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CLINICO-PATHOLOGICAL EFFECTS OF *TRYPANOSOMA EVANSI* ON NATURALLY INFECTED CAMELS

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ABSTRACT

Trypanosomiasis in camels is one of the most important diseases, widely distributed all over the world. A total of 176 blood samples were collected from camels (112 samples from camels in Cairo abattoir and 64 from animals imported from Sudan). Results revealed that out of 112 camels in Cairo abattoir, 5 (4.46%) were harbour *T. evansi* parasite between RBCs and 107 (95.54%) were negative. Out of 64 blood samples collected from imported camels, 14 (21.88%) were infected and 50 (78.12%) were negative, have no parasite in their blood film. Hematological parameters included hemoglobin level (Hb gm/dl), total leucocytic count (TLC) and differential leucocytic count (DLC) were estimated. Serum biochemical analysis included enzymatic liver functions (ALT, AST, ALP and GGT) were estimated also in samples taken from normal and infected camels. Blood samples from *T. evansi* infected camels showed marked decrease in hemoglobin concentration. The leucogram results showed significant increase in total leucocytic count (TLC) mainly Eosinophils, Neutrophils and monocytes in infected group compared to non-infected group.

Regarding the liver enzymes, the results showed increased levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and GGT ($P \leq 0.05$) in infected camels compared to the control group. Creatinine and blood urea nitrogen were significantly increased in *Trypanosoma* infected camels compared to normal ones.

From the present study it can be concluded that the above mentioned biochemical, parasitological and hematological parameters are mostly affected due to infection with *T. evansi* and may constitute important indices for disease prognosis.

Key wards:

Camels, *Trypanosoma,* heamatological examination, biochemical examinations.

INTRODUCTION

Trypanosoma evansi (T. evansi) is the causative agent of camel trypanosomiasis which called Surra disease. **Elhaig *et al.* (2013)** considered that disease as one of the most widely spread endemic protozoan disease of camels and different domestic animals all over the world**.** According to the last Official Egyptian Veterinary reports, 267,000 camels live in Egypt belonging to the one humped species Camelus dromedarius **(Abdel-Rady,2008).**

Trypanosomes are blood and tissue parasites of order Kinetoplastida and family *Trypanosomatidae* which are found in different mammals including humans **(Dyary *et al.,* 2014).** They are mainly transmitted by biting of insects (Tabanus, Stomoxys and Liperosia) in which they undergo a biological cycle. The disease characterized by high mortality, morbidity and anemia which had been a consistent finding in infected animals **(Dyary *et al.,* 2014).** Camel *trypanosomiasis* occurs in acute and chronic forms. The acute form is usually fatal, whereas the chronic form is more common and associated with secondary infection **(Olaho-Mukani and Mahamat, 2000).** Chronic Infection is usually, characterized by intermittent fever, anemia, lymphadenopathy and weight loss **(Osório *et al.,*2008).**

During infection, trypanosomes persist in the host blood stream, evading immune responses and invading vital body organs such as liver, spleen and kidneys **(Pays *et al.,*2014 and Takeet andFagbemi,2009).** So the present study was aiming to investigate some biochemical and hematological parameters that may associated with such disease.

MATERIAL AND METHODS

Samples:

A total of 176 blood samples (with and without EDTA as anticoagulant) was collected from 112 camels present in Cairo abattoirs originated from El-Haram region and 64 camels imported from Sudan and equipped for purchase by Hagana in Haram region.

Parasitological Examination:

Fresh thin smears were made using glass slides immediately after collection of whole blood **(Khan *et al.,* 2013; Asi *et al.,* 2014).** All the smears were air dried, fixed with absolute methanol and finally stained with Giemsa stain according to **El-Dakhly *et al.* (2013).**

Hematological and biochemical studies:

For hematological and biochemical studies, blood samples were collected with and without anticoagulant (EDTA; 1mg/ml). Serum was separated from each blood sample which that collected without anticoagulant.

The whole blood samples were used to determine different hematological parameters according to **Gad and El-Maddawy (2014).**Total erythrocytic counts (TEC), Total leucocytic counts (TLC), hemoglobin concentration (Hb) and differential leucocytic count (DLC) were estimated as previously described by **Ghaffar *et al.* (2014).**

Different serum biochemical parameters such as various enzymeslikealanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma (GGT) were determined according to **Doumas *et al.* (1973) and Hussain *et al*. (2014)** and assayed using reagent kits supplied by StanBio Laboratories incorporation, USA.

