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CLINICAL, PARASITOLOGICAL, SEROLOGICAL, AND MOLECULAR DIAGNOSIS OF TRYPANOSOMIASIS IN CAMELS (*CAMELUS DROMEDARIUS*)

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ABSTRACT

Trypanosomiasis the most prevalent disease of camels, this study was carried out 106 one-humped camel (*Camelus dromedarius*) in Aswan. indicated 18 camels suffered from clinical signs of Trypanosomiasis as weakness, rough coat, Pale mucous membrane and anemia, Emaciation and thinning of the hump and prominent ribs, fever, watery eye, and Diarrhea, and by Microscopic examination Trypanosoma showed in 7 stained blood film of camels (6.6%), out from 106 serum samples were 13 (12.26%) camels give positive results by Formol Gel Test including only one sample positive by blood film test. When using PCR test only 15 camels of them were tested and (13) 86.7 % of them were found to be positive for the presence of TBR genome. When tested by Rhode Trypanozoon antigenic type (RoTat1.2) PCR targets all the 15 samples were negative that may be due to the Trypanosomes may be *Trypanosoma evansi* type B (lacks of RoTat genome) which may newly introduced to Egypt or may the sequence assorted variety of the RoTat1.2 gene in trypanosoma in Egypt detected from camels, especially in relationship with parasite long perseverance in camels because of the chronic period of the disease. Therefore, TBR-PCR was the more specific and sensitive method of the all methods used during this study. The suspected camel showed significant decreases in total erythrocytes count (TEC), hemoglobin (Hb), and a non significant increase in total leucocyte count (TLC), There was a significant increase in Alanine Amino Transferase (ALT), Total protein, globulin show a significant increase while glucose, Aspartate Amino Transferase (AST) level didn't differ significantly.

Key words: Trypanosomiasis – *Trypanosoma evansi* - clinical examination - microscopic examination - formol gel test - PCR – TBR1.2 - RoTat1.2 - hematological and biochemical findings - camels (*Camelus dromedarius*).

INTRODUCTION

Historically, at 1880 in India was the first detection of *Trypanosoma evansi* in blood of camels and horses by Evans, (Leadbeater *et al., 2000).* The Trypanosomiasis distributed throughout tropical and subtropical regions of the world. In Africa, camels were the most vital host, while in Central and South America the steed was primarily influenced. In Asia, a much more extensive scope of the hosts, including dairy cattle, wild ox and pigs. And the incubation period of surra varies from 5 - 60 days and may extend to 3 months and the parasite appears in the blood before the 14th day of infection. (Eyob *et al.*, 2013). The Salivated trypanosomes were passed to the recipient in the saliva of the tsetse fly (*Glossina spp.*). (Ian *et al., 2*004), and transmitted mechanically by biting insects, (Desquesnes *et al.,* 2013). Clinically camels manifested by fever, anorexia, edema and die; the chronic form was characterized by severe emaciation, intermittent fever, marked generalized muscular atrophy, pale mucous membranes and occasionally abdominal edema, and Sweat odor of urinary ketone, (Higgin, 1983). Anemia appears to be a major component of the pathology of Surra disease, and its continuation initiate anoxic conditions (Enwezor and Sackey, 2005).

The diagnosis of camel Trypanosomiasis still unsatisfactory up to date, because of the non-pathognomonic signs, (Dargantes *et al.,* 2005). The routine microscopic examination requires sensitivity, and serological examination requires specificity or sensitivity, and the use the molecular detection PCR test is a more trusted technique for diagnosis and epidemiological studies, (Gonzales *et al.,* 2007).

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Various techniques have been utilized to identify non-particular humoral antibodies present in animals infected with trypanosomiasis. Like formol-gel, mercuric chloride precipitation test, and thymol turbidity test, and are thought to be old technique despite the fact that the formol-test may even now have some utilization in the field since it is easy to proceed. most tests rely on the high amount of serum globulins after taking the infection. (OIE, 2012).

