories (1-4 years, 5-8 years, 9-12 years and > 13 years). The ages of camels were estimated according to their dentations and verified through information provided by their owners.

Blood collection: A total of 201 camel blood samples were obtained from the jugular vein punctured using 16 gauge needle of apparently healthy camels. The collected samples were kept in an icebox containing ice packs and immediately transported to the Parasitological laboratory of the department of Life Sciences, The Islamia University of Bahawalpur. From each camel, 15 ml blood sample was collected with and without anticoagulant. Heparin was used as anticoagulant. One of which containing heparin used to determination of haematology and biochemical test, other test tube was left uninterrupted for clotting of blood and serum separation for testing and isolation of *Toxoplasma gondii*. Blood samples were centrifuged for 10-15 minutes at about 3500 rpm to separate the plasma that was stored at –20°C until assayed for biochemical test.

Serological analysis

Latex agglutination test (LAT): The collected serum samples were examined for the presence of antibodies against *Toxoplasma gondi* by direct agglutination test (Antec Diagnostic. Product TM, UK) following the procedure of the manufacturer. The *Toxo*-latex reagent is a suspension of polysterine latex particals coated with soluble *T. gondii* antigen. The serum and latex reagent were mixed. A positive result was expressed by agglutination. The blood collected in heparined tubes was run on Sysmex KX-21 automatic blood cell counter to record red blood cell (RBC), white BWBC, Hb, PCV, MCV, MCH, MCHC, PLT, P-LCR, NEUT, MXD, LYM, RDW, HCT, PLT and PDW following the methods described by Benjamin (1978). The biochemical parameters included in the study were ALT, AST, cholesterol, bilirubin, sugar, urea and uric acid. Serum isolated from each sample was run on SACA-19100 automated biochemical analyzer.

Ethical considerations: This research study was approved by the animal ethical committee of the Faculty of Sciences, the Islamia University of Bahawalpur. All efforts were made to minimize animal suffering during the course of the work. Informed written permission was got from all camel farmers who engaged in the study.

Data analyses: The data collected from field and laboratory investigations were entered and coded using Microsoft Excel, Office 2007 and analyzed using MINITAB version 13.1. Descriptive statistic was used to sum up the data. The seroprevalence results of LAT tests and haematological and biochemical parameters were compared using the Chi-squared test and Student's t-test respectively. Significant differences were considered when $P \leq 0.05$.

RESULTS AND DISCUSSION

Overall prevalence of toxoplasmosis: In present study out of total 201 hosts observed by the latex agglutination test (LAT), 36 were positive and the overall prevalence percentage of toxoplasmosis was 17.91 %. Most of the relevant previous studies described the clinical findings

of toxoplasmosis in sheep, goats and cattle species (Lashari and Tasawar, 2010, Tasawar *et al*., 2011, 2013), but few studies have described the clinical picture of this disease in camels. In Iran Sadrebazzaz *et al*. (2006); Khamesipour *et al*. (2014) examined 120 and 122 apparently healthy camels and found that 4.2, 6.60% were infected with *Toxoplasma gondii* respectively.

The highest 61.7, 67 and 69% seroprevelence for toxoplasmosis in camel using the LAT serologically was detected in the Butana plains, mid-eastern Sudan and Czech Republic (Manal, 2003; Elamin *et al*., 1992; Bartova *et al*., 2017) respectively. In Iraq Mahmoud *et al.* (2014) examined 360 apparently healthy camels and found that 60.83% were infected with toxoplasmosis. In other study at Al-Najaf province of Iraq Al-Mudhfer and Kshash (2012) reported 20.35% were infected with toxoplasmosis. In contrast only 6.5% of the examined camel were harboring with *T. gondii* in camels of Al-Qadisiya governorate in Iraq (Al-Hindawe, 2006). The difference in seroprevalence rates around the world depends on many factors: type of breeding and management practices, zoohygienic status, age of examined animals and geographical region (Zhao *et al*., 2011). Higher prevalence could be due to free access of cats to the enclosure. Also it differs because of difference in serodiagnostic tests used (Vesco *et al*., 2007).

Relationship between age and toxoplasmosis in camels revealed that the parasite had significantly ($P<0.05$) highest prevalence 31.25% in age group of ≥ 13years and lowest prevalence 10.52% in age group of 9-12 years (Table 1). Basheir *et al*. (2012) reported that age is an important factor in the prevalence of *T. gondii* in camels. The prevalence increased as the age of camels increased. Progressive increase of *T. gondii* with age suggests a continuous exposure to the organism in the environment as reported in past (Basheir *et al*., 2012). Similar results have been reported in other domesticated animals in different areas of the world (Ivana *et al*., 2006; Tasawar *et al*., 2011, 2013). Some reports are contradicted to the present study (Lashari and Tasawar, 2010). The differences may be due to the management system and health practices have a significant reason on the incidence of blood borne parasites (Vesco *et al.,* 2007).

