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ORIGINAL ARTICLE

Diagnosis of Toxocara sp. from Lions by PCR technique

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ABSTRACT
This study was conducted on Asiatic lions *Panthera leoleo* (Linnaues, 1758) kept in Al-Zawraa park in Baghdad city the capital of Iraq. The fecal samples of 28 Asiatic lions (4 male, 7 female and 17 cubs) were collected for three stages pre-treatment , during treatment and post treatment that programmed with anthelminthic therapy (Overmix one tablet per 10 kg) for eight months from March to October 2017.Morphological examination of the eggs and worms was described and delineated as *Toxocara canis* and *Toxocaris leonina* after study of differential characteristic between them. DNA extraction were applied for adult worms by using Organic extraction depending on chemical material. Polymeriaze chain reaction were performed to confirm the diagnosis of species of *Toxocara* by using specific primers for *T. canis*. The target gene was ITS-2 its number from NCBI was AB110034. PCR technique produce 380bp of fragment of target gene ITS-2 gen confirming *Toxocara canis* in lions.
Keywords: Lion, *Toxocara canis*, *T. leonina*, molecular, organic method.

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INTRODUCTION

There are scanty in the survey of parasites in the lions in all the world, however few reported for groups of wild animals as Bengal tigers, jaguars, pumas, lynx and lions from various zoological gardens from different part of world from time to time [9]. Opara *et al*., [10] revealed that lion infected with Ascaris sp. 50% in The Zoological Garden, Nekede Owerri, Southeast Nigeria, but they not delineated the species of Ascaris. Okulewicz *et al*., [8] they reviewed the infection of wild animals with *Toxocara canis*, *T. cati* and *Toxocaris leonina*.In Iraq, our country there aren't find any survey about lions except one study of protozoa parasites in the fecal samples of different animals domestic and wild in Al-Zawraa zoo [11]). So, the current study considered the first time in Iraq.

The aims of the current study are: first- determination the infection of *Toxocara* sp. in lions in three stages pre-treatment, during treatment and post treatment that programmed with anthelminthic therapy .Second-classification study between *Toxocara* sp. and *Toxocaris leonina* Third- Molecular study to confirm the species of *Toxocara*.

MATERIALS AND METHODS

This study was conducted during March to October 2017 on Asiatic lions *Panthera leoleo* (Linnaues, 1758) kept in Al-Zawraa park in Baghdad city the capital of Iraq.

Collection of samples

The fecal samples of 28 Asiatic lions (4 male, 7 female and 17 cubs) were collected for three stages pre-treatment , during treatment and post treatment that programmed with anthelminthic therapy (Albendazole 10mg/ kg + Praziquantel 2mg/kg).The dose is repeated every 10 days during treatment and then returned every three months post treatment

these three stages schedule for eight months from March to October 2017. All the samples were transferred to the workplace Iraq Natural History Research Center and Museum/University of Baghdad.

Flotation method was applied for all fecal samples as Thienpont *et al*., [13] by using sheather's solution was prepared by Tom [14]. Individual parasites were repeatedly washed in physiologicalsaline, ph 7.3 and put it in alcohol 70%(figure1). Many of worms (male & female) were put in Formalin Alcohol Azocarmine Lactophenol (FAAL) [1] for morphologic study. Morphological examination of the worms was described according to Bowman [2].

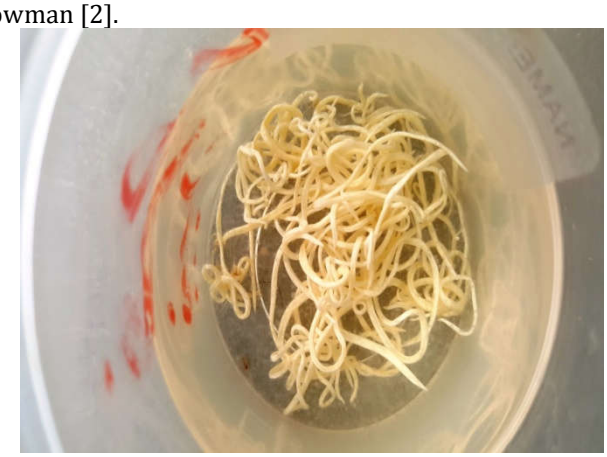

Figure 1: Adult worms of *Toxocarasp* .