RoTat 1.2 VSG "variable surface glycoprotein" is available in all *T. evansi* however not in *T. brucei* (Claes *et al.,* 2004). *T. evansi* are contained homogeneous DNA mini-circles, and they loss a part of the kDNA maxi-circles which described as *T. evansi* type B and a special primers to give positive results by the molecular detection, and all the results assures the presence of *T. evansi* type B isolates, *T. brucei*, and *T. evansi* type A without RoTat 1.2 gene in Kenyan isolates. (Njiru *et al.,* 2006).

The clinical and non-clinical cases were positive by the DNA genome (TBR) while the clinical cases only detected by ITS and RoTat primers. (Elhaig *et al.,* 2013).

Determination of the hematological and biochemical changes, (Hb %), PCV and RBCs count were significantly decreased, TLC, lymphocytes, and Monocytes demonstrated a significant increase. (Hilali *et al.,* 2006), and Hematological analysis revealed a significant decrease the TEC, Hb% and PCV, However, the TLC and differential Leucocytes did not differ except for Eosinophils value which was significantly increased in Trypanosoma infected camels. (Eljalii *et al.,* 2015).

Liver function tests uncovered a significant increase in the action of lactate dehydrogenase catalyst (LDH), globulin, total bilirubin, and indirect bilirubin while alkaline phosphatase enzyme (ALP) indicated a significant decrease. Kidney function tests showed a marked decrease of both creatinine and urea. (Hilali *et al.,* 2006). The Plasma total protein, albumin, globulin, cholesterol and urea concentrations were insignificantly decreased. (Omer *et al.,* 2007). This study aimed to Investigate *Trypanosoma evansi* affecting camels in early stages, to estimate the haemato-biochemical values of camels infested and suspected with Trypanosomiasis, and also to evaluate a simple PCR-based technique for field diagnosis of *Trypanosoma* infection in camels, at Aswan governorate.

MATERIALS AND METHODS

1. Animals and samples:

A total number of (106) one humped camels (*Camelus dromedarius*) from 4 localities in Aswan (Daraw, Aswan west Village, Sohil west Village, and Abu elreish village.) was carried out From January 2017 to December 2017.

Table 1: Showing number of examined camels in each area.

Locations / Animals	Daraw		Aswan west		Sohil west		Abu elreish		Total animals
	Male	She- camel	Male	She- camel	Male	She- camel	male	She- camel	
NO.	69	2	9	5	7	1	8	5	106

Camels in Daraw imported from Sudan and these animals not resident and sold, While camels in Aswan west village, Sohil west village, and Abu elreish village were resident and used to ride tourists, camels in villages grazing with other animals like sheep and goat.

Age of the examined camels were 5 to 8 years old except 7 camels were 3 years old.

Whole blood samples of camels were collected by puncturing jugular vein into 2ml Ethylene Diamine tetra-acetic acid (EDTA) coated vacutainers tubes. Then kept in a cooler box and transported for lab activities. And another one in plain test tube to obtain serum.

METHODES

Clinical examination:

Camels were subjected to clinical examination according to the methods described by (Higgin, 1983).

Microscopic examination of blood smears:

It was carried out according to (Ijaz *et al.,* 1998)

Formol gel test:
The formol gel test (FGT) was carried according to. (OIE, 2012) by adding two drops of concentrated formalin solution (37 % formaldehyde) to 1 ml of serum

Molecular diagnosis: (PCR TEST)
The test carried out at the ARRI (animal reproduction research Institute) bio- technology unit.

All chemicals and reagents were molecular biology grade and reagents were prepared according to (Sambrook and Russell, 2001). Finally, the DNA amplification carried out according to (Sarataphan *et al.,* 2007). (Masiga *et al*., 1992) (Konnai *et al.,* 2009), (Salim *et al.,* 2011).

Heamatological and biochemical parameters:
Total Erythrocyte Count (TEC) and Total Leukocyte Count (TLC) by Neubauer's haemocytometer and differential leucocytic count two blood smears were taken from each blood sample and stained with Giemsa (Bancroft *et al.,* 1996). Whereas the concentrations of serum total protein (Biuret Method) and hemoglobin concentration (Hb), albumin concentrations, ALT, AST by using commercial test kits (spectrum-diagnostic) and spectrophotometer according to (Brit, 1967), (Kaplan *et al.,* 1983), (IFCC, 1986), (ECCLS, 1989), (Tietz, 1990), (Young, 1990), (Tietz *et al.,* 1994), (Young, 1995), (Tietz *et al.,* 1995), (Burtis, 1999), (Young, 2001).

