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Clinicopathological and Cytological Studies on Naturally Infected Camels and Experimentally Infected Rats with *Trypanosoma evansi*

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Abstract: Haemoparasitic diseases like trypanosomiasis have an adverse impact on health, productivity and working capacity of camels in many camel-rearing regions in the world. In the present study, a total of 70 camels were examined for the presence of *Trypanosoma evansi (T. evansi)* parasite. The antioxidant status, coagulation disorders and cytological changes associated with *T. evansi* infection as well as the hematological and biochemical changes were studied in both naturally infected camels and experimentally infected rats. The results revealed significant increases in the activities of antioxidant enzymes and prolonged increases in the values of prothrombin time (PT) and activated partial thromboplastin time (APTT) which were seen in both camels and rats. Anemia and significant leucocytosis were observed in both camels and rats. Serum biochemical parameters showed significant increases in the activities of hepatic enzymes in both of them. In contrast to camel results, there was an increase of blood urea nitrogen (BUN) and serum creatinine concentrations in experimentally infected rats. Different cytological changes associated with *T. evansi* infection were observed in both camels and rats.

Key words: Trypanosoma evansi · Clinicopathological · Antioxidant status · Coagulation disorders · Cytological

INTRODUCTION

In Egypt, camels take a principal position. Besides being a mean of meat and milk production, they are used for transportation of crops and do other farm works; also, their hair, wool and hides are used. According to the last Official Egyptian Veterinary reports, 267,000 camels live in Egypt belonging to the one humped species *Camelus dromedarius* [1]. Haemoparasitic diseases like trypanosomiasis have adverse impact on health, productivity and working capacity of camels in large areas of Africa [2]. Trypanosoma evansi (*T. evansi*) the cause of trypanosomiasis (Surra) constitutes one of the major veterinary problems worldwide. The disease causes significant morbidity and mortality in camels [3]. Camel trypanosomiasis occurs in chronic and acute forms. The acute form is usually fatal, whereas the chronic form is more common and associated with secondary infection [4]. Anemia appears to be a major component of the pathology of Surra disease, its development and persistence in the course of the disease induce anoxic conditions, which manifest signs of dysfunction in various organs. This is followed by the release of large quantities of cytoplasmic and mitochondrial enzymes, especially aspartate amino transferase (AST) and alanine amino transferase (ALT) [3]. Recently, the study of *T. evansi* infection has been approached from the perspective of oxidative stress generated in the host [5]. The oxidative stress results in oxidation products and tissue damage [6]. Other studies have been approached from the perspective of coagulopathies associated with acute phase of *T. evansi* infection [7]. The current study was designed to evaluate coagulation disorders in chronic (naturally infected camels) and acute (experimentally infected rats) phases of trypanosomiasis, to study antioxidant status and to study the cytological changes associated with *T. evansi* infection as well as the hematological and biochemical alterations.

MATERIALS AND METHODS

A total of 70 samples were collected from camels which obtained from two abattoirs located in Giza and Cairo governorates. From each camel, blood sample and

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liver specimen were collected. The blood sample which obtained from each camel (Jugular vein) was divided into three parts. The first part was collected in heparin-containing tube for rat inoculation and hemogram evaluation. The second part was collected in sodium citrate-containing tube, its plasma was taken after centrifugation and kept deep frozen until analysis of PT and APTT as coagulation monitoring tests according to Feldman *et al*. [8]. The last part was collected in clean centrifuge tube and was allowed to clot, then centrifuged for serum separation. The clear non-hemolysed supernatant serum was harvested for biochemical studies and ELISA test.

Detection of *T. evansi* antibodies in camel serum was carried out using ELISA according to Zweygarth *et al*. [9] to identify the positive cases. For antigen preparation, *T. evansi* was obtained from naturally infected camel then the parasite was expanded by inoculating 0.5 ml of infected camel blood into two adult albino rats. When the parasitemia reached 10^6-10^8 Trypanosoma ml^{-1}, the blood was extracted by cardiac puncture using EDTA as anticoagulant. *T. evansi* parasites were purified by anion exchange chromatography using DEAE-cellulose as described by Lanham and Godfrey [10]. The eluted parasites were washed three times by centrifugation at 1475 x g for 20 min, in 20 mM phosphate buffer pH 7.2, containing 1% glucose. Then *T. evansi* parasites were resuspended in 2 ml of 20 mM phosphate buffer saline (PBS) pH 7.2, and sonicated four times for 1 min. at a time. The resulting homogenate was centrifuged at 7000 x g for 15 min. and the supernatant was stored at-20°C and used as antigen for ELISA as described by Voller *et al*. [11] and Luckins [12].