DNA extraction

About one centimeter tissues of adult worms were sent to the molecular laboratory for DNA extraction and PCR technique. Two methods of DNA extraction were applied , first one by using gSYNC DNA Extraction Kit (Geneaid) for 8 samples. Second one by using Organic extraction depending on chemical material of 5 samples of adult worm tissues.

Chemical material

1-Stain buffer extraction.
2-Protenase K (10ml/ml).
3-Phenol-chloroform isoamyl alcohol
4-Saturated ethanol
5-70% ethanol.
6-TE buffer

Genotyping:

Polymeriaze chain reaction were performed to confirm the diagnosis of species of *Toxocara* by using specific primers for *T. canis*. The target gene was ITS-2 its number from NCBI was AB110034 (Khademvatan et al., 2013), the sequences of these primers have been listed as bellow:
(F): 5′-AGTATGATGGGCGCGCCAAT-3′ and (R): 5′-TAGTTTCTTTTCCTCCGCT-3′ [4]. Primers from ALPHA Canadian company.

RESULTS AND DISCUSSION

A total of 28 fecal samples were collected from lions once per week, that mean about 896 samples per eight months. The result of first week was 100% positive for *Toxocara* sp. infection, that encourage to start with treatment programmed anihelminths. This high prevalence of infection can be explained by the favorable climatic conditions, which support prolonged survival of the eggs in the environment; in addition of the feeding management and improper disposal of feces. This result is similar to Khatun *et al* [6] who revealed to Indian Lion was infected with *Toxascarisleonina*100%, in Rangpur Recreational Garden and Zoo in Bangladesh. However, Virendra *et al*. [16] revealed to *Toxocara* sp. in lions in Nandan Van Zoo, Raipur without delineated the species may be the difficult of diagnosis the species.
The current study recorded two species of Ascarides in Asiatic lions *Panthera leoleo* that was lineated as *Toxocara* sp. and *Toxocaris leonina* by morphological study and then molecular study confirmed the specie of *Toxocara* was *T. canis*. These findings of the presence of two parasites in lions were similar to Okulewicz *et al.,* [9] who suggest that these parasite species do not have genetic barriers preventing them from settlement in different definitive hosts and thus have increased chance of survival.

Morphological study

Classification of species of Ascarides was lineated as *Toxocara* sp. and *Toxocarisleonina* according to differentiated characteristics like shape of the head, the cervical alae, the esophagus, the tail and the egg, in table 1. Approximately 90 % of eggs measured were of similar size, so the measurements of eggs not benefit in the differentiation of *Toxocara* sp. [15].

Table.1. Identification characters between *Toxocara* sp. and *Toxocaris leonina*in lion.

organ	*Toxocara sp.*	*Toxocaris leonina*
The head	resembles an arrowhead	resembles a spear
The cervical alae	elliptical and broad fig.2	longer and considerably narrower fig.3
The esophagus	It has ventriculus that intercalated between the esophagus and the intestine fig.4	No ventriculus, the esophagus connects to the intestine directly fig.5
The tail	The tail of the male is fingerlike projection fig.6	The tail of the male conical tapers gradually without fingerlike projection fig.7
The egg	Spherical shape , thick, rough, pitted shell, dark brown to black granular contents, un segmented and usually occupying the whole of the shell. Medium sized about 30X30.5 mm. fig.8	Oval, thick,smooth and colorless shell, yellowish brown and granular contents, un segmented and occupying only part of the shell. Medium sized about 40.5 X 40.9 mm.fig.9