Statistical Analysis
The Significance of the results was evaluated using Analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS) computer programs (2002).

RESULTS

Examination of 106 camels in this study revealed 88 (83 %) camels were apparently healthy, and 18 (17%) camels showed clinical abnormalities, loss of condition, weakness, depression, and a rough coat., pale mucous membrane and anemia, emaciation and thinning of the hump and prominent ribs, some cases show a rise in body temperature and watery eye, diarrhea with the hindquarters soiled with feces. As in photo (1).

By microscopic examination founded 7 (6.6%) positive Smears 6 of them were from the 18 clinically suspected camels and one from the 88 apparently healthy camels as in photo (2).

By using FGT result revealed 13 (12.26%) serum sample give positive result by Formol gel test, 2 serum samples were positive from the 18 clinically manifested camels and the 11 serum samples from the 88 apparently healthy camels. And there was only one sample give positive result by both microscopic examination and Formol gel test.

Table 2: Comparative evaluation of the techniques used for diagnosis of Trypanosoma during different seasons.

Season	Summer	Autumn	Winter	Spring	Total
No. of examined animals	22	35	20	29	106
Clinical examination (suspected)	2 (1.9%)	6 (5.6%)	3 (2.8%)	7 (6.6 %)	18 (17%)
Microscopic examination (positive)	1 (0.9%)	4 (3.8%)	0	2(1.9%)	7 (6.6%)
Formol gel test (positive)	1 (0.9%)	6 (5.6%)	2 (1.9%)	4 (3.8%)	13 (12.26%)

15 samples includes (5 positive samples by microscopic examination including the only one positive sample by both microscopic examination and formol gel test and 10 samples were negative by microscopic examination and 4 of them were positive by formol gel test,), examined for PCR total genomic DNA of camels blood samples were amplified using TBR1, and RoTat1.2 primers. A DNA fragment of 164 bp., and 151 bp. was amplified. The TBR-PCR revealed that 13 (86.7%) samples were positive for infection photo (3). All samples which were positive to *T. evansi* infection by blood film (5 samples including the positive sample by FGT) were positive by PCR while the remaining positives

by PCR (8 samples) were negative by blood film, and 2 samples of them were positive by FGT While Amplification of the RoTat 1.2 primer (in the same 15 samples) was carried out. The results revealed all samples were negative (0 %) photo (4).

Table 3: Comparative evaluation of the techniques used for diagnosis of Trypanosoma.

Test	No. of examined camels	positive	Rate	Negative
Clinical examination	106	18	17%	88
Microscopical examination	106	7	6.6%	99
Formol gel test	106	13	12.26%	93
TBR-PCR	15	13	86.6%	2
RoTat-PCR	15	0	0%	15

43 samples (including the 5 positive samples by blood film) were subjected to the determination of TEC, TLC, Leuckocytic count, and Hb%. The data of the mean standard error of the mean for TEC, TLC differential leuckocytic count and Hb % in suspected and healthy camels with *Trypanosoma evansi* in the table (4), revealed that decrease in Hb%, TEC, and increase in TLC, Neutrophils and Eosinophils.

Table 4: Heamatological parameters in suspected camels.

