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Molecular detection of *Trypanosoma* species in sheep and goats in Mosul city

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Abstract

In this study, we examined blood samples of 385 sheep and goats of different ages, sexes, and sources under routine microscopic examination of the blood smear (wet, thin, thick, buffy coat layer smears) to detect *Trypanosoma*. Results show that 81 samples were positive. These samples are succumbed to the molecular detection of *Trypanosoma* and other species by the extraction of parasitic DNA this parasitic DNA is detected in samples using KIN1, KIN2, and AITSF, AITSR primers. After that, conventional polymerase chain reaction was applied, and the results showed that 81 samples had a positive reaction in using KIN1 and KIN2 primers, while the positive samples were 76 when using AITSF, AITSR primers. Moreover, results showed a high rate of infection in sheep as compared with goats using both pairs of primers and two species of *Trypanosoma* in sheep and goats. Molecular was recorded, which include *T. conglense* and *T. vivax*. Animals more than 1-2 years old group showed a high rate of infection as compared with other ages group, and females have recorded a high rate of infection as compared with males. According to the source of animals, imported animals showed a high infective rate compared to native ones. This study is the first recorded *Trypanosoma* species in small ruminants in Mosul city.

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Introduction

Trypanosomosis is a protozoal microorganism caused by unicellular flagellated protozoa. It is classified with the Protozoa Sub-kingdom, Sarcomastigophora Phylum, Kinetoplastida Order, *Trypanosomatidae* Family, and *Trypanosoma* Genus: the genus *Trypanosoma* has two main groups: Stercoraria and Salivaria, which is found in blood, different body tissues, and fluids of the vertebrates (1). There are three subgenera of *Trypanosoma* that affect health: Vivax, *congolense*, and *Brucei* (2). *Trypanosoma* is transmitted by flies' bites because the infective stages of the parasites are found in the mouth of the infected insect vector. *Trypanosome's* species, namely: *T. congolense*, *T. vivax*, and *T. brucei* affects small and large ruminants (3). The intensity of the *Trypanosoma* infection depends on the animal's *spp*, age of animals, and *Trypanosomes spp*, so the pathogenesis of Trypanosomosis differs according to the *spp* causing the disease (4). The main clinical signs are fever, anemia, loss of body condition, enlargement of lymph nodes, and abortion in pregnant animals. Diagnosis of *Trypanosoma* occurs by classical methods of microscopical examination of the blood smear (5). The microscopic examination does not detect the species and does not investigate multiple infections (6). PCR has pliable the amplification of specific DNA sequences. This method can be developed to detect many types of parasites. (7). The current study has been done because no molecular studies of detection the species of *Trypanosoma* in sheep and goats in Nineveh province.

Materials and methods

Animals and samples

Three hundred and eighty-five sheep and goats with different ages, sex, and source were examined under routine

microscopic examination of the stained blood smear (wet, thin, thick and buffy coat layer smears) to detect of *Trypanosoma*. There were 81 positive samples. Then, molecular detection was done. Five ml of blood was collected from the jugular vein of each animal. The blood was transported to a tube which contained EDTA then stored in -20 ºC.

DNA extraction and PCR protocol

DNA was extracted from the blood by using DNA blood extraction kit (Qiagen), the method of DNA extraction was done according to the manufacturer's instructions manuals. The first step of PCR was done to detect the *Trypanosoma* group. KIN primers universal Trypanosome can be used according to (8). PCR was performed in 25 ul volumes. The second step can be done according to Alex *et al.* (9) using AITSF and AITSR primers (Table 1). The amplification condition of PCR was done using a thermocycler (Table1). The final PCR products were separated using 1.5% agarose gel electrophoresis. Bands observed corresponded to *T. congolense (Kilifi/Forest and Savannah);* 650-800 bp, *T. brucei;* 520-540 bp, *T. simiae;* 440-500 bp, *T. Godfrey;* 320-400 bp, and *T. vivax;* 290-400 bp (9) (Figures 1 and 2).