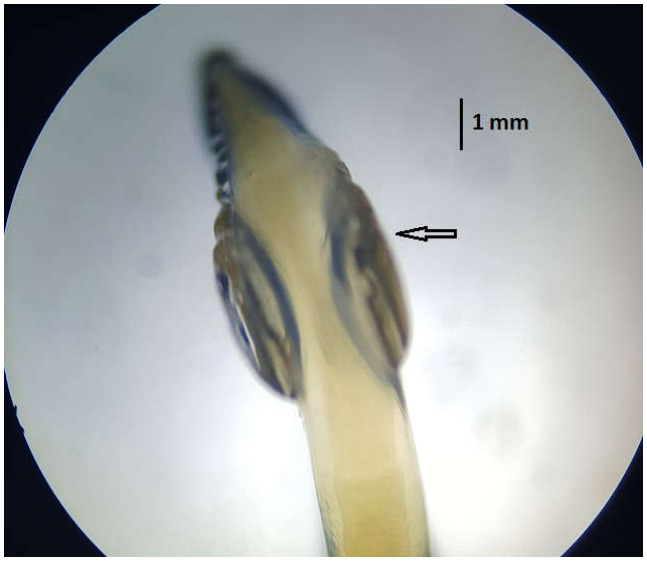

Figure 2:Anterior end appeared the head and the cervical ala of *Toxocara canis*.

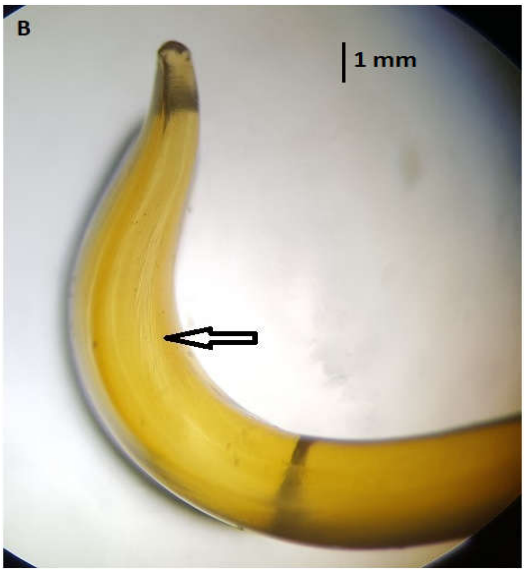

Figure 3: Anterior end appearedthe head and the cervical alae in A. male and B. female of *Toxocaris leonina*.

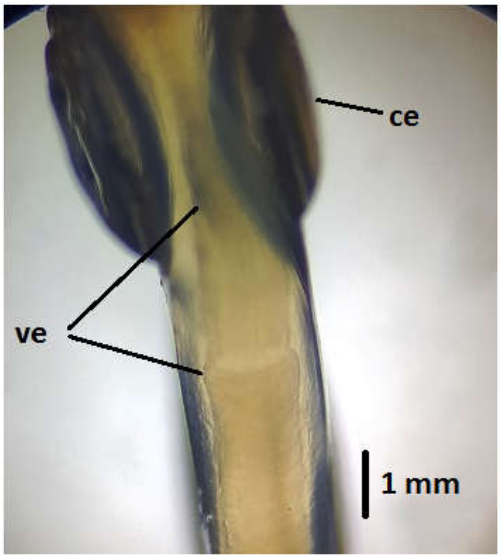

Figure 4:*Toxocaracanis* has ventriculus that intercalated between the esophagus and the intestine, ce=cervical alae, ve=ventriculus.

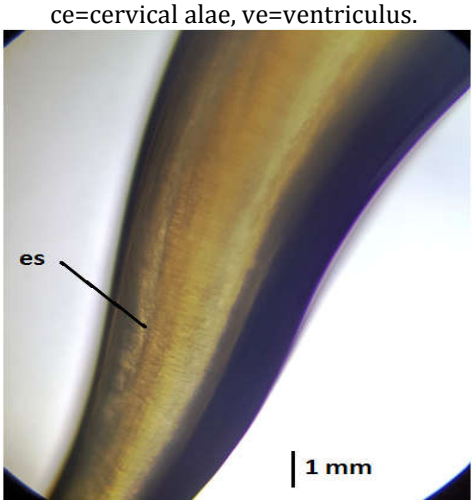

Figure 5:The esophagus connects to the intestine directly without a ventriculus in *Toxocaris leonina*.

