Molecular study of *Trypanosoma spp* in camels of Al- Diwaniyah province in Iraq

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ABSTRACT

Due to the economic importance of the camels and because of the scarcity of studies that death with it in Iraq, our study aimed to investigate *Trypanosoma spp* by molecular methods. The current study was conducted during the period from September 2017 to the month March 2018 and a total of 200 samples were collected randomly from camels included (125) females and (75) males and two groups of ages (150) sample > 1 years and(50) sample <1year) some of them have clinical signs and some did not show any symptoms from the slaughterhouse of Al- Diwaniya province. This study was designed to diagnose this parasite were initially diagnosed by microscopic examination and was examined by geimesa stain method and the results were positive of (76/200) (38%) *Trypanosoma spp*. The prevalence of Trypanosomiasis in females (57/125) (45.6%) while males (19/75) (25.3%) and in all ages. The highest incidence in months (September, October and March) (88.3%, 96.2% and 75%), respectively. Secondly the diagnosis of *Trypanosoma* in 50 blood samples positive microscopically using molecular techniques, which included polymerase chain reaction (conventional PCR) .The results (10/50)(20%) *Trpanosoma spp* and (7/50)(14%) *T. evansi* then DNA sequencing analysis and detecting genetic relationships (phylogenic analysis).

Key words : PCR, *Trypanosoma*, camels , Geimsea stain.

دراسة جزيئية لطفيلي التريبانوسوما في الجمال في محافظة الديوانية في العراق

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الخلاصـــة

نظرا للأهمية الاقتصادية للابل وبسبب قلة الدراسات التي تتعلق بها في العراق هدفت الدراسة لتحري عن طفيلي *Trypanosoma spp* التريبانوسوما بالطرق الجزيئية . أجريت هذه الدراسة خلال الفترة الممتدة من شهر أيلول 2017 ولغاية شهر اذار 2018 تم جمع 200 عينة دم عشوائية من الابل (125) اناث و(75) ذكور وبمجموعتين من الاعمار(150)عينه اكبر من سنه و(50) عينه اصغر من سنه ومنها ظهرت عليها علامات سريرية ومنها لم تظهر عليها اي اعراض من مجزرة محافظة الديوانية .صممت هذه الدراسة لتشخيص هذا الطفيلي اولا بالفحص المجهري باستخدام صبغة كمزة(geimesa stain) وكانت النتائج 76(38%) تريبانوسوما .وبينت الدراسة بأن أعلى نسب للإصابة *Trypanosoma spp* بداء المثقبيات ظهرت في الاناث(57/125) (45.6%) اما الذكور(75/ 19) (25.3 %) وبمختلف الاعمار. حيث سجلت اعلى نسبة اصابه في الاشهر (ايلول ,تشرين الاول واذار) (88.3% , 96.2% و75%) على التوالي حسب وقت الدراسة . وثانيا تشخيص الطفيلي التريبانوسوما في 50 عينة دم موجبة بالفحص المجهري باستخدام التقنيات الجزيئية والتي تضمنت تفاعل السلسلة البلمرة الاعتيادي (Conventional PCR) وكانت النتائج كالاتي *T. evansi and Trpanosoma spp* وبالنسب 10(20 %) ,7(14%) على التوالي. ومن ثم استخدام طريقة تحليل ترتيب النيوكليتيدات (sequencing analysis) وكذلك تم تحديد العلاقات الوراثية التطورية (التحليل الفيلوجيني) (phylogenetic analysis).

الكلمات المفتاحية : تفاعل سلسلة البلمرة, التريبانوسوما, الجمال, صبغة كمزا.