For rat inoculation, thirty five Albino rats (250 ± 20 g) from the Animal House of Faculty of Veterinary Medicine, Cairo University were used. They had free access to tap water and standard rat chow. Rats were randomly divided into two groups. Group I composed of 12 rats were kept as uninfected controls. Group II composed of 23 rats were injected intra-peritoneally with 0.5 ml of infected camel blood [13]. The current study continued for 21 days through which, the wet and Giemsa-stained thin blood smears were prepared daily from the tail vein of infected rats to assess infectivity [14].

Samples (blood and tissues including liver, spleen and kidney) were taken on the day 7 and 21 post infection (p.i) from both control and infected rat groups. Six rats in group II died on the day 10 (p.i). The rest of rats were anaesthetized with diethyl ether and decapitated on the day 21 (p.i).

Hematological parameters including erythrocyte count (RBCs), packed cell volume (PCV), hemoglobin concentration (Hb), total leucocyte count (TLC) and differential leucocytic count (DLC) were done according to Feldman *et al*. [8].

Serum biochemical analysis including the measurement of serum total proteins, albumin, creatinine concentrations and BUN were carried according to Dumas and Biggs [15], Tabacco *et al*. [16] and Fabiny and Ertingshausen [17], respectively. The enzymatic activities of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) were done according to Reitman and Frankel [18], Tietz [19], Dumas and Biggs [15] and Doumas *et al*. [20], respectively and assayed using reagent kits supplied by StanBio Laboratories incorporation, USA.

The activities of antioxidant enzymes including glucose-6-phosphate dehydrogenase (G6PDH) and glutathione reductase (GR) were done on plasma samples according to Kornberg [21] and Goldberg and Spooner [22], respectively and assayed using Biodiagnostic commercial reagent kits.

All the above mentioned parameters were carried on both naturally infected camels and experimentally infected rats. Impression smears from liver of naturally infected camels and from liver, spleen and kidney of experimentally infected rats at 7^{th} and 21^{st} days (p.i) were taken after cutting the removed mass into two halves to obtain a freshly cutted surface. The cutted surface was blotted on clean paper towel. The surface was made small enough to make several rows of imprints, stained by field stain [23], washed and then examined under the microscope [24].

Statistical Analysis: Values were expressed as mean ± SD. Statistical comparisons between the means of different experimentally infected rat groups were made with completely randomized two ways ANOVA "Student-Newman-Keuls test" by COSTAT program version one. A probability "P" value of <0.05 was assumed for statistical significance. On the other hand, Student's T test was carried out to assess the significance of mean difference between *T. evansi* negative and positive camel groups by using SPSS ® program version sixteen.

RESULTS AND DISCUSSION

ELISA Results: Results of ELISA revealed that 45 (64.3%) camels gave positive results for circulating antibodies to *T. evansi*. According to these results, samples of camel were divided into *T.evansi* positive group and *T. evansi* negative group.

Table 1: Hematological and serum biochemical parameters of *T. evansi* negative and *T. evansi* positive groups.

	Unit	*T. evansi* negative	*T. evansi* positive
PCV	%	25.20±0.79	22.67±0.58*
Hb	g/dl	12.97±0.40	9.68±0.20*
RBCs	$\times10^6/\mu l$	8.48±0.81	6.07±0.40*
MCV	Fl	26.45±5.34	37.39±1.75*
MCHC	g%	51.45±0.31	42.72±1.46*
PT	Sec.	113.33±2.08	119.33±2.52*
APTT	Sec.	12.16±0.89	18.49±0.59*
TLC	$\times10^3/\mu l$	10.33±0.96	15.60±0.66*
Neut.	$\times10^3/\mu l$	7.15±0.72	11.11±0.34*
Lym.	$\times10^3/\mu l$	2.07±0.19	3.01±0.30
Eosin.	$\times10^3/\mu l$	0.41±0.04	0.52±0.01*
Mono.	$\times10^3/\mu l$	0.10±0.01	0.56±0.01*
T.prot.	g/dl	7.75±0.48	7.64±0.49
Albumin	g/dl	3.70±0.36	2.76±0.25*
globulins	g/dl	4.05±0.16	4.88±0.28*
A/G	-	0.91±0.07	0.56±0.05*
ALT	U/L	11.33±1.53	51.00±7.94*
AST	U/L	30.00±2.00	71.33±10.97*
ALP	U/L	85.00±5.00	134.00±21.17*
GGT	U/L	24.33±4.04	48.33±7.64*
BUN	mg/dl	26.00±2.00	24.67±1.53
Creatinine	mg/dl	0.47±0.10	0.47±0.11
GR	U/L	115.33±12.22	127.00±16.09*
G6PDH	mu/ml	29.33±5.51	38.67±6.11*

* represents significant difference between *T. evansi* negative and *T. evansi* positive camel groups at probability P< 0.05.

Table 2: Hematological and serum biochemical parameters of different experimentally infected rat groups.