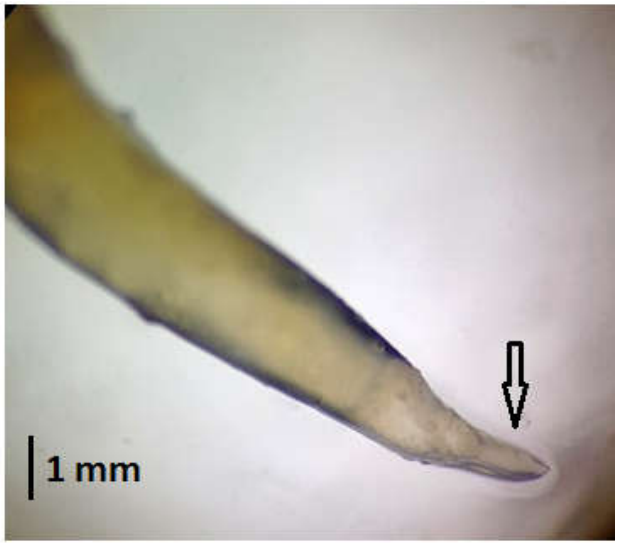

Figure 6: The tail end of the male of *Toxocara canis* has finger like projection.

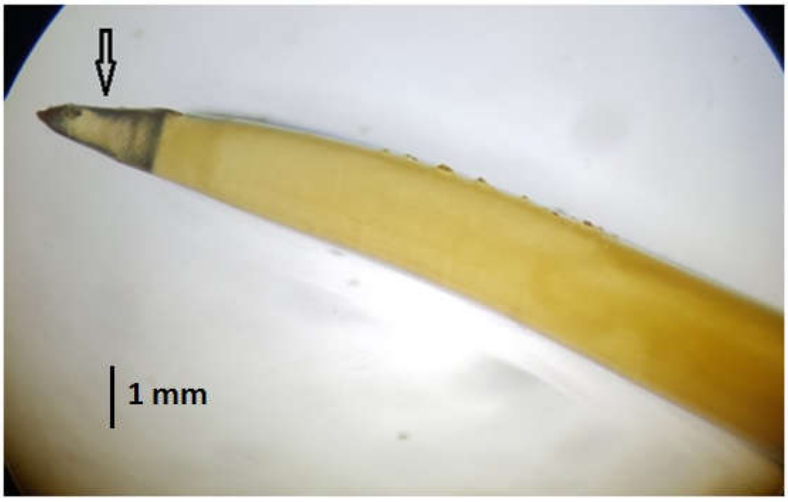

Figure 7:The tail of the male of *T. leonina* tapers gradually without fingerlike projection.

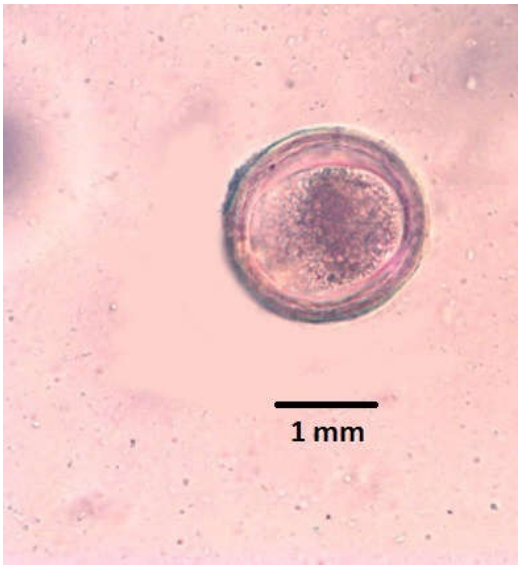

Figure 8: Egg of *Toxocaracanis* in lion.