Introduction

One humped and two humped camels make up nearly 25 million animals (1). Camels are considered to be one of the best farm that animals andcan tolerated the harsh conditions in the arid parts of the world because of their unique adaptive physiological characteristics Therefore, requires good management and the control programs of diseases (2). Camel is an important multi-use animal used for transportation and production of milk, meats and wools since ancient times (3). Many parasitic diseases can infect camels and thus affect on their health and cause anemia ,the wasting, fever and death in heavy infection therefore camel medicine has a long history in the world and Iraq (4). These diseases are transmitted from camel to camel by vectors such as *Tabanus* and *Stomoxy* (5,6). *T. evansi* is the main cause of the surra disease in camels and is one of the most important blood parasites that causes severe losses of economic, so it is considered the most important economic aspect in the camel breeding fields in the world (7,8). Surra disease caused high morbidities and mortalities (9). The acute form of the Trypanosomiasis is almost always fatal during a few weeks, while common chronic form is evidence by anemia, emaciation, frequent fever, edema, conjunctivitis, the abortions, enlargement of lymph nodes and lacrimation (9). So far there are no vaccine available for Trypanosomiasis (10). The most common methods for diagnosis of blood parasites in camels are checking the blood electron microscopy and then DNA extraction for is confirm diagnosis by the use of technologist (PCR) with analysis sequence (1,11). There are no control programs to prevent blood parasites on a large scale (12). Molecular tools and material increase become completed part of studying the epidemiology and diagnosis of *Trypanosoma* (13). This study was conducted to investigate the parasites of the local Iraqi camels in Al-Diwaniyah governorate through the use microscopic examination , PCR technology, DNA sequence and Phylogenetic tree.

Materials and Methods

Collection of blood samples

Total of 200 blood samples were collected from the jugular vein from each camel some of these camels was suffered from weakness, anaemia , liver pale and other asymptomatic camels in the house slaughter in Al-Diwaniyah province during the period from September – 2017 to the end of march – 2018 and data included animal's age (>1 year to 2-7 year and <1year) and animal's sex (male and female) .These samples kept at tubes (EDTA) to make slides and put in deep freeze under (20-) °C.

Microscopic examination

A drop of blood was taken and placed on the microscopic glass slide and spread by other slide then leave in air to dray fixed by absolute methanol from (5-10)minute then stain with Giemsa stain to 30 minute then wash by water and leave to dry after drying they examined under oil immersion Len of light microscope according to (2).

Primers

Specific primers was provided by IDT(Canada) as follow:

Table (1): Primers used in this study with their Sequence and PCR product Size:

Genes	Primers sequence (5'-3')		Product size (bp)	Source
Trypanosoma spp. **18ribosomal RNA gene**	**F**	**CCGGAAGTTCACCGATATTG**	**480-757bp**	**14**
	R	**TGCTGCGTTCTTCAACGAA**		
Trypanosoma evansi **VSG gene**	**F**	**GCGCGGATTCTTTGCAGACGA**	**257bp**	**15**
	R	**TGCAGACACTGGAATGTTACT**		

Molecular examination

Genomics DNA of camels blood samples were extracted by using Column-pure blood Genomics DNA Mini Kits ,Applied Biological Materials (Abm) Canada by using conventional PCR technique was performed to detect of *Trypanosoma* under PCR Thermocycler conditions as table(2):

Table (2): PCR program amplify.

Genes	Initial denaturation	Cycle condition x30			Finally extension	Hold	Cycle No.
		Denatura-tion	Annealing	Exten-sion			
Trypanosoma spp.	95°C /120 sec	95°C /30 sec	58°C/30 sec	72°C/60 sec	72°C/60 sec	4°C	35
Trypanosoma evansi	94°C /280 sec	94°C /120 sec	58/60 sec	72°C/60 sec	72°C/60 sec	4°C	40

DNA sequencing Analysis

DNA sequencing technique was carried out for Phylogenetic relationship analysis study of genes (VSG gene and 18ribosomal RNA gene) for (*Trpanosoma evansi* and *Trypanosoma spp*) respectively in camels with NCBI-Gen Bank Global. PCR product carried out DNA sequence by (suol – South Korea).

Statistical analysis

Statistical analysis was achieved by using Chi Square tests (X^2) at $p \leq 0.05$ was used to analyze differences rate among samples, sex and different studied ages (1**6**).

Result

The results of microscopic examination

The results show that of the total 200 samples of camels blood examined microscopically found that 76(38%) samples infected with *Trypanosoma spp* as table (3,4) and figure (1,2) .