	Unit	Control	7th day (p.i)	21st day (p.i)	LSD
PCV	%	40±2 ^{a}	31±1 ^{b}	22±2 ^{c}	5.45
Hb	g/dl	13.97±0.64 ^{a}	15.08±0.47 ^{b}	16.13±0.44 ^{c}	1.04
RBCs	$\times10^6/\mu l$	5.41±0.50 ^{a}	3.82±0.15 ^{b}	2.49±0.30 ^{c}	0.69
MCV	Fl	74.75±4.04 ^{a}	81.09±0.61 ^{a}	89.99±5.51 ^{ab}	7.92
MCHC	g%	34.81±0.87 ^{a}	38.15±0.47 ^{b}	41.51±2.72 ^{c}	3.34
PT	Sec.	23.37±0.67 ^{a}	25.67±0.58 ^{b}	29.33±1.53 ^{c}	2.03
APTT	Sec.	24.33±1.26 ^{a}	28.43±1.01 ^{b}	37.00±1.41 ^{c}	2.47
TLC	$\times10^3/\mu l$	4.63±0.81 ^{a}	6.77±0.51 ^{b}	8.87±0.35 ^{c}	1.17
Neut.	$\times10^3/\mu l$	2.59±0.45 ^{a}	2.71±0.20 ^{a}	2.40±0.10 ^{a}	0.59
Lym.	$\times10^3/\mu l$	1.85±0.32 ^{a}	3.86±0.27 ^{b}	6.21±0.25 ^{c}	0.56
Eosin.	$\times10^3/\mu l$	0.13±0.02 ^{a}	0.14±0.01 ^{b}	0.18±0.01 ^{c}	0.03
Mono.	$\times10^3/\mu l$	0.04±0.01 ^{a}	0.06±0.01 ^{b}	0.08±0.01 ^{c}	0.01
T.prot.	g/dl	6.67±0.47 ^{a}	8.05±0.21 ^{b}	8.43±0.50 ^{bc}	1.15
Albumin	g/dl	3.67±0.06 ^{a}	4.00±0.28 ^{a}	4.23±0.45 ^{a}	0.81
globulins	g/dl	3.00±0.44 ^{a}	4.05±0.07 ^{b}	4.20±0.10 ^{bc}	0.73
A/G	-	1.23±0.16 ^{a}	0.99±0.09 ^{a}	1.00±0.10 ^{a}	0.32
ALT	U/L	27±7 ^{a}	68±11 ^{b}	160±36 ^{c}	20.95
AST	U/L	30±5 ^{a}	88±11 ^{b}	203±6 ^{c}	18
ALP	U/L	68±8 ^{a}	138±11 ^{b}	156±26 ^{bc}	45.2
GGT	U/L	28±3 ^{a}	67±16 ^{b}	109±10 ^{c}	25.18
BUN	mg/dl	21±7 ^{a}	115±7 ^{b}	133±18 ^{bc}	31.54
Creatinine	mg/dl	1.43±0.21 ^{a}	2.15±0.21 ^{b}	2.57±0.25 ^{bc}	0.58
GR	U/L	116±16 ^{a}	166±37 ^{a}	186±13 ^{ab}	54.2
G6PDH	mu/ml	26±2 ^{a}	46±5 ^{b}	52±4 ^{bc}	8.74

LSD represents least significant difference between different groups at probability P< 0.05.

Means with different superscripts (a,b,c) within a raw are significantly different at P< 0.05.

Clinicopathological Findings

Erythrogram: Mean values of the erythrogram [packed cell volume (PCV %), hemoglobin concentration (Hb), erythrocytes count (RBCs), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC)] of different camel and rat groups are illustrated in tables 1, 2.

Comparing the mean values of *T. evansi* positive group of camel with those values of *T. evansi* negative group, PCV, Hb concentration, RBCs and MCHC values showed significant decreases while, MCV values revealed significant increases. During the microscopical examination of the stained blood film, hypochromacia, poikilocytosis and anisocytosis were observed in addition to the appearance of parasite in positive group (Fig. 1, a-b).

From the above mentioned data the macrocytic hypochromic anemia (regenerative anemia) was determined by MCV and MCHC values. This anemia may be attributed to the enhanced oxidation of the erythrocytes which carried out by membrane injury, osmotic fragility and destruction of the cell during chronic *T. evansi* infection in camels [25,26].

Erythrogram results of experimentally infected rats revealed the presence of hemolytic anemia which determined by the increases of Hb concentration and MCHC values. Microscopical examination of the stained blood films at the 7th day (p.i) showed the presence of *T. evansi*, while that at the 21st day (p.i) showed severe aggregation of *T. evansi*. Furthermore presence of polychromacia and stomatocyte were seen in 7th day (p.i) and become more pronounced at 21st day (p.i) (plate1, c-d).

