Open Veterinary Journal, (2022), Vol. 12(5): 688–692
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)

Short Communication
DOI: *10.5455/OVJ.2022.v12.i5.13*

Submitted: 20/04/2022 Accepted: 14/08/2022 Published: 16/09/2022

Diagnostic study of trypanosomiasis of cats in Mosul, Iraq

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Abstract

Background: Trypanosomiasis is a zoonotic parasitic disease endemic in Iraq but with limited information about its occurrence in cats.

Aim: This study was designed to detect *Trypanosoma* spp. in cats using microscopic examination by Giemsa stain and conventional polymerase chain reaction (PCR) technique in Mosul, Iraq.

Methods: A total of 120 blood samples from cats were microscopically examined using Giemsa stain. Only 35 positive blood samples were examined by the conventional PCR technique. Hematological changes were also reported.

Results: The infection rate of *Trypanosoma* spp. was 34.2% (41 out of 120). Results of conventional PCR technique for the positive 35 blood samples indicated 31.4% as *Trypanosoma* spp. and 20% *Trypanosoma evansi*. This study showed that the infection in younger cats was significantly more than in older cats, with significant differences between females and males. Affected cats suffered from fever, dullness, pale mucous membranes, emaciation, muco-purulent ocular discharge, anorexia, incoordination, and anemia. Results of the blood picture indicated increase in total leukocyte count and decrease in hemoglobin concentration, packed cell volume, and total red blood cells.

Conclusion: *Trypanosoma* spp. and *T. evansi* infection in Mosul of Iraq is reported for the first time in cats, and younger cats were more affected than older cats.

Keywords: Trypanosomiasis, Cats, *Trypanosoma* spp, *Trypanosoma evansi*, PCR.

Introduction

Trypanosomiasis is considered as one of the most common and important diseases that affect human and many animals including cats (Panigrahi *et al.*, 2015). Flies such as *Stomaxys*, *Tabanus*, and *Triatomids* bugs spread the disease, and some recent resources showed that the disease can be transmitted by ingestion of dead animals infected recently with trypanosomosis (Nwoha, 2013). Clinical signs start to appear on affected cats after 2–3 weeks of flies' bite, initially with a local skin inflammatory reaction (Chancre) appearing at the site of biting. The size of Chancre is depending on several factors such as the immune status of the affected cat, virulence of *Trypanosoma* spp., and inoculation dose of *Trypanosoma* species. The parasites are dividing and multiplying inside Chancre and then enter the adjacent lymph nodes then the lymph vessels and finally the bloodstream (parasitemia) (Aloba *et al.*, 2022). Clinical signs are varies depending on the severity of the disease and *Trypanosoma* spp. which include anorexia, anemia, fever, enlarged superficial lymph nodes, conjunctivitis as well as edema of limbs (Zenad and Radhy, 2020). The disease has three forms; acute, subacute, and chronic, where the acute form is highly fatal. The disease is common in cats as they are more exposed to vectors (Solikhah, 2021).

There are many techniques used for the diagnosis of *Trypanosoma* spp. including wet smear. The ideal way to diagnose *Trypanosoma* parasite in cats is using a thin blood smear stained with one of Romanowsky stains as Giemsa stain and examined under microscope (magnification of 1,000×) (Solikhah *et al.*, 2019). In addition, for accurate diagnosis, polymerase chain reaction (PCR) technique is considered as a very good procedure for the diagnosis of trypanosomiasis (Njiru *et al.*, 2005).

The current study was aimed to provide information about the trypanosomiasis of cats in Mosul city of Iraq using microscopical examination and molecular methods.

Material and Methods

Animals and samples collection

A total number of 120 blood samples were collected from the cephalic vein of young cats (≤1 year) and old cats (>1 year), 53 males and 67 females, from different regions in Mosul city of Iraq. All blood sample were microscopically examined using blood smears stained with Giemsa (Soulsby, 1986; Konnai *et al.*, 2009; Prasad *et al.*, 2015; Salvioni *et al.*, 2021). Positive samples were stored at −20°C until PCR tested. Clinical signs were recorded for each cat.

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Molecular examination

Genomic DNAs from 35 positive blood samples were extracted by using column pure blood genomics DNA mini kits (Alanazi, 2018). Applied biological materials by using conventional PCR technique was performed for the detection of *Trypanosoma* spp. and *Trypanosoma evansi* (Table 1).

Hematological examination

Hematological examination was used for hemoglobin concentration (HB), packed cell volume (PCV), total red blood cells (TRBC), MCV, MCHC, and total leukocyte count (TLC). Parasitemia were calculated according to Da Silva *et al*. (2010) and Al-thuwaini (2021).

Statistical analysis

Statistical analysis done by using chi-square test in IBM-SPSS statistics version 19 program (Leech *et al.*, 2007).

Ethical approval

The ethical approval was issued by the Institutional Animal Care and Use Committee (UM.VET.2021.16) of the Faculty of Veterinary Medicine, University of Mosul on the 2nd of September 2021.