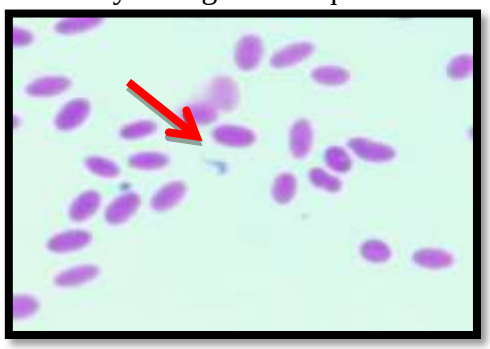


Fig-1 *Trypanosoma sp*. appear between camel RBCs (thin smear) 100x.

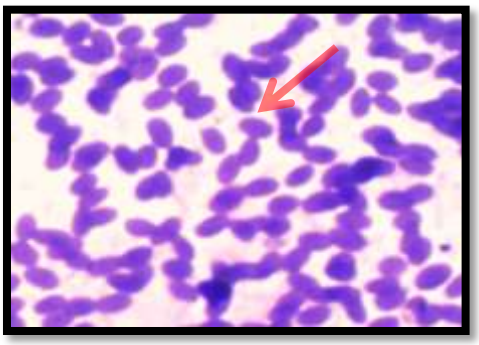


Fig-2 *Trypanosoma sp*. appear between camel RBCs (thick smear) 100x.

Table -3 The prevalence of *Trypanosoma spp* infection in camels according to the microscopic examination and sex .

Sex	No.	*Trypanosoma sp* infection	
		+	%
Males	75	19	25.3 b
Females	125	57	45.6 a
Total	200	76	38

Different letter = significant difference at $p < 0.05$

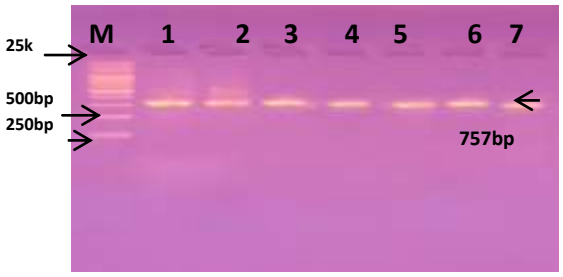
Table -4 The prevalence of *Trypanosome spp* infection in camels according to the microscopic examination and age.

Animals age	No.	*Trypanosoma sp* infection	
		+	%
< 1	50	14	28 [a]
> 1	150	62	41.33[a]
Total	200	76	38

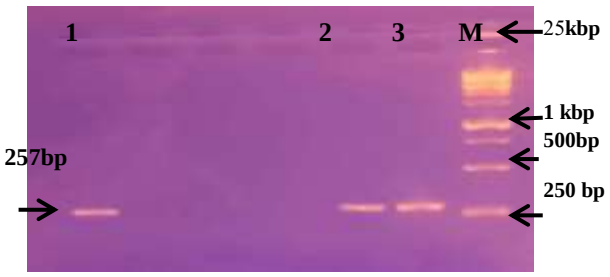
Similar letter = no significant difference at $p < 0.05$

Result of Molecular diagnosis (PCR) technique

50 microscopically detect samples were examined by PCR technique 10(20%) *Trypanosoma spp* and 7(14%).*T. evansi* .



Figure(3): Agarose gel electrophoresis image that show PCR product that amplified fragment of small subunit ribosomal RNA gene in *Trypanosoma spp*. from camel blood samples. Where M: Marker (250bp-25kbp) and lane (1-7)some positive samples for *Trypanosoma spp*. at 757bp product size.



Figure(4): Agarose gel electrophoresis image that show PCR product that amplified fragment of small subunit ribosomal RNA gene in *Trypanosoma evansi* from camel blood samples. Where M: Marker (250bp-25kbp) and lane (1-3) some positive samples for *Trypanosoma evansi* at 257bp product size.