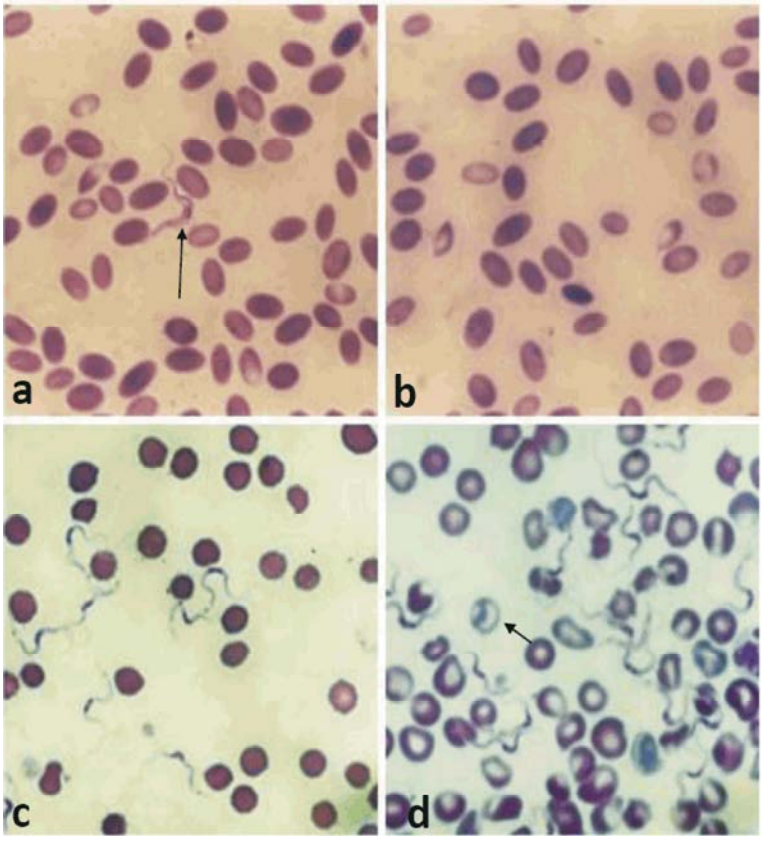

Fig. 1a-d: a. Blood film of naturally infected camel showed the presence of *T. evansi* parasite (arrow). (Giemsa stain x 1000)
b. Blood film of naturally infected camel showed the presence of hypochromacia, poikilocytosis and anisocytosis in *T. evansi* positive group. (Giemsa stain x 1000)
c. Blood film of experimentally infected rat at the 7th day (p.i) showed the presence of *T. evansi* parasite (Giemsa stain x 1000)
d. Blood film of experimentally infected rat at the 21st day (p.i) showed severe aggregation of *T. evansi* parasite with the presence of stomatocyte (arrow) and polychromacia. (Giemsa stain x 1000)

This anemia may be attributed to increased susceptibility of red blood cell membrane to oxidative damage probably as a result of depletion of reduced glutathione on the surface of the red blood cell leading to acute hemolysis due to the proliferation of *T. evansi* parasites [27].

Values of coagulation monitoring tests of naturally infected camels and experimentally infected rats including prothrombin time (PT) which used to evaluate the extrinsic factors (VII) and common pathways (X, V, II, and I) and the activated partial thromboplastin time (APTT) which used to evaluate the intrinsic pathway (XII, XI, IX and VIII) showed significant increases. The prolongation of PT and APTT values which observed indicating severe liver affection; as liver synthesizes all of the known coagulation factors except factor VIII so that, any decreases in one of these factors, PT and APTT will become abnormally prolonged [8, 28]. This liver affection was confirmed biochemically by increases of hepatic enzyme activities and cytologically by hepatic fatty degeneration and necrosis.

Leukogram: Mean values of the leukogram [total leucocyte count (TLC), neutrophil, lymphocyte, eosinophil and monocyte counts] of different camel and rat groups are illustrated in tables 1, 2.

Comparing mean values of camel *T. evansi* positive group with those values of negative group showed significant leucocytosis, neutrophilia, monocytosis and eosnophilia. These changes occur as a result of an increase in the activity of the mononuclear phagocytic system. The eosinophilia observed is a feature of parasitic infections and is associated with immediate-type hypersensitivity reactions.

Similar results were observed in experimentally infected rats except neutrophilia was replaced by lymphocytosis. This may account for the generalized lymphoid tissue hyperplasia characteristic of *Trypanosoma evansi* infections as in the acute phase of the disease; lymph nodes and spleen are remarkably reactive while in chronic infection the immune system becomes depleted of lymphoid cells [27, 29].

All the above mentioned changes of the hemogram (erythrogram and leukogram) were more pronounced in the experimentally infected rats at the end of the experiment.