Statics / Parameter	TEC (c.m.m)		Hb% (gm%)		TLC (c.m.m)		Lymphocyte		Neutrophils		Monocytes		Eosinophils		Basophiles	
No.	**Suspected** 6	Apparently Healthy 37	**suspected**	Apparently Healthy	**suspected**.	Apparently Healthy	**suspected**	Apparently Healthy	**suspected**	Apparently Healthy	**suspected**	Apparently Healthy	**suspected**	Apparently Healthy	**suspected**	Apparently Healthy
Min.	4	3.9	7.8	8.8	5.8	4	16	10	45	36	14	6	2	0	0	0
Max.	6.7	12.07	11	15.7	25	22	34	40	68	74	20	26	4	12	0	2
Mean	5.36	6.93	9.26	11.77	14.66	12	24	23.86	57	55.68	16.66	16.73	2.5	3.27	0	0.46
SD	0.95	2.05	1.2	1.75	6.28	4.72	8.19	7.72	10	10.63	2.07	4.7	0.83	2.34	0	0.69
SEM	0.38	0.34	0.49	0.29	2.57	0.78	3.35	1.27	4.09	1.75	0.84	0.77	0.34	0.38	0	0.11

Biochemical findings: The same 43 samples were subjected to the determination of serum proteins components (µg/dl) and liver enzymes (AST, ALT) and blood glucose level. The data of the mean ± standard error of the mean for serum proteins components (µg/dl) and liver enzymes (AST, ALT) and blood glucose in healthy and suspected camels with *Trypanosoma evansi* in the table (5), the result revealed a decrease in blood glucose level, AST and increase in ALT level, total protein, albumin, and globulin amounts.

Table 5: Biochemical parameters in suspected camels**.**

Statics / Parameter	Glucose (mg/dl)		ALT (u/l)		AST (u/l)		Total protein (g/dl)		Albumin		Globulin		Albumin/ globulin ratio	
No.	**Suspected** 6	Apparently Healthy 37	**Suspected** 6	Apparently Healthy 37	**Suspected** 6	Apparently Healthy 37	**Suspected** 6	Apparently Healthy 37	**Suspected** 6	Apparently Healthy 37	**Suspected** 6	Apparently Healthy 37	**Suspected** 6	Apparently Healthy 37
Min.	58	81	8.5	0.58	7.6	9.43	7.3	3	1.8	0.2	4.96	1.8	0.29	0.029
Max.	149	149	57.1	49.8	44.2	370	9.3	8	4.24	3.2	6.29	6.9	0.79	0.94
Mean	107	117	37.08	12.09	31.23	86.15	8.2	6.33	2.61	1.56	5.58	4.81	0.48	0.35
SD	29.18	15.17	16.22	12.34	14	83.11	0.83	0.92	0.89	0.7	0.54	0.91	0.18	0.21
SEM	11.91	2.52	6.62	2.05	5.7	13.85	0.34	0.15	0.37	0.11	0.22	0.15	0.075	0.03

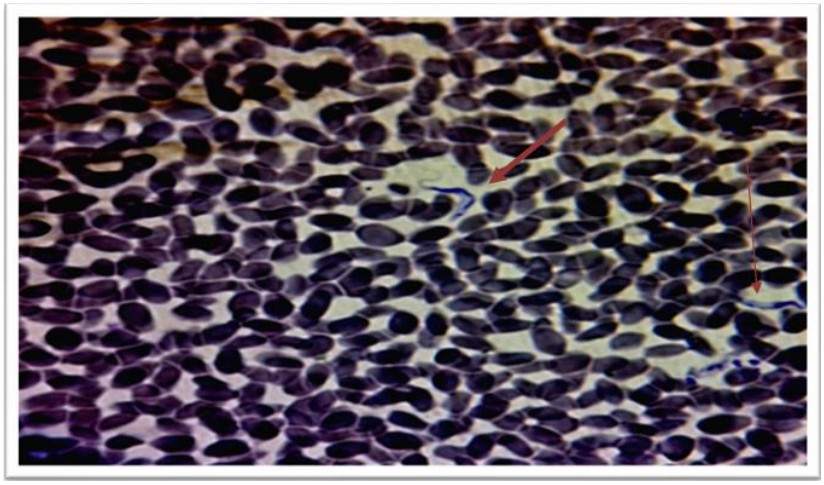

Photo 1: *Trypanosoma evansi* by microscopic examination.