Table 1: Primers and amplification condition which used in this study

Primer	Sequence	Amplification condition	Ref
Kin1	5'-GCGTTCAAAGATTGGGCAAT-3'	94 ˚C for 3min, then 94 ˚C 45 seconds, 68 ˚C 60 seconds, 72 ˚C 60 seconds for 35 cycle, final extension 72°C (10 min)	8
Kin2	5'-CGCCCGAAAGTTCACC-3'		
AITSF	5'-CGGAAGTTCACCGATATTGC-3'	95˚C for 10 min, 37 cycles: 95˚C for 30 sec, annealing at 60˚C for 1 min, 72˚C for 2 min, final extension at 72˚C for 10 min.	9
AITSR	5'-AGGAAGCCAAGTCATCCATC -3'		

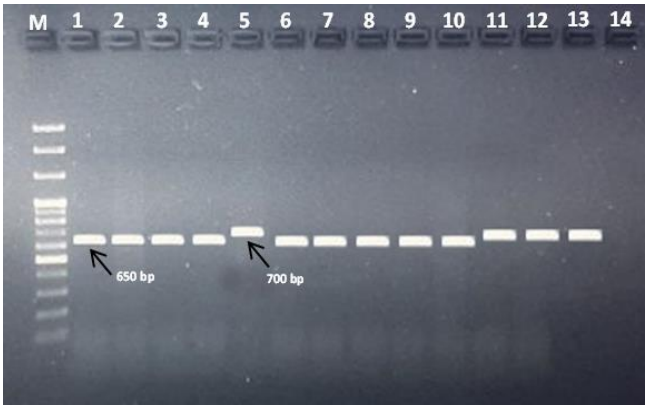

Figure 1: Amplification of KIN1 and KIN2 primers, M=marker, 1-13 (650-700bp) positive to *Trypanosoma*, 14 Negative control.

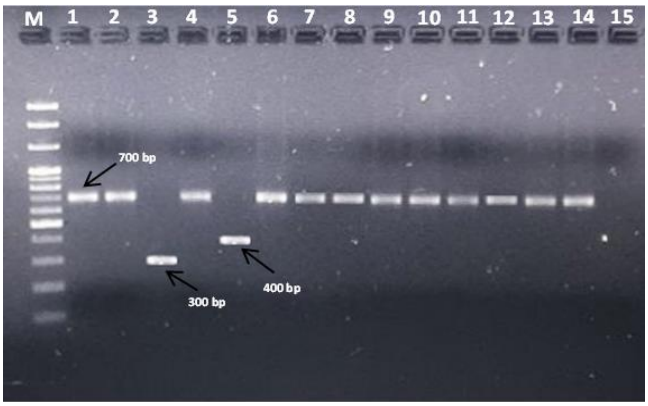

Figure 2: Amplification of AITSF and AITSR primers, M=marker, 1,2,4,6-14 positive to *T. conglense* (700bp) 3,5 positive to *T. vivax*. (300-400bp) 15 negative control.

Results

Polymerase Chain Reaction results showed 100% of infection using Kin1 and Kin2 primers, while the percentage of infection was 93.8% using AITSF and AITSR primers in the same samples (Table 2). The PCR recorded a high infection rate in sheep and goats using both pairs of primers and AITSF, AITSR showed two species of Trypanosoma in sheep and goats (Table 3). According to age, the high prevalence rate of infection in sheep and goats is more than 1-2 years old, while the animals recorded a lower infection prevalence for more than two years (Table 4). Females of sheep and goats recorded high prevalence rate of infection with trypanosoma than males (Table 5). The high prevalence rate of infection with T. congolense in imported sheep and goats when compared with native once (Table 6).