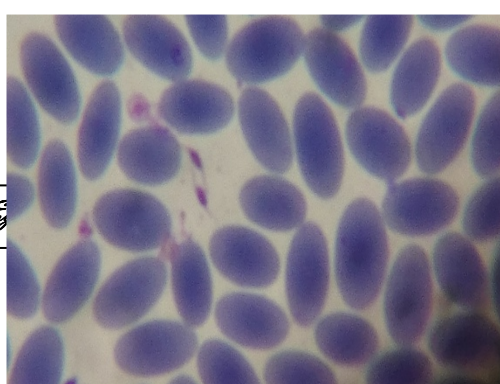
Serum biochemical analysis included Creatinine and blood urea nitrogen (BUN) were carried out according to **Fabiny and Ertingshausen (1971) and Tabacco *et al*. (1979)** respectively.

<u>Statistical analysis:</u>

The data regarding hematological and serum biochemical parameters were analyzed by Student's t-test, using SPSS, at $P \leq 0.05$. 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

Results revealed that out of 112 blood samples collected from camels in Cairo abattoir 5 (4.46%) were presumed to be positive for *T. evansi* infection between RBCs. Fig.(1,2) However, 107 (95.54%) blood smears were negative (Table 1). Moreover, out of 64 blood samples collected from living imported camels, 14 (21.88%) were presumed to be infected. However, 50 (78.12%) blood smears were negative (Table 1).



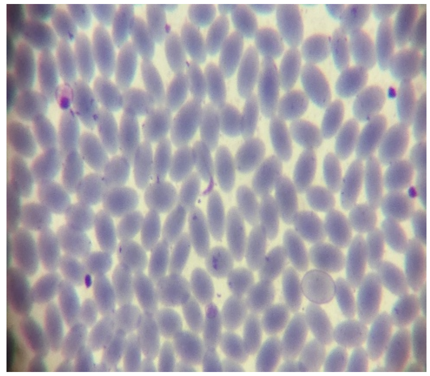


Fig. (1, 2): The *Trypanosoma* species parasite in intercellular space of blood camel.

Table (1): prevalence of *Trypanosoma evansi* in different camel groups.

Parameters	Total	normal camels	infected camels
Slaughtered	**112**	**107 (95.54%)**	**5 (4.46%)**
Imported	**64**	**50 (78.12%)**	**14 (21.88%)**

The data in (Table 2) demonstrated the difference in hematological parameters in the investigated infected and non-infected camels. The results showed drastic decrease ($P \leq 0.05$) in hemoglobin which ranged from 12.97±0.40 gm/dl in healthy camels to 6.68±0.20 gm/dl in infected camels. In the contrary, TLC increased significantly ($P \leq 0.05$) among infected camels to reach a mean of 10.60± 0.66 x10^3/µl while in healthy camel was 8.33± 0.96 x10^3/µl. Regarding the results of Neutrophils, Eosinophils, Monocytes and Basophils count, results showed relative increase in infected than non-infected camels. Lymphocytes showed decrease in infected than non-infected camels as shown in (Table 2).

Table (2): Hematological parameters of infected and normal camels (Mean± S.E.).

Parameter	normal camels	infected camels
HB (gm/dl)	12.97±0.40	6.68± 0.20*
TLC ($\times10^3$ /µl)	8.33± 0.96	10.60± 0.66*
Neutrophils (%)	31.57± 0.32	42.06± 1.34*
Lymphocytes (%)	49.07± 0.19*	42.45± 0.30
Eosinophils (%)	2.02± 0.04	4.05± 0.01*
Monocytes (%)	1.35± 0.01	4.56± 0.01*
Basophils (%)	0.01	0.50± 0.00*

***Values in *Trypanosoma* infected group are significantly different than their corresponding in the normal group at P≤0.05.**

Regarding to serum biochemical parameters illustrated in Table (3), the liver enzymes result showed increased levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and GGT (P≤0.05) in infected camels compared to the non-infected group. Creatinine and blood urea nitrogen also were significantly increased (P≤0.05) in *Trypanosoma* infected camels compared to non-infected one.