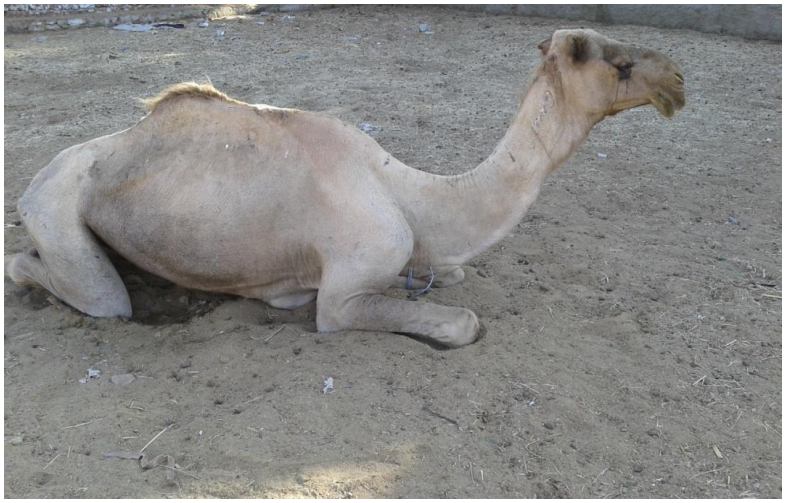

Photo 2: Animal clinically suffering from trypanosomiasis.

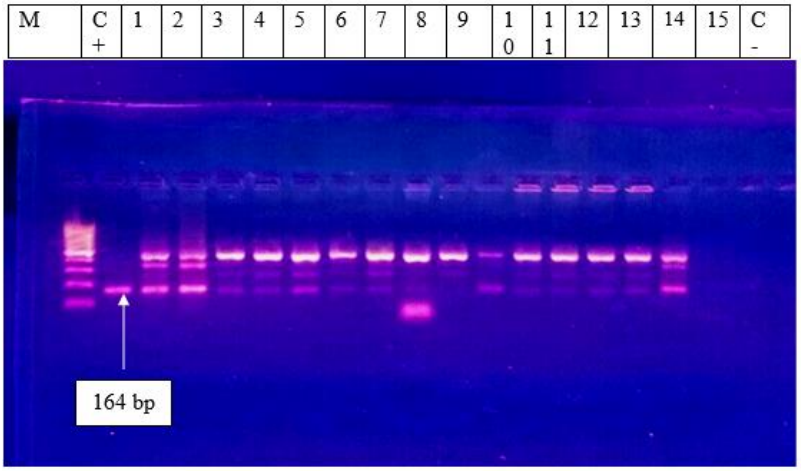

Photo 3: TBR- PCR results: Agarose gel electrophoresis of amplified *T. evansi* DNA (164 bp PCR products). M: 100 bp DNA ladder as a standard marker, Lane (1, 2, 3, 4, 5, 6, 7, 10, 11, 12, 13, 14 and 15) Positive PCR products. Lane (8, and 9) Negative PCR products.

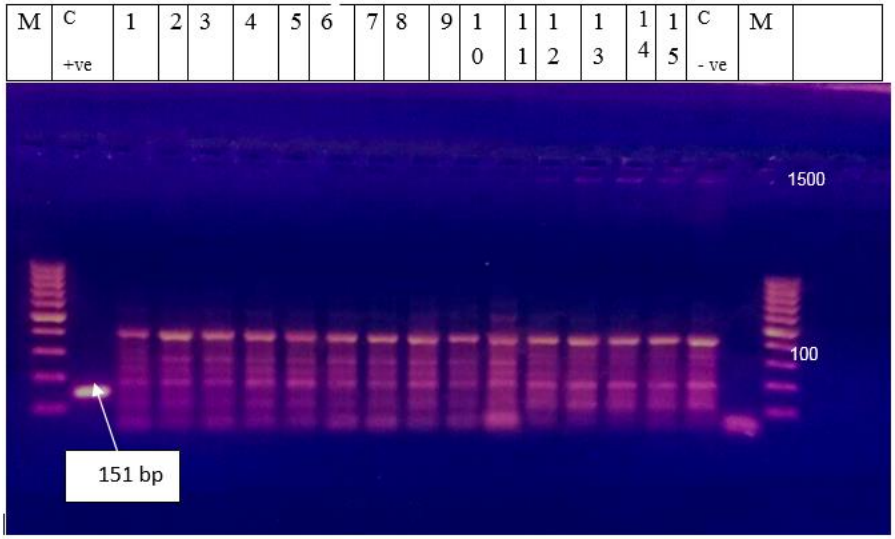

Photo 4: RoTat 1.2-PCR results: Agarose gel electrophoresis of amplified *T. evansi* DNA (151 bp PCR products). M: 100 bp DNA ladder as a standard marker, all samples were negative (giving several non- specific bands) including the 5 positive samples with microscopical examination and all the TBR primer positive samples.