Results and Discussion

The microscopic examination of blood smears stained with Giemsa in this study showed that the infection rate of *Trypanosoma* spp. was 34.2% (41 out of 120 cats). The infection rate was significantly ($p < 0.05$) higher in males (49.1%) compared with that in females (22.4%) (Table 2). The highest rate of infection was reported in younger cats (≤1 years). Statistical analysis showed significant differences between ages (Table 3). All 35 microscopically positive samples were examined by PCR technique and showed that the infection rate of *Trypanosoma* spp. was 31.4% (11 out of 35 cats), and with *T. evansi* was 20% (7 out of 35 cats). Positive bands were at 480 bp for *Trypanosoma* spp. and 151 bp for *T. evansi* (Figs. 1 and 2).

The infected cats with *Trypanosoma* spp. were suffering from pale mucous membranes, fever, dullness, anorexia, emaciation, muco-purulent ocular discharge, incoordination and anemia (Fig. 3). In addition, there was decrease in TRBC, HB, PCV, with a macrocytic hypochromic anemia type and increase in TLC due to increase of lymphocytes, eosinophils, basophils, monocytes and increase in parasitemia (Table 4).

The infectious rate of *Trypanosoma* infection in the current study was higher than that reported in other studies such as in Southern Louisiana and US with infection rates of 7.3% and 11.4%, respectively (Zecca *et al.*, 2020; Eric *et al.*, 2021). The difference of the infection rate of *Trypanosoma* spp. might be due to different efficacy of the control programs and sensitivity of diagnostic tests (Alanazi, 2018). The results of molecular methods are more sensitive and many researchers revealed *Trypanosoma* spp. using the PCR technique (Faraj *et al.*, 2015; Eric *et al.*, 2021).

Table 1. PCR protocol used to detect *Trypanosoma* spp. and *T. evansi* in DNA samples.

Target gene	*Primer sequences 5'-3'*	*Size of product/bp*	*Initial denaturation °C/ minutes.*	*Amplification (40cycle)*			*Final extension °C/ minutes.*	*Product Co.*
				Denaturation °C/seconds.	*Annealing °C/ seconds.*	*Extension °C/ seconds.*		
ITS-1 *gene*	*F(5'CCGGAAGTTCACCGATATTG-3')* *R(5'TGCTGCGTTCTTCAACGAA-3 ')*	*480*	*95/2*	*95/30*	*58/30*	*72/60*	*72/5*	*Bioingentech*
RoTat 1.2 VSG	*F(5' CTGAAGAGGTTGGAAATGGAGAAG-3')* *R(5' GTTTCGGTGGTTCTGTTGTTGTTA-3')*	*151*	*95/2*	*95/30*	*58/30*	*72/60*	*72/5*	*Bioingentech*

(ITS gene): specific for genus *Trypanosoma* (Njiru *et al.*, 2005); (Rotat VSG gene): specific for *Trypanosoma evansi* (Konnai *et al.*, 2009).

Table 2. The infections rate of *Trypanosome* spp. according to the sex.

Sex	No. examined cats	No. infected cats	Infectious rate%
Male	53	26	49.1*
Female	67	15	22.4*
Total	120	41	34.2

(*): Significant value ($p < 0.05$).

Table 3. Infections rate with *Trypanosoma* spp according to the age.

Age	No. examined cats	No. infected cats	Infection rate%
≤1 year	75	34	45.3*
>1 year	45	7	15.6*
Total	120	41	34.2

*Significant value ($p < 0.05$).

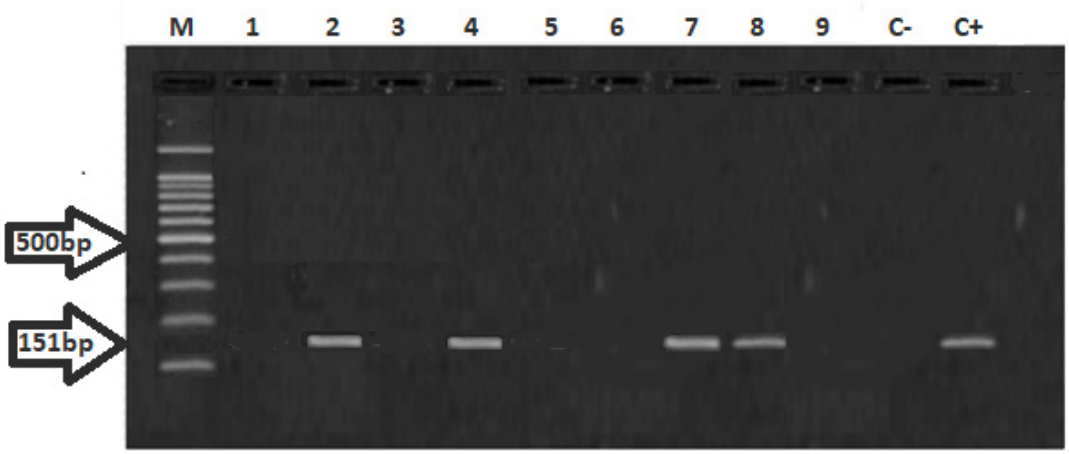

Fig. 1. Gel electrophoresis by using (ITS1) showing M: molecular size marker 100–1,200 base pair. Lane C+: *Trypanosoma* sp. DNA positive control. Lane C−: negative PCR control. Lanes 1–4: template DNA of *Trypanosoma* sp. at 480 bp isolated from cats.