Results of DNA Sequencing

Four purified PCR products samples were analyzed by the use of sequencing method in order to obtain the nucleotide sets of genes and recorded in gene bank The phylogenic tree was using neighbor join boot strab 1000 radiation tree. The results of analysis showed the maximum homology of nucleotide sequences and accession numbers are (MH571705, MH595480, MH697863 and MH697864).

Results of Phylogenetic Tree Construction

between Iraqi *Trypanosoma spp* isolated strains and world strain range (93-99 %). while between the local *T. evansi* isolated strains and Iraqi strains range (99- 100 %).

Figure -3 The Phylogenic tree of 18s rRNA of *Trypanosoma spp*.

Discussion

The study results showed that the Iraqi camels were infected with *Trypanosoma spp* in Al- Diwaniyah province in Iraq, the microscopic examination of the blood smears show that the *Trypanosoma* appear between RBCs this result is in agreement with (16). The prevalence of *Trypanosoma* according to the sex of animals, the number of infected females (57/125)(45.6%) and the number of infected males (19/75) (25.3%) of the total 200 samples. There are significant difference at $p < 0.05$. The results of the present study are similar to the results recorded by (17, 18, 19) these recorded the highest infection of females more than males suspected to infection from males. But disagree with (20) recorded (23%) in Shalatin city, Red sea and (21) recorded (23%) in Iran, These reported the higher infection was found in males more than females. The differences could be due to the several effects such as the many of males of camels were using for meat slaughter while females of camels were nearly old age because using breeding and milk production, therefore stress during gestation and milk production performance them more susceptible *Trypanosome* spp. While according to the age of animals, the number of infected (<1) year (14/50)(28%) and the number of infected camels (>1) year (62/150)(41.33%). There are no significant difference at $p < 0.05$. In this study show *Trypanosome* can infected camels in all age with the increasing of animal age and agreement with (17,19) they found that the highest prevalence rate at all age especially in camels more 1year. (22) reported that the expected of the young animal to the colostrum that contains the antibodies make them more resistant . But disagree with (18) recorded (11/38) (28.9%) in young camels in Nigeria. This could be attributed to the owners and nomads who preferring to graze the animals in open fields' because the open field increases the risk of infection because animals become more exposed to vector bites and small aged animals take protection via the colostrum and from mother's immunity and different age animals groups were breed together permitting transmission of infection from adults animals (carriers) to susceptible young animals and other reason such as differences in diagnostic techniques, location and climatic conditions.

The results of this study show the increase prevalence of blood parasites in the beginning of spring and summer to last autumn and decrease in winter with significant difference at $p < 0.05$. The results correspond with (23). The distribution of the blood parasites diseases may be due to season of vectors and the climate condition that effect on distribution vectors especially hard ticks and biting flies.

ISSN 2072-3875

The Prevalence of Trypanosomiasis in camels by using PCR

Molecular methods are more sensitive and specific diagnostic tools PCR done depend on specific primers and positive samples microscopically with show can be infected camels by *Trypanosoma spp* and also many researcher diagnosed *Trypanosoma spp* by PCR as (3,15,24,25) with vitiation in percentages of researchers with our percentage can be explained by the following reasons, such as that the parasite *Trypanosoma* has the ability to change from the same itself by (VSGS) thus making it difficult to identify and may amount of parasite very little in size in sample compare with the researchers that the parasite was in part in the sample more than the presence in the sample quantity taken in this study. The difference between the results of microscopy and molecular examination is due to the fact that in most cases chronic infections and the amount of DNA were low and the genetic variation of *Trypanosoma*, so the difference in results.

Conclusions and Recommendations

The results showed that *Trypanosoma spp* were distributed in camels in Al-Diwaniyah provinces according to Giemsa stain method and PCR technique. Molecular and phylogenetic diagnostics are more accurate in determining the species of parasites. The scientific methods that would develop the production of livestock to raise the economic level of the country through the production of vaccines and the use of preventive treatments to prevent the spread of these diseases. For new research studies are directed to know if these parasites have the potential transmission to humans and study to detect the pathogenic effects on humans because farmers and owners are in continue contact with the animal.

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ISSN 2072-3875