DISCUSSION

These clinical signs were similar to those described by (Higgin, 1983).

Microscopic examination of stained blood smear revealed that the total infected camels was 7 (6.6%) similar to (Abd-Elmaleck *et al.*, 2014), differ to (Mahmoud M., 2008), It is important to mention that because of microscopic low prevalence (6.6%), detection of *Trypanosoma evansi* in the blood of examined animal is suitable only in acute infection where the number of trypanosomes in blood is very high (parasitaemia). On the other hand in chronic infection trypanosomes in blood are scanty or completely absent, so the microscopic examination is not successful at this stage due to the result revealed that TBR-PCR high prevalence (86.6%) similar opinion was mentioned by (Chaudhary *et al.,* 2010, and Shahzad *et al.,* 2010).

All the microscopic confirmed camels samples were negative (except one were positive) in the Formol Gel Test, this serological test showed poor to slight accordance with any other diagnostic test for surra applied in the present study. Similar to (Chappuis, 2005), (Aslam *et al.,* 2010). The apparently poor specificity of the FGT is explained by the fact that it detects, in a non-specific way, high concentrations of plasma immunoglobulins that can be due to any acute or chronic infection causing hyper gammaglobulinemia. (Tehseen *et al.,* 2015).

The highest infection rate of Trypanosoma in the study area were in spring and autumn which may be had an occasional variety during the rainy season and in the early dry season due to the rapid spread of insects similar to results mentioned by (Higgin, 1983), (Mahran, 2004), (Mahmoud M., 2008), When using molecular method (PCR) in this study TBR-PCR (86.6%) was the more specific and sensitive method of the all methods used during this study, TBR primers can be utilized routinely as an indication for early detection of animal reservoirs in the acute and chronic phases, that helps in rapid treatment of diseased animals and/or disposal of reservoirs of infection (Fernández *et al*., 2009, Pruvot *et al*., 2010).

However, this study revealed that many camels were negative by blood examination but positive by TBR-PCR, which may be related to low parasitaemia and/or low sensitivity of the stained blood smear technique and indicates that low parasitaemia might be due to early infections, chronic infection and/or lower strain virulence. The high proportion of non-clinical infection of *T. evansi* among the investigated camels was in agreement with the findings in the Upper Egypt of (Mahran, 2004).

Generally, the percentage of detection and the sensitivity of the PCR is variable depending upon the primers employed, which are determined by the number of copies and the homology of the primers with the target sequence (Fernández *et al.,* 2009).

The negative samples RoTat1.2 PCR targets in this study agreed with some previous studies (Elhaig *et al.,* 2013), and these negative results may be due to the trypanosomes may be

Trypanosoma evansi type B (lacks of RoTat genome)which detected in Sudan (Salim *et al*., 2011) or may the sequence assorted variety of the RoTat1.2 gene in Egyptian trypanosome detected from camels, especially in relationship with parasite long perseverance in camels because of the chronic period of the disease (Amer *et al*., 2011).

The suspected camel showed significant decreases in total erythrocytes count (TEC), hemoglobin (Hb), and a non significant increase in total leucocyte count (TLC), There was a significant increase in Alanine Amino Transferase (ALT), Total protein, globulin show a significant increase while glucose, Aspartate Amino Transferase (AST) level didn't differ significantly in suspected camels than healthy ones. The hematological and biochemical parameters changes are similar to (Hilali *et al.*, 2006), (Eljalii *et al.*, 2015), and differ to (Omer *et al.*, 2007).