Table 2: Showed infection with *Trypanosoma* using two pairs of primers

Primer	No	No +ve	infection %
Kin1/Kin2	81	81	100
AITSF/AITSR	81	76	93.8

Discussion

Trypanosomiasis in animals is a significant problem to livestock development. Some rural areas of Africa affected by this disease. During the last few years, several researchers have detected many species in farm animals (10). In this study, the 81 samples detected using the routine microscopic method revealed that it could be positive using Kin1 and Kin2 primers, which can detect *the Trypanosoma* group, and 76 from 81 samples gave a

positive result using AITSF and AITSR primers by the PCR technique. This primer recorded two species of *Trypanosoma* in sheep and goats with a high infective rate in sheep as compared with goats. Konnai *et al.* (11) reported that 18.3% percentage of infection in livestock of trypanosomes by using KIN primers and, he studied these primers' ability to investigate and differentiate the *Trypanosoma* using a single PCR (12). In this study, only two spp of *Trypanosoma* were recorded in sheep, including *T. conglense* and *T. vivax*, so *T. conglense* is predominant. Several studies take several spp of *Trypanosoma* in small ruminants (13) showed that *T. vivax* was the most dominant *Trypanosoma* in animals with an infection rate reached 20.91 %. *T. simiae* was rarely detected, only two goats and one sheep. At the same time, Bourzat and Gouteux (14) showed *T. vivax* with a high infection rate with clinical signs in animals, the increased prevalence of *T. vivax* approved by Authié *et al.* (15), which may result from the virulent of *Trypanosoma*, which is low and better controlled by animals, or from the mechanical transmission which has not recorded in the other spp, except *T. congolense*. The lower prevalence of *T. congolense* "forest type" when compared with *T. vivax* in domestic animals approved by Sidibe *et al.* (16); Bengaly *et al.*, (17), which is due to the increased parasitemia in *T. congolense* with anemia which leads to the death of the animal. Another study showed that *T. vivax*, *T. brucei*, and *T. congolense* were absent in sheep but present in goats with low infection rates.

Table 3: Infection rate of *Trypanosoma* species in sheep and goats

Type	No	No +ve (%)						
		Trypanosoma	*T. congolense*	*T. vivax*	*T. bruci*	*T. simiae*	*T. godfrey*	AITSF/AITSR
Sheep	55	55 (67.9)	43 (56.6)	9 (11.8)	0 (0)	0 (0)	0 (0)	52 (68.4)
Goats	26	26 (32.1)	21 (27.6)	3 (4)	0 (0)	0 (0)	0 (0)	24 (31.6)

Table 4: Results of infection of *Trypanosoma* species in sheep and goats according to age

Age	Animals	No +ve (%)					
		Trypanosoma	*T. congolense*	*T. vivax*	*T. bruci*	*T. simiae*	*T. godfrey*
Less than one year	Sheep	10	7 (70%)	2 (20%)	0 (0)	0 (0)	0 (0)
	Goats	7	4 (57%)	2 (28%)	0 (0)	0 (0)	0 (0)
< 1-2 years	Sheep	38	32 (84.2%)	6 (15.7%)	0 (0)	0 (0)	0 (0)
	Goats	16	16 (100%)	0 (%)	0 (0)	0 (0)	0 (0)
> 2 years	Sheep	7	4 (57.14%)	1 (14.2%)	0 (0)	0 (0)	0 (0)
	Goats	3	1 (33.3%)	1 (33.3%)	0 (0)	0 (0)	0 (0)

Table 5: Results of infection rate of *Trypanosoma* species in sheep and goats according to gender

Gender	Animals	No +ve (%)					
		Trypanosoma	*T.congolense*	*T.vivax*	*T.bruci*	*T. simiae*	*T. godfrey*
Male	Sheep	19	13 (68.4%)	3 (15.7%)	0 (0%)	0 (0%)	0 (0%)
	Goats	9	6 (66.6%)	1 (11.1%)	0 (0%)	0 (0%)	0 (0%)
Female	Sheep	36	30 (83.3%)	6 (16.6%)	0 (0%)	0 (0%)	0 (0%)
	Goats	17	15 (88.2%)	2 (11.7%)	0 (0%)	0 (0%)	0 (0%)

Table 6: Results of infection of *Trypanosoma* species in sheep and goats according to the source of animals

Source	Animals	No +ve (%)					
		Trypanosoma	*T. congolense*	*T. vivax*	*T. bruci*	*T. simiae*	*T. godfrey*
Native	Sheep	20	14 (70%)	3 (15%)	0 (0%)	0 (0%)	0 (0%)
	Goats	10	7 (70%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)
Imported	Sheep	35	29 (82.8%)	6 (17.1%)	0 (0%)	0 (0%)	0 (0%)
	Goats	16	14 (87%)	2 (12.5%)	0 (0%)	0 (0%)	0 (0%)