Serum Biochemical Evaluation: Statistical analysis of different serum biochemical parameters of different camel and rat groups are illustrated in tables 1, 2.

In naturally infected camels, comparing mean values of *T. evansi* positive group to those of the negative group, the results showed significant increases in the activities of antioxidant enzymes (G6PDH and GR). BUN, serum creatinine and serum total proteins concentrations showed no significant changes with significant decrease in A/G ratio which attributed to the presence of significant hypoalbuminemia and hyperglobulinemia. This hypoalbuminemia and hyperglobulinemia may be resulted from severe degenerative changes in liver [30, 31] which was supported cytologically by the presence of hepatic necrosis. Significant increases in the activities of hepatic enzymes which observed may be attributed partly to cellular damage caused by the trypanosomes lysis or probably resulted from host destruction of trypanosomes [3]. The reported increase in these enzymes activities, especially AST, is not surprising as it is indicative of liver damage which was supported cytologically by its fatty degeneration and necrosis.

In experimentally infected rats, comparing the mean values of experimentally infected groups to those of the control ones showed similar serum biochemical parameters to that observed in naturally infected camels; significant increases in the activities of hepatic enzymes (ALT, AST ALP and GGT) were observed as a result of hepatic damage which was supported cytologically by the present fatty degeneration which followed by hepatic necrosis.

The difference in the results between camels and rats was the increases in serum total proteins and globulin concentrations. These increases may be a result of hemolysis which is secondary to *T. evansi* infection. Increase synthesis of immunoglobulin in response to infection may also result in the observed increases in protein globulins concentrations [27]. In addition, there was an increase of BUN and serum creatinine concentrations, these increases may be attributed to renal affection which was confirmed cytologically by the presence of renal casts as a result of renal tubular damage as well as granulomatous lesions.

In the current study, there are significant increases in the activities of antioxidant enzymes (G6PDH and GR) in both naturally infected camels and experimentally infected rats at the 7^{th} day (p.i) and at the 21^{st} day (p.i), these enzymes belonged to the first line of cell defense against the oxidative stress (OS) [5]. The OS is characterized by increasing the production of free radicals including hydroxyl, superoxide, hydroperoxides and malondialdehyde radicals. These free radicals have been reported to act as cytotoxic agents so; the erythrocyte is

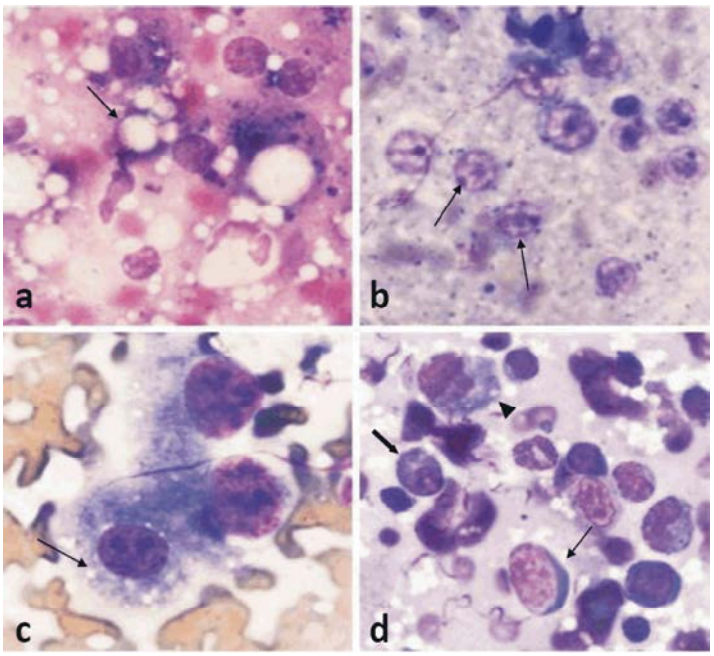

Fig. 2a-d: a. Hepatic smear of naturally infected camel showed fatty degeneration in which the hepatocytes contain round, sharp and delineated vacuoles (arrow). (Field stain x 1000)
b. Hepatic smear of naturally infected camel showed necrotized hepatocytes (arrows) with poorly delineated, lightly colored and lacy appearing areas in the cytoplasm. (Field stain x 1000)
c. Hepatic smear of experimentally infected rat at the 7th day (p.i) showed the hepatocyte cytoplasm becomes slightly vacuolated (arrow) and deeply stained basophilic with coarse nuclear chromatin. (Field stain x 1000)
d. Hepatic smear of experimentally infected rat at the 21st day (p.i) showed severe aggregation of reactive lymphocytes (thin arrow), plasma cells (thick arrow) and Kupffer cells (head arrow) with massive infiltration of *T. evansi* parasites . (Field stain x 1000)

under constant exposure to free radicals during the infection [6]. However, red cells have a potent antioxidant protection that modifies free radicals into less reactive intermediates before cellular biomolecules damage [32]. So, increases in these activities considered as a way of defense against the oxidative stress resulted from *T. evansi* infection.