Table (3): Serum biochemical parameters of naturally infected camels and non–infected camels (Mean ±SE).

Parameter	Healthy camels	infected camels
ALT (u/l)	12.69± 0.82	40.09± 4.40 *
AST (u/l)	113.44± 4.25	145.14± 10.87*
ALP (u/l)	82.77± 7.23	121.63± 7.44*
GGT (u/l)	24.33± 4.04	89.33± 5.64*
Creatinine (mg/dl)	0.27± 0.10	0.47± 0.11*
BUN (mg/dl)	16.00± 2.00	24.67± 1.53*

*** Values in *Trypanosoma* infected group are significantly different than their corresponding in the healthy group at P≤0.05**

DISCUSSION

Surra is one of the most important diseases among camels that cause serious economic losses **(Enwezor and Sackey, 2005).** In the present study, decreased Hb concentration and increased TLC counts in *T. evansi* in naturally infected camel compared to non- infected camels were observed. The lower Hb concentration was considered as indication for anemia caused by the disease as previously reported by **Padmaja (2012) and Eyob and Matios (2013).** Anemia is considered as a major and important indicator of *trypanosoma* infection in camel. The anemia occurs due to parasitic infection which causes large number of erythrocyte to be removed due to mononuclear phagocytic response in spleen and lymph nodes **(Eyob and Matios, 2013).** Moreover, Anemia appears to be predominantly caused by hemolysis associated with decreased life span of erythrocytes and extensive erythrophagocytosis **(Habila *et al.,* 2012).** Erythrophagocytosis in trypanosomiasis is likely to be caused by cell damage and increased rate of removal of red cells from the circulation due to hemolytic factors released by dying trypanosomes, immune complexes bound to RBCs, together with fever and mechanical damage to RBCs by trypanosomes **(Yusuf *et al.,* 2012).**

Finally, Mechanisms involved in the development of anemia are seemingly to includ hemolysis, free fatty acids, immunologic mechanisms, hemodilution, coagulation disorders, depression of erythrogenesis and release of trypanosomal sialidase **(Megahed *et al.,* 2012).** Themostimportant oxidativeenzyme duringtrypanosomiasisissialidase.

Sialic acid in erythrocyte surface membrane is hydrolysed by sialidase **(Sallau *et al.,* 2008).** Leucocytosis is thought to be a result of increased activity of the mononuclear phagocytic system during trypanosomiasis. These results are similar to the findings of **(Padmaja 2012).** Contrary results were reported by **Hussain *et al.,* (2016) and Oparah *et al.* (2017).**

However the Trypanosomiasis affected camels showed higher neutrophil, higher Eosinophil and lower Lymphocyte than the healthy control group.This is in agreement with the findings of **Hussain *et al.* (2016)** and may be due to initial enhanced immunological response followed by immunosuppressive effect of trypanosomiasis , influenced with the ever changingvariable surface glycoprotein of the infecting trypanosomes **(Adeyemi and Sulaiman, 2012).**

Significantly increased values of different hepatic enzymes such as ALT, AST and GGT were also recorded. These increased values of hepatic enzymes could be due to centrilobular degeneration as a result of the hypoxia and severe oxidative stress induced by parasitic infection. Similar changes also have been reported in camels due to trypanosomiasis **(Dagnachew *et al.*,2014 and Sivajothi *et al.*,2015).** Moreover, ALP activity was significantly increased in infected camels. These findings were in accordance with the study of **Seleim *et al.* (2003).** The significantly elevated levels of ALP in infected cattle could be attributed to some factors as the ALP is a leakage enzyme usually found in the bone, the intestines and predominantly in the liver **(Girling *et al.,* 2015).** The elevated levels of ALP in the infected cattle, therefore, suggest a probable invasion of the vital body organs and inflammation particularly of the intestines and liver **(Girling *et al.,* 2015).** The high levels of ALP in the infected group may also be due to the destruction of trypanosomes by the host immune system **(Enwezor and Sackey, 2005).** Several studies had reported significantly elevated ALP levels in both clinical and sub-clinical *trypanosoma* infections **(Oluyomi and Sulaiman, 2012).**

Increased levels of serum creatinine during *trypanosoma* infections may be linked to damage to host tissues or renal malfunction as reported by **Abenga and Anosa (2005).**

The infected animals were apparently healthy and equipped for purchase by Hagana in Haram region until the blood film revealed positive cases. So blood film is highly recommended.