Franco *et al.* (18) and Daniel *et al.* (19) showed that the *T. vivax*, *T. congolense*, and *T. brucei* cause the infection in domestic sheep and goats, and *T. vivax* is dominant. The cause of mechanical transmission or decrease a time of development cycle in the tsetse-fly. Wayo *et al.* (20) showed that the most dominant *spp* of *Trypanosomes* was *T. congolense* in ruminants, and it approved the role of small ruminants as reservoirs of parasites. The reason for

the difference between the results of the two pairs of primers could be the fact that sheep and goats may infect with other *spp* of Trypanosome (21), which records the infection of sheep with *T. evansi*.

Gael *et al.* (10) showed 5 *Trypanosoma spp* that infected goats (*T. vivax*, *T. simiae*, *T. simiae T. savo*, *T. congolense*, and *T. brucei*) and two spp that infected sheep (*T. simiae* and *T. theileri*) with increased trypanosome infection in both. Consequently, goats are more resistant to *Trypanosoma* than sheep, as Ng'ayo *et al.* (22) suggested. Sheep have a higher rate of infection when compared to goats and pigs. Findings show they are symmetrical with other studies that record that sheep are more infected than goats naturally (23). Another study showed that sheep were highly infected with *T. congolense* Kilifi and *T. brucei*, while pigs and goats were highly infected with *T. vivax*. (22), while Bedaso *et al.* (24) recorded 2 cases of *T. vivax* 1 sheep and one goat, rare of *T. brucei*, while Kiran and Idris (25) recorded a high prevalence rate among sheep was then the prevalence of the disease among goats.

Conclusions

Trypanosoma was affected by sheep and goats. *T. conglense* is more dominant than other species of these parasites in sheep and goats in Nineveh province.

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Conflict of interest

The authors declare no conflict of interest in the manuscript.

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التقصي الجزيئي عن أنواع المثقبيات في الضأن والماعز في مدينة الموصل

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الخلاصة

تم من خلال هذه الدراسة فحص عينات دم ٣٨٥ رأساً من الضأن والماعز من مختلف الأعمار والأجناس والمصدر والتي تم فحصها تحت الفحص المجهري الروتيني من خلال فحص المسحات الدموية (الرطبة، الخفيفة، السميكة، مسحة طبقة الخلايا اللمفية) وذلك للكشف عن طفيلي المثقبيات والتي أظهرت أن ٨١ عينة كانت موجبة، تم إخضاع هذه العينات الموجبة للكشف الجزيئي عن طفيلي المثقبيات والأنواع عن طريق استخلاص الحمض النووي الطفيلي ، ثم تم الكشف عن هذا الحمض النووي في العينات باستخدام بادئات KIN1 و KIN2 و AITSF و AITSR ثم تم تطبيق تفاعل السلسلة المتبلمرة التقليدي، وأظهرت النتائج أن ٨١ عينة أعطت تفاعلًا إيجابيًا باستخدام بادئات KIN1 و KIN2 بينما عند استخدام البادئات AITSF، AITSR كان عدد العينات الموجبة ٧٦. أظهرت النتائج إصابة عالية في الضأن عند مقارنتها بالماعز باستخدام زوج من البادئات فضلا عن انه تم الكشف الجزيئي عن نوعين من المثقبيات في الأغنام والماعز *T. conglense* و*T. vivax*. أظهرت الحيوانات التي يزيد عمرها عن ١-٢ سنوات نسبة مرتفعة للإصابة عند مقارنتها مع الفئات العمرية الأخرى، وسجلت الإناث نسبة عالية للإصابة عند مقارنتها بالذكور. وفقًا لمصدر الحيوانات، أظهرت الحيوانات المستوردة معدل إصابة مرتفعًا عند مقارنتها بالحيوانات المحلية. وتعد هذه الدراسة هي الأولى من نوعها للكشف عن أنواع المثقبيات في المجترات الصغيرة في مدينة الموصل.