The mean and (±SEM) of haematological data obtained from 60 *Toxoplasma gondii* positive and 60 *Toxoplasma gondii* negative camels is shown in Table 2. Blood is an important index for several metabolic processes in the body that may in one animal species vary due to age, physiological condition and environmental factors. In the present study a significant ($P<0.05$) decrease was observed in the values of RBC, HGB (g/dL), HCT, NEUT (%), MCV (f L) and RDW (f L), whereas values of WBC ($x10^3/\mu L$), MCH (Pg), MCHC (g/dL), LYM (%), MXD (%), LYM ($x10^3/\mu L$), MXD ($x10^3/\mu L$), NEUT ($x10^3/\mu L$), PDW (fL), MPV (fL), P-LCR (%) were significantly increased in infected camels in comparison with non infected camels. Similar results were documented by Sazmand *et al*. (2011); Ahmed *et al.* (2004); Chaudhari *et al*., (1996); Partani (1994); Coles (1986); Raisinghani and Lodha (1980) in camel.

Ahmad *et al*. (2004) reported leukopaenia in equines toxoplasmosis. The variation in the results of WBC in present and prior studies could be due to differences in handling the samples (Salaheldin *et al*., 1979). Generally the WBC count in the camel was more as all the other livestock (Salaheldin *et al*., 1979). In the present study, the difference in total leukocyte count of normal and infected camels was significantly ($P < 0.05$) different. The neutrophils comprised 67.9±16.4 in normal and 49.4±13.1% in infected camels. The present results are similar with findings recorded by Bush (1991). Leukopineia is well documented in camel toxoplasmosis (Chaudhari *et al*., 1996).

The values of red blood cell counts were lower than that reported means of 9.686 ± 0.276 and 9.056 ± 0.161 9.83 in normal and infected camels, respectively (Ahmad *et al*., 2004); 9.20 ± 0.31 in normal and 7.50 ± 0.40 million mm^3 in infected camel (Nadim and Soliman, 1967), 9.20 ± 0.31 and 7.50 ± 0.40 in normal and 4.52 million mm^3 in infected camels (Jatkar and Mohan, 1971). This could be due to variation in techniques, nutrition, breed and environmental conditions (Salaheldin *et al*., 1979).

The mean value of Hb in infected camels decreased considerably in the present study and are in agreement with those reported in past (Farooq *et al*., 2011; Ahmad *et al.*, 2004; Jatkar and Mohan, 1971; Nadim and Soliman, 1967). The haemoglobin results in non infected camels in the present study agree with those reported by Salaheldin *et al*. (1979) and Lakhotia *et al*. (1964) in Sudanese and Indian camels, respectively but these values are lower than those reported by Soliman and Shaker (1967) and Chaudhari *et al*. (1996).

In the present study the results of haematocrit (HCT) in normal and infected camels were 45.7±13.1 and 43.6±11.3% respectively. Hussein *et al*. (2010) reported HCT value 26.43 ± 0.69% in normal camels. Ahmad *et al*. (2004) reported in normal and infected camel 27.662 ± 0.726 and 26.423 ± 0.726%, respectively. Jatkar and Mohan (1971) reported lower HCT values of 28.94 and 21.90 %, respectively for normal and infected camels. Raisinghani and Lodha (1980) recorded reduction in HCT of 66.20 percent in camels infected with *Trypanosoma evansi*. The lower values of erythrocytes, haemoglobin were reported in different parasitic infestation which causes large number of erythrocyte to be removed due to mononuclear phagocytic response in spleen and haemal lymph nodes. This removal of erythrocytes also leads to decrease in haematocrit ultimately resulting to severe anoxic conditions (Atmaca

et al., 2015). Since *Toxoplasma gondii* infection in camel may last for many years, the differences in the duration of the disease at which they entered into the present study and those studies in the past may account for part in least for differences in packed cell volume observed in the present study and those reported in past. The present values of platelet (PLT) are in line with those recorded in past in different breeds Majahim, Maghatir and Najdi camels. While Hussein *et al*., 2012 reported higher values of platelets platelet count: 212.17 ± 10.76 ($x10^9$/L) than present study. Various platelet indices like platlets distribution cell ration (PDW), Mean platelet volume (MPV), Platelet large cell ratio (P-LCR) were investigated with higher values in camels infected with *Toxoplasma gondii* than the normal camels in present study. The same platlets indices have been reported high in camel infected with *Trypanosoma evansi* in racing camel in Dubai (Chaudhari *et al*., 1996; Ahmad *et al*., 2004).

A red cell width (RDW) value was low in infected camel than the non infected camels of the present study. RDW is a mathematical quantity of anisocytosis (Ahmad *et al*., 2004). Modern automated cell counters are able to evaluate the volume of red cells very easily and accurately. RDW is a useful tool in differentiating iron deficiency anemia and parasitic anemia (Ahmad *et al*., 2004).