All the above mentioned changes which observed in different serum biochemical parameters were more pronounced in the experimentally infected rat at the end of the experiment.

Cytological Findings

Camel Cytological Findings

Liver Cytology: Impression smears from naturally infected camels showed fatty degeneration of the liver; hepatocytes contain rounded, sharp delineated vacuoles which push the nucleus to a side (Fig. 2, a). These vacuoles may be varied in size from small to large ballooning vacuoles causing hepatocytes distention. Mixed infiltrations of large numbers of lymphocytes beside Kupffer cells (macrophages) with fibroblasts were also seen. Other hepatic smears showed completely necrotized hepatocytes with poorly delineated, lightly colored and lacy appearing areas in their cytoplasm (Fig. 2, b).

From the above description, the observed fatty degeneration which manifested by lipid accumulation inside hepatocytes may be due to tissue hypoxia resulted from the present anemia and vascular damage [3, 33]. The observed necrosis of hepatocytes is probably due to liberation of *T. evansi* toxins in plasma and tissue fluids. These toxins induce an increase of lysosomal secretion followed by autolysis of the cell [34].

Renal Cytology: Renal impression smears from naturally infected camels showed normal renal tubular epithelial cells with abundant light blue cytoplasm and round nuclei. Renal tubular epithelial cells often remain together as recognizable tubule fragment of various sizes without presence of any abnormal finding.

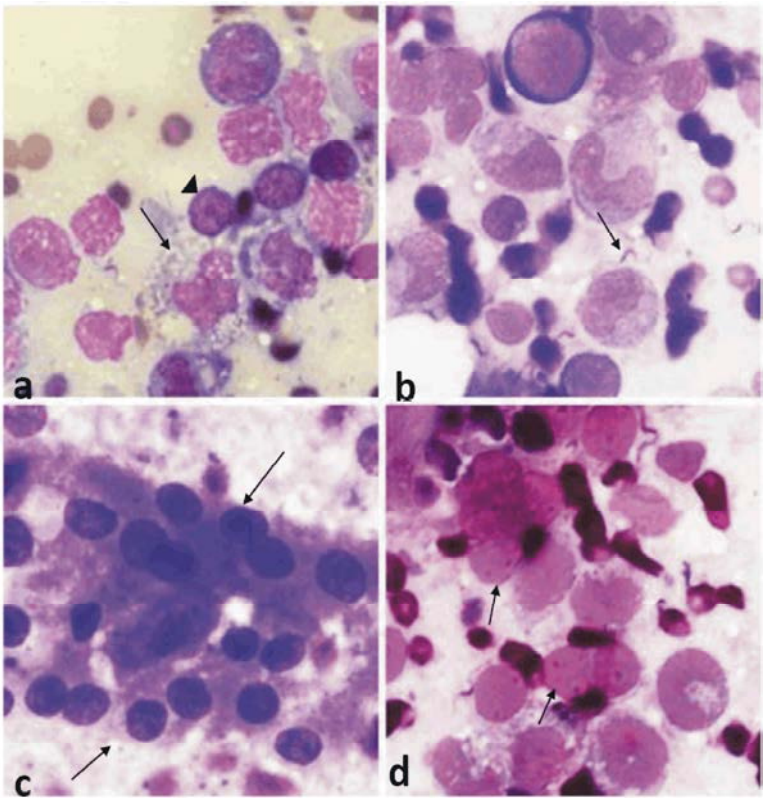

Fig. 3a-d: a. Splenic smear of experimentally infected rat at the 7th day (p.i) showed the presence of histocyte (arrow) and reactive lymphocyte. (Field stain x 1000)
b. Splenic smear of experimentally infected rat at the 21st day (p.i.) showed severe aggregation of histocytes with the appearance of T. evansi parasites (arrow). (Field stain x 1000)
c. Renal smear of experimentally infected rat at the 7th day (p.i) showed the presence of renal cast (arrow). (Field stain x 1000)
d. Renal smear of experimentally infected rat at the 21st day (p.i.) showed coarse nuclear chromatin with two or three prominent nucleoli (arrow). (Field stain x 1000)

Rat Cytological Findings

Liver Cytology: Impression smears from experimentally infected rats at the 7th day (p.i) showed slight vacuolation of hepatocytes cytoplasm became and stained deeply basophilic with coarse nuclear chromatin. Considerable infiltration of lymphocytes within the hepatic smear was also observed (Fig. 2, c). At the 21st day (p.i) severe aggregation of reactive lymphocytes, plasma cells and Kupffer cells with massive infiltration of *T. evansi* parasites was noticed (Fig. 2, d).