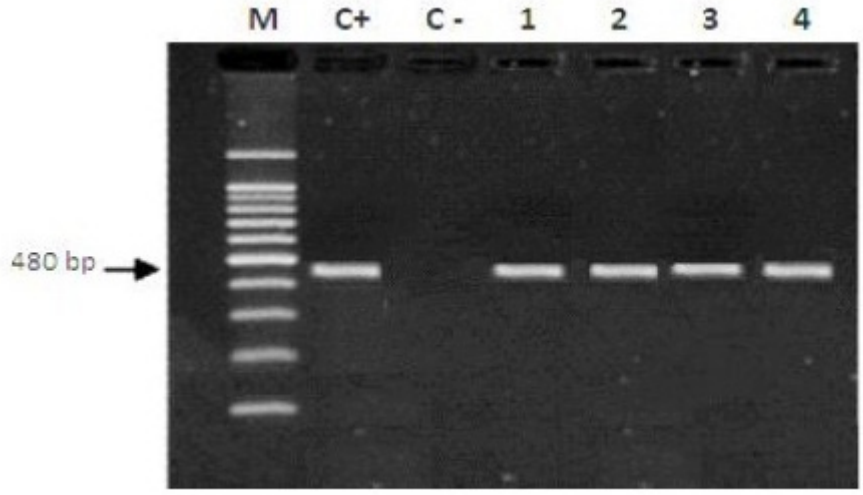

Fig. 2. Gel electrophoresis using RoTat VSG of *T. evansi*. M: molecular size marker 100–1,200 base pair. Lane C+: *T. evansi* DNA positive control. Lane C −: negative PCR control. Lanes 2,4,7,8: template DNA of *T. evansi* isolated from cats at 151 bp. Lanes 1,3,5,6,9: negative DNA samples.

Fig. 3. Cat suffers from some clinical trypanosomiasis such as pale mucous membranes, fever, dullness, anorexia, emaciation, muco-purulent ocular discharge, incoordination, and anemia.

Table 4. Hematologic parameters of control and infected cats with trypanosomiasis in 10 cats.

Parameters	Control cats (mean ± SE)	Infected cats (mean ± SE)
TRBc($_{x}10^{6}$/microliter)	6.0 ± 0.46	4.20 ± 0.40
HB (g/dl)	10.07 ± 0.77	8.55 ± 0.35
PCV (%)	34.80 ± 1.00	27.40 ± 0.69
MCV/fl	57.35 ± 2.11	54.78 ± 2.40
MCHC (g/dl)	34.44 ± 1.56	30.09 ± 1.36
Parasitemia (%)	---------	13.57 ± 5.91
TLC ($_{x}10^{3}$/microliter)	9.10 ± 0.55	7.85 ± 0.85
Neutrophils (%)	65.78 ± 0.18	58.50 ± 0.26
Lymphocyte (%)	25.35 ± 0.13	30.15 ± 0.33
Eosinophil (%)	5.11 ± 0.05	7.70 ± 0.03
Basophils (%)	0.06 ± 0.03	0.09 ± 0.02
Monocytes (%)	4.45 ± 0.3 5	4.68 ± 1.85

On the other hand, the infection rate was lower in older ages as that could be due to the older animals may produce better immunity against the parasite infection (Eric *et al.*, 2021).

The current study showed that there was an increase in the infection rate in males than females. These results are similar to the results of Faraj *et al*. (2015) and in contrast with Lauricella *et al*. (2010) who reported that both males and females were affected in similar rates.

The clinical signs observed in infected cats were similar to other studies (Gurtler *et al.*, 2007). Change in blood values were observed in affected cats compared to control cats. There was a decrease in TRBC, HB concentration and PCV that causes anemia, similar to others studies done on cats (Da silva *et al.*, 2009). The increase in WBCs was due to increase in lymphocytes, eosinophils, basophils, and monocytes, and decrease of neutrophils was reported, as reported in other studies (Al-Badrani, 2012; Marwa and Alobaidii, 2022). The reason for this decrease could be a result of secondary bacterial infection (Da silva *et al.*, 2009). However, Chaudhary and Iqbal (2000) found increase in the levels of neutrophils in camels infected with *T. evansi.*

Author contributions

NHM and MAA: designed, photographed, and supervised this study. NHM and DAM: collected laboratory samples, conducted the practical part of the study and assisted in data analysis. NHM and MAA:

contributed to the drafting of the manuscript. All authors contributed to the conduct of the study and discussed the results to a satisfactory scientific conclusion.

Acknowledgments

The authors would like to thank the University of Mosul, College of Veterinary Medicine.

Conflict of interest

The authors declare that there is no conflict of interest.

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