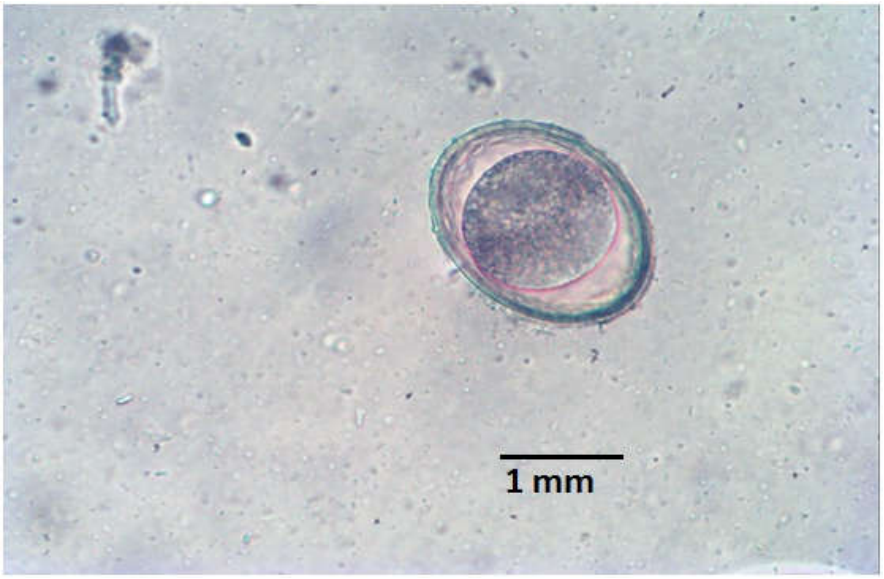

Figure 9: Egg of *Toxocaris leonina* in lion.

Molecular study

The result of first method of DNA extraction by using gSYNC DNA Extraction Kit (Geneaid)was negative, that contrast with Hadi and Kawan [3]. This resultmay be due to the lack of validity of the kit.

The result of second one by using Organic extraction depending on chemical material was positive (figure 10) which obtained 100ng a pure DNA that purity was 1.6 for PCR amplification. This finding was similar to Sambrook [12] who revealed to the purity range of DNA was between 1.6 - 2.

The PCR was applied to amplify the targeted fragment which was specified by using specific primers that were designed manually in the *T. canis* specific part of ITS2 Sequences. The result of fragment size amplified was 380 bp in ITS2 gene in figure 11. This result was similar to Khademvatan *et al.,* [5] who revealed that the positive control of *T. cani s*consist of 380 bp. However, Moudgil *et al.,* [7] confirmed that Asiatic lions (*Panthera leopersica*) kept at Zoological Park, Chhatbir, Punjab, India were infected only with *Toxocaris leonina*.

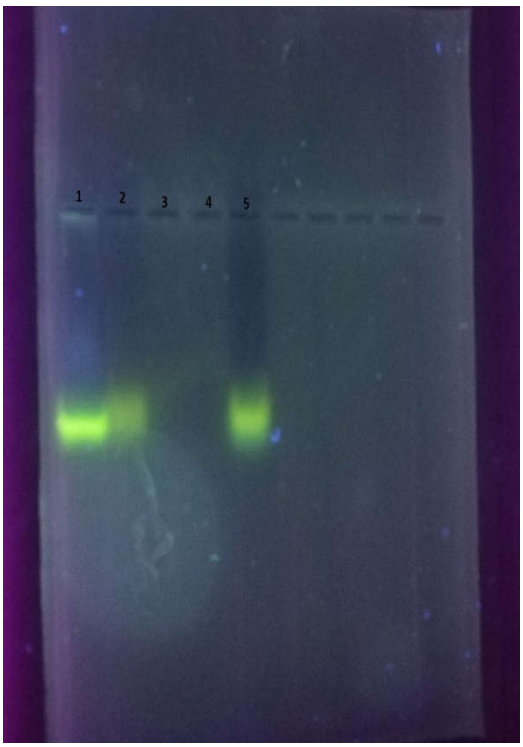

Figure 10:DNA extraction from adult worm of *Toxocara sp.* by Organic method.

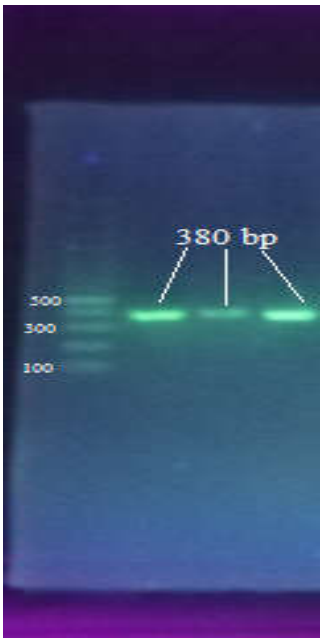

Figure 11:PCR technique produce 380bp of fragment of target gene ITS-2 gen confirming *Toxocara canis* in lions.

CONCLUSION

It may be concluded from the current study that Asiatic lions *Panthera leoleo* (Linnaues, 1758) which kept in Al-Zawraa park in Baghdad city/Iraq were infected with *Toxocara canis* and *Toxocaris leonina.* These findings were delineated by morphological and molecular studies.

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