REFERECES

Abdel-Rady, A. (2008): Epidemiological studies enzyme linked immunosorbent assay. Tropical (parasitological, serological and molecular Animal Health and Production,9:53-62.(Techniques of *Trypanosoma evansi* infection in 13. Pathak, K., Y. Singh, N. Meirvenne and M. Kapoor, camels (Camelus dromedarius) in Egypt. Veterinary 1997. Evaluation of various diagnostic techniques for World, 1 (11): 325 - 328.

Abenga, J. N. and Anosa, V. O. (2005): Serum total proteins and creatinine levels in experimental Gambian trypanosomosis of vervet monkeys. African J. Biotechnol. ;4 (2):187-190.

Adeyemi, O.S. and Sulaiman, F.A. (2012): Biochemical and morphological changes in *Trypanosoma brucei* infected rats treated with homidium chloride and diminazene aceturate. Journal of basic and clinical physiology and pharmacology; 23(4):179 -183.

ASI, M.N.;Lodhi, L.A.; Mughal,M.N.;Abbas,G.;Muhammad, G.and Saqib,M.(2014): Nutritional secondary hyperparathyroidism in an African lion cub (Pantheraleo). Pakistan Vet. J., 34: 554-556.

Dagnachew, S.; Terefe, G.; Abebe, G.; Barry, D.J. and Goddeeris, B.M. (2014): Comparative biochemical changes in young Zebu cattle experimentally infected with *Trypanosoma vivax* from tsetse infested and non-tsetse infested areas of northwest Ethiopia. Vet. Parasitol. **;** 205:451- 459.

Doumas, B.T.; Perry, B. W.; Sasse, E. A. and Straumfjord, J.V. (1973): Clinical Chemistry,19: 984-993.

Dyary, H.O.; Arif, A.H.; Sharma, R.S.K. and Rasedee, A. (2014): Antitrypanosomal and cytotoxic activities of selected medicinal plants and effect of Cordylineterminalis on trypanosomal nuclear and kinetoplast replication. Pak. Vet. J.**;** 34: 444 - 448.

El-Dakhly, K.H. M.; El-Habashi, N.; El-Nahass, E.; Sakai, H. and Yanai, T. (2013): Toxoplasmosis in the eastern grey kangaroo, Macropus giganteus and the cape hyrax, Procavis capensis, in Japan. Pakistan Vet. J.**;** 33: 529 - 531.

Elhaig, M.M.; Youssef, A.I. and El-Gayar, A.K. (2013): Molecular and parasitological detection of *trypanosoma evansi* in camels in Ismailia, Egypt. Vet. Parasitol. **;** 198: 214-218.

Enwezor, F.N. and Sackey, A. K. (2005): Camel trypanosomosis-a review. Vet Arhiv; 75: 439-452.

Eyob, E. and Matios, L. (2013): Review on camel trypanosomosis (Surra) due to *Trypanosoma evansi*, Epidemiology and host response. J. Vet. Med. Anim. Hlth.**;** 5: 334 -343.

Fabiny, D. L. and Ertingshausen, G. (1971): Automated reaction- rate method for determination of serum creatinine. Clinical Chemistry, 17:696- 700.

Gad, S.B. and El-Maddawy, Z.K. (2014): Silymarin improves pancreatic endocrine function in rats. Pakistan Vet. J.**;** 34: 214 -218.

Ghaffar, A.; Ashraf, S.; Hussain, R.; Hussain, T.; Shafique, M.; Noreen, S. and Aslam, S. (2014): Clinic hematological disparities induced by triazophos (organophosphate) in Japanese quail. Pakistan Vet. J.**;** 34: 257-259.

Girling, S.J. ; Campbell-Palmer, R. ; Pizzi, R. ; Fraser, M.A. ; Cracknell, J. ; Arnemo, J. *et al.* (2015): Hematology and serum biochemistry parameters and variations in the Eurasian beaver (*Castor fiber*) PLoS One. ; 10:1.