Liver is considered one of the most important visceral organs that affected by *T. evansi* infection resulting in its necrosis. Fatty degeneration followed by necrosis of hepatocytes was considered the common cytological findings associated with *T. evansi* infection as a result of nutritional impairment and local asphyxia [35].

Spleen Cytology: Cytological evaluation of splenic smears from experimentally infected rats at the 7th day (p.i) showed splenic hyperplasia (Fig. 3, a) as a result of increased reactive lymphocyte, plasma cell and histocyte (macrophage) numbers. At the 21st day (p.i), splenic hyperplasia became more pronounced with aggregation of histocytes (granulomatous lesions). *T. evansi* parasites were also noticed in the examined smears (Fig. 3, b).

Among the lymphatic tissues, spleen is the most important organ that serves as a first line of defense mechanism. Formation of granulomatous lesions due to aggregation of histocytes may be considered the initial splenic response to *T. evansi* challenge [35, 36].

Renal Cytology: Smears obtained from kidney of experimentally infected rats at the 7th day (p.i) showed, the presence of renal casts due to the renal tubular damage

(Fig. 3, c) and accumulation of few macrophages with coarse nuclear chromatin. At the 21st day (p.i) renal tubular epithelium showed, the presence of coarse nuclear chromatin with two or three prominent nucleoli (Fig. 3, d). Other areas showed severe infiltration with reactive lymphocytes and macrophages (granulomatous lesions) as well as the presence of *T. evansi* parasites.

Toxins which liberated by the parasites are likely to impair the function of the kidney. In such cases, the renal cast formation as well as granulomatous lesions indicated non-functional kidney especially at the late stage of *T. evansi* infection [35].

CONCLUSION

From the present study, it is concluded that changes associated with *T. evansi* experimental infection (rats) were supported the changes of natural infection (camels) in antioxidant status, coagulation disorders and cytological changes as well as the biochemical changes. On the other hand, hematological changes of experimentally infected rats showed hemolytic anemia with lymphocytosis due to the acute phase of the disease while, in naturally infected camel macrocytic hypochromic anemia was observed due to the chronicity of the disease.

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REFERENCES

1. Abdel-Rady, A., 2008. Epidemiological studies (parasitological, serological and molecular techniques) of Trypanosoma evansi infection in camels (Camelus dromedarius) in Egypt. Veterinary World, 1(11): 325-328.
2. Mihret, A. and G. Mano, 2007. Bovine trypanosomosis in three districts of East Gojjan zone bordering the blue, Nile River in Ethiopia. J. Infect Developing Countries, 1(3): 321-325.
3. Enwezor, F. and A. Sackey, 2005. Camel trypanosomosis-a review. Veterinarski Arhiv, 75(5): 439-452.
4. Olaho-Mukani, W. and H. Mahamat, 2000. Trypanosomiasis in the dromedary camel. In: Gahlot, T.K. (Ed.), Selected Topics on Camelids. The Camelid Publishers Chandan Sagar Well Bikaner India, pp: 255-270.
5. Portillo, R., 2010. Anaemia as the main actor in the infections due to Trypanosoma evansi. http://www.scitopics.com/Anaemia_as_the_main_actor.
6. Saleh, M.A., M. El Salahy and A. Samera, 2009. Oxidative stress in blood of camels (Camelus dromedarius) naturally infected with Trypanosoma evansi. Veterinary Parasitology, 162: 192-199.
7. Dakshinkar, N.P., V.M. Dhoot, S.V. Uphadhye, G.R. Bhojne, D.B. Sarode and S.W. Kolte, 2002. Trypansomiasis in a jungle cat. Indian Veterinary Journal, 79(1): 66-67.
8. Feldman, B.F., J.G. Zinkl and N.C. Jain, 2000. Schalm's Veterinary Hematology. 5th Ed Lea and Febiger Philadelphia U.S.A.
9. Zweygarth, E., C. Sabwa and D. Rottcher, 1986. An enzyme-linked immunosorbent assay for the detection of antibodies to Trypanosoma evansi in camels (Camelus dromedaries) using Peroxidase conjucated protein A. Annals of Tropical Medicine and Parasitology, 37: 105-106.
10. Lanham, S.M. and D.G. Godfrey, 1970. Isolation of salivarian Trypanosomes from man and other mammals using DEAE-celulose. Experimental Parasitology, 28: 521-534.
11. Voller, A., D.E. Bidwell and A.A. Bartlett, 1975. Serological study on human Trypanosoma rhodesiense infections using a micro-scale enzyme-linked immunosorbent assay. Tropical medicine and Parasitololgy, 26: 247-251.
12. Luckins, A.G., 1977. Detection of antibodies in trypanosome infected cattle by means of microplate enzyme linked immunosorbent assay. Tropical Animal Health and Production, 9: 53-62.
13. Pathak, K., Y. Singh, N. Meirvenne and M. Kapoor, 1997. Evaluation of various diagnostic techniques for Trypansoma evansi infections in naturally infected camels. Veterinary Parasitology, 69: 49-54.
14. The Office International des epizooties (OIE), 2000. Manual of Standards for Diagnostic Tests and Vaccines. 4th Ed.
15. Dumas, B.T. and H.G. Biggs, 1972. Standard methods of clinical chemistry. Academic Press New York, 7: 175.