The mean±SEM values of biochemical parameters in Cholistani camels naturally infected with *T. gondii* and non–infected camels are presented in Table 3. Toxoplasmosis causes severe and progressive damage to the liver (Atmaca *et al*., 2015), resulting in alterations in liver metabolism. A significant (P<0.05) reduction was seen in the values of sugar (µmol/L) and AST(µ/L) whereas values of urea (µmol/L), uric acid(µmol/L), bilirubin (µmol/L), ALT (µ/L) and cholestrol (mmol/L) were significantly (P<0.05) elevated in infected camels.

The values of sugar (µmol/L) and AST are in agreement with previous studies conducted by Raisinghani and Lodha (1980) in camel. This is a common finding in *Toxoplasma gondii* and is reported due to excessive utilization of blood glucose by the parasites for their metabolism (Anosa, 1988). Increased metabolic rate caused by fever and hepatocyte degeneration could also be a reason for hypoglycemia in trypanosomiasis (Cadioli *et al*., 2006).

Whereas several studies have showed elevation of ALT, urea (µmol/L), uric acid (µmol/L), bilirubin (µmol/L) and cholestrol (mmol/L) activities in *T. gondii* infected mice, dogs, goats and camels (Amany *et al*., 2010; Atmaca *et al*., 2015). Increased plasma ALT activities reflect impairment of the liver. When the liver is impaired the liver cells release the enzymes into the blood raising the enzyme activities during toxoplasmosis (Sowjanya *et al*., 2013; Atmaca *et al*., 2015).

In conclusion, this study showed that *T. gondii* was more prevalent in old animals and toxoplasmosis induces significant (P<0.05 decreases in erythrocyte, haemogloin, haematocrit and neutrophils concentrations whereas, increases leukocytes, lymphoid and mean platelets volume in infected and non infected camels. Moreover the increased concentration was observed in hepatic enzyme activities, such as ALT, urea, bilirubin, and cholestrol. These findings showed that blood parameters can be used to study the pathogenesis of toxoplasmosis in camels.

Table 1. Relationship between age and toxoplasmosis in Cholistani camels of Bahawalpur during February, 2015 to December, 2015.

Age(years)	No of hosts observed	No of hosts positive	Prevalence (%)
1 – 4	68	11	16.17
5 – 8	98	18	18.36
9 – 12	19	2	10.52
> 13	16	5	31.25

Table 2. The mean (±SEM) serum haematological parameters of infected and non–infected dromedary camels with *Toxoplasma gondii* during February, 2015 to December, 2015.

Hematological parameters	She-camels	
	***Toxoplasma gondii* non infected hosts**	***Toxoplasma gondii* infected hosts**
WBC ($x10^3$/µL)	17.6±5.8[a]	19.4±6.0[b]
RBC ($x10^6$ /µL)	8.3±2.2[a]	5.8±1.7[b]
HGB (g/dL)	11.6±3.0[a]	11.3±2.1[b]
HCT (%)	45.7±13.1[a]	43.6±11.3[b]
MCV (fL)	55.6±14.2[a]	55.9±10.5[b]
MCH (Pg)	14.1±2.6[a]	14.5±1.3[a]
MCHC (g/dL)	26.0±6.0[a]	26.7±5.8[a]
PLT (x 10^3/µL)	125.2±27.5	125.6±17.4
LYM (%)	23.4±15.3[a]	28.4±15.0 [b]
MXD (%)	10.0±13.3[a]	22.4±5.9[b]
NEUT (%)	67.9±16.4	49.4±13.1
LYM (x 10^3/µL)	4.6±3.3[a]	5.9±4.4[b]
MXD (x 10^3/µL)	1.5±0.7[a]	1.6±0.7[b]
NEUT (x 10^3/µL)	11.6±4.5	12.0±4.1
RDW (f L)	31.1±11.2[a]	27.5±14.8[b]
PDW (f L)	4.0±4.2[a]	4.1±4.1[a]
MPV (f L)	2.9±3.0[a]	3.1±3.0[b]
P-LCR (%)	2.6±3.4 [a]	2.9±3.5[b]

The values in a row with different superscripts are significantly different (P < 0.05)

Table 3. The mean (±SEM) serum biochemical parameters of infected and non–infected dromedary camels with *Toxoplasma gondii* during February, 2015 to December, 2015

Biochemical parameters	*Toxoplasma gondii* non infected hosts	*Toxoplasma gondii* infected hosts
Urea (µmol/L)	25.48±1.35 [a]	47.39±2.97 [b]
Uric acid (µmol/L)	0.4245±0.032[a]	0.4845±0.046[a]
Sugar (µmol/L)	75.77 ± 2.52[a]	66.71± 2.92[b]
Bilirubin (µmol/L)	0.2961±0.015[a]	0.3629±0.051[b]
ALT (µ/L)	32.48±2.75[a]	38.03±1.88[b]
AST (µ/L)	9.402±0.56[a]	3.460±0.32[b]
Cholestrol (mmol/L)	25.097±0.90[a]	43.32±2.87[b]

The values in a row with different superscripts are significantly different (P < 0.05)

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