Habila, N.;Inuwa,M. H.;Aimola, I.A.;Udeh, M. U. and Haruna, E.(2012): Pathogenic mechanisms of *Trypanosoma evansi* infections. Res. Vet. Sci.; 93: 13-17.

Hussain, R.; Khan, A.; Mahmood, F.; Rehan, S. and Ali, F. (2014): Clinico-hematological and tissue changes induced by butachlor in male Japanese quail (Coturnix japonica).Pestic. Biochem. Physiol.**;** 109: 58 - 63.

Hussain,R.;Khan, A.;Abbas,R. Z.; Ghaffar,A.;Abbas,G.and Ali, F.(2016): Clinico-Hematological and Biochemical Studies on Naturally Infected Camels with Trypanosomiasis. Pakistan Journal of Zoology; 48 (2).

Khan, S.A.; Alauddin, M.; Hassan, M.M.; Islam, S.K.M.A.; Hossain, M.B.; Shaik at, A.H. ; Islam, M.N.; Debnath, N.C. and Hoque, M.A.(2013): Comparative performance and hemato-biochemical profile of Jinding ducks in different production systems of Bangladesh. Pakistan Vet. J.; 33: 113-116.

Megahed, G. A.; Abd Ellah, M. R. and Abdel-Rady, A. (2012): Comparative biochemical studies on natural *Trypanosoma evansi* infection in she-camels. Comp. Clin. Pathol. ; 21: 1121-1124.

Olaho-Mukani, W. and Mahamat, H. (2000): Trypanosomiasis in the dromedary camel. In: Gahlot, st renal tubular epithelium showed, the presence of T.K. (Ed.), Selected Topics on Camelids. The Camelid Publishers Chandan Sagar Well Bikaner India, nucleoli: 255-270.

Oluyomi, S. A. and Sulaiman,F. A.(2012): Biochemical and morphological changes in *Trypanosoma brucei brucei*-infected rats treated with homidum chloride and diminazene aceturate. J. Basic Clin. Physiol. Pharmacol; 23:179-183.

Oparah, Q. N.; Lawal, I. A. and Abdullahi, U.S. (2017): Hematological Indices in *Trypanosoma Brucei* (Federe Isolate) Infected Nigerian Donkeys (Equus Asinus) Treated with Homidium and Isometamidium Chloride. Macedonian Veterinary Review; 40 (1):73 - 82.

Osório, A.L.A.R.; Madruga, C.R.; Desquesnes, M.; Soares, C. O.; Ribeiro, L.R.R. and Da Costa, S. C. G. (2008):*Trypanosoma* (*Duttonella*) *vivax*: Its biology, epidemiology, pathogenesis, and introduction in the new world - a review. Mem. Inst. Oswaldo Cruz.; 103:1-13.

Padmaja, K. (2012): Haemato-biochemical studies and therapy of trypanosomiasis in camels. Vet. World, 5: 356-358.

Pays, E.; Vanhollebeke, B.;Uzureau, P.; Lecordier, L. and Pérez-Morga D. (2014): The molecular arms race between African trypanosomes and humans. Nat. Rev. Microbiol. ; 12 (8):575-584.

Sallau, A.B.; Ibrahim,M. A.;Salihu, A. and Yusuf, I. A. (2008): Bloodstream form of *Trypanosoma evansi* contains galactosidase. Middle-East J. Sci. Res.; 3: 49-52.

Seleim, R.S.; Tos, A.; Mohamed, S.R.; Nada, H.S. and Gobran, R.A. (2003): Elisa and other tests in the diagnosis of pasteurella multo-cida infection in camels. Proceedings of the International Conference on Agriculture Research for Development; 8-10.

Sivajothi, S.; Rayulu, V .C. and Sudhakara, B. R. (2015): Hematological and biochemical changes in experimental *Trypanosoma evansi* infection in rabbits. J. Parasit. Dis.; 39 (2):216 -220.

Tabacco, A.; Meiattini, F.; Moda, E. and Tarli, E. (1979): Simplified enzymic/colorimetric serum urea nitrogen determination. Clinical Chemistry, 25: 336 -337.

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

Yusuf, A. B.; Umar, I. A. and Nok, A. J. (2012): Effects of methanol extract of Vernoniaamygdalina leaf on survival and some biochemical parameters in acute *Trypanosoma brucei* infection. African Journal of Biochemisty Research. 150-158.