CONCLUSION

Finally, according to this study we can say that trypanosomiasis in camels was prevalent in Aswan, either by clinical and non-clinical cases and therefore, reliable diagnosis should be used for rapid treatment or control of the disease., the clinical examination of camels suspected with trypanosoma not a reliable method, the Microscopic examination frequently used for diagnosis of trypanosoma infections had low sensitivity. and depends on the acuteness of the case, high parasitaemia and the good technician skills, while the use of FGT as a field test for diagnosis of trypanosomiasis in camels, it is easy to apply, but it is non-specific technique because it depends on the presence of high amount immunoglobulin's which may occur in any other chronic disease. The TBR-PCR test is the more reliable and easy technique for diagnosis of *Trypanosoma evansi* (more specific and sensitive method). The all negative samples obtained by using RoTaT1.2- PCR test revealed presence of *Trypanosoma evansi* type B which lack to VSG which recently detected in Kenya, Ethiopia, and Sudan, or it may be any other trypanosomes except *T. evansi* type A. and Camel blood is consider as a mirror of the physiological or adverse conditions the heamatological and biochemical parameters changed due to the infection camels by trypanosomiasis. And recommended that Should Use of a simple PCR-based technique for routine field diagnosis of *Trypanosoma* for early detection, treatment, and control. Need for more investigation of *T. evansi* by using of suitable PCR test primers to determine the presence of *T. evansi* type B isolates, *T. b. brucei* and presence of *T. evansi* type A without RoTat 1.2 gene in Egyptian isolates.

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التشخيص السريري والطفيلي والمصلّي والجزيئي لداء التريبانوسوما في الإبل (الجمال وحيدة السنم)

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داء التريبانوسوما هومن أكثر الأمراض انتشاراً في الإبل ، وقد أجريت هذه الدراسة على ١٠٦ جمل ذو سنم واحد (Camelus dromedarius) في أسوان. أظهرت النتائج ان ١٨جمل (١٧٪) من الإبل بها بعض الأعراض السريرية لمرض التريبانوسوما مثل الضعف ، والأغشية المخاطية الشاحبة ، وفقر الدم ، وضمور السنام والأضلاع البارزة ، والحمى ، والعين المائي ، والإسهال ، وكذلك بالفحص المجهري ظهرت المثقبيات في ٧ افلام من الدم الملون من الجمال (٦,٦ ٪) ، في حين أن الأمصال اعطى ١٣ جمل (١٢,٢٦ ٪) من الإبل نتائج إيجابية باستخدام FGT (اختبار هلام الفورمالين) بما في ذلك عينة واحدة فقط إيجابية عن طريق اختبار فيلم الدم. عند استخدام اختبار PCR تم اختبار ١٥ جمل ووجد أن (١٣) ٨٦,٧٪ منها إيجابية لوجود جينوم TBR. كانت جميع العينات (١٥ عينة) سلبية باختبار RoTat1.2 ، وقد تكون نتائج PCR بسبب التريبانوسومات قد تكون نوع من المثقبيات B evansi (نقص جينوم RoTat) والذي قد يكون تم إدخاله حديثًا إلى مصر أو قد يكون متسلسلًا متنوعًا من RoTat1.2. او تم اكتشاف الجين في التريبانوسوم المصري من الجمال ، خاصة مع طول المدة في الحالات المزمنة من المرض. لذلك ، كان TBR-PCR الطريقة الأكثر تحديدًا وحساسية لكل الطرق المستخدمة أثناء هذه الدراسة.

أظهر الجمل المصاب انخفاضًا ملحوظًا في TEC عدد خلايا الدم الحمراء والهيموجلوبين ، وزيادة كبيرة في عدد خلايا الدم البيضاء TLC ، الايزينوفيل. كان هناك انخفاض كبير في تركيزات الجلوكوز ، انزيمات الكبد من مستويات ألانين أمينو ترانساميناز ومستويات الاسبرتات الامينية الناقلة (AST ،ALT) ، البروتين الكلي ، تظهر الجلوبيولين زيادة كبيرة في حين أن مستوى الألبومين لم يختلف بشكل كبير.