16. Fabiny, D.L. and G. Ertingshausen, 1971. Automated reaction-rate method for determination of serum creatinine. Clinical Chemistry, 17: 696-700.
17. Tabacco, A., F. Meiattini, E. Moda and E. Tarli, 1979. Simplified enzymic/colorimetric serum urea nitrogen determination. Clinical Chemistry, 25: 336-337.
18. Reitman, S. and S. Frankel, 1957. A colorimeteric method for determination of oxaloacetic transaminase and serum glutamic pyruvic transaminase. American Journal of Clinical Pathology, 28: 56-63.
19. Tietz, N.W., 1986. Text Book of Clinical Chemistry. Philadelphia: WB Saunders.
20. Doumas, B.T., B.W. Perry, E.A. Sasse and J.V. Straumfjord, 1973. Clinical Chemistry, 19: 984-993.
21. Kornberg, A., 1955. Methods in Enzymology. Academic Press New York, pp: 323.
22. Goldberg, D.M. and R.J. Spooner, 1983. In Methods of Enzymatic Analysis. Bergmeyen HV 3rd Ed Verlog. Chemie. Deerfield beach F.I., 3: 258-265.
23. Tankeyul, B., C. Lamon, S. Kuptamethi and K. Chooparnya, 1987. The reliability of field's stains as a hematological staining. Journal of the Medical Association of Thailand, 70(3): 136-41.
24. Rick, L.C., D.T. Ronald and H.M. James, 1999. Diagnostic cytology and hematology of the dog and cat. 2nd Ed pupli John As U.S.A.
25. 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. Veterinary Parasitology, 130: 163-168.
26. Akanji, M.A., O.S. Adeyemi, S.O. Oguntoye and F. Sulyman, 2009. Psidium guajava extract reduces trypanosomosis associated lipid peroxidation and raises glutathione concentrations in infected animals. EXCLI Journal, 8: 148-54.
27. Sulaiman, F.A. and O.S. Adeyemi, 2010. Changes in haematological indices and protein concentrations in Trypansoma brucei infected rats treated with homidium chloride and diminazene aceturate. EXCLI Journal, 9: 39-45.
28. Da Silva, S., P. Wolkmer, M. Costa, A. Zanette, B. Oliveira, T. Gressler, A. Otto, M. Santurio, A. Lopes and G. Monteiro, 2010. Clotting disturbances in Trypanosoma evansi infected cats. Comparative Clinical Pathology, 19: 207-210.
29. Abubakar, A., B. Iliyasu, A.B. Yusuf, A.C. Igweh, N.A. Onyekwelu, B.A. Shamaki, D.O. Afolayan and E.O. Ogbadoyi, 2005. Antitrypanosomal and haematological effects of selected Nigerian medicinal plants in Wister rats. Biokemistri, 17: 95-9.
30. Chaudhary, Z.I. and J. Iqbal, 2000. Incidence, biochemical and haematological alterations induced by natural trypanosomosis in racing dromedary camels. Acta Tropica, 77: 209-213.
31. Ahmed, S., AA. Butt, M. Muhammad and M.Z. Khan, 2004. Haematobiochemical studies on the haemoparasitized camels. International Journal of Agriculture and Biology, 6(2): 331-334.
32. Mousa, H.M., O.H. Omer, B.H. Ali, N. Al-Wabel and S.M. Ahmed, 2006. Antioxidant levels in tissues of young and adult camels (Camelus dromedarius). Journal of Physiology and Biochemistry, 62(3): 213-218.
33. Derakhshanfar, A., A. Mozaffari and A. Zadeh, 2010. An Outbreak of trypanosomiasis (Surra) in camels in the southern fars Province of Iran: Clinical, hematological and pathological findings. Research Journal of Parasitology, 5: 23-26.
34. Dargantes, A., R. Campbell, D. Copeman and S. Reid, 2005. Experimental Trypanosoma evansi infection in the goat. II. Pathology. Journal of Comparative Pathology, 133: 267-276.
35. Uche, U. and T. Jones, 1992. Pathology of experimental Trypanosoma evansi infection in rabbits. Journal of Comparative Pathology, 106: 299-30.
36. Biswas, D., A. Choudhury and K. Misra1, 2001. Histopathology of Trypanosoma (Trypanozoon) evansi infection in Bandicoot rat. I. Visceral Organs. Experimental Parasitology, 99: 148-159.