Isolation and identification of some parasites that transmitted by Earthworms

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Abstract

Total of (106) specimens of Earthworms were collected from Baghdad area examined for different species of parasites since of they act as intermediate or reservoir hosts. There are one or more species of the following parasites Two nematodes *Ascaridia* sp., *Toxcara* sp.; plants nematodes *Heterodera* sp. and oocyst of protozoa *Monocystis* sp. Were recovered from body cavity; the total rate of infection was 20.7% for the first time in Iraq. The result showed that sedimentation method the best method for isolation the parasites, *Ascaridia* sp. is most prevalent parasite in these infestations.

عزل وتشخيص بعض الطفيليات التي تنقلها ديدان الأرض

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الخلاصة

تم جمع وفحص 106 عينة من ديدان الأرض من منطقة بغداد للتحري عن وجود الطفيليات وقد وجدت بيوض اثنين من الديدان الاسطوانية هما *Ascaridia sp., Toxocara* sp. وبيوض الديدان الخيطية النباتية *Heterodera* sp. وأكياس الاوالي *Monocystis* sp. وذلك في التجويف الجسمي لديدان الأرض وبنسبة إجمالية 20.7 % وتم وصف بيوض الطفيليات ورسمها وتصويرها. وتعد التسجيل الأول للطفيليات المعزولة من ديدان الأرض. أظهرت النتائج إن أفضل طريقة للعزل هي طريقة الترسيب وان الديدان الخيطية *Ascaridi* sp. هي الأكثر وجوداً بين بقية الطفيليات.

Introduction

Earthworms– also called angleworm there are more than 1,800 species of terrestrial worms of the class Oligochaeta – Phylum Annelida, in particular, members of the genus Lumbricus. Earthworms occurs in virtually all soils of the world in which the moisture and organic content are sufficient to sustain them (1).

The earthworm body is divided into ring- like segments, as many as 150 in L. Terrestris. Some internal organs, including the excretory organs, are duplicated in each segment. Between segments 32 and 37 is the clitellum, a slightly bulged, discolored organ that produces a cocoon for enclosing the earth worm s eggs. The body is tapered at both ends, with the tail end the blunter of the two (2).

Earthworms cannot see or hear, but they are sensitive to both light and vibrations. Their food consists of decaying organisms; as they eat, however, earthworms also ingest large amounts of soil, sand, and tiny pebbles. It has been estimated that an earthworm

ingest and discards its own weight in food and soil every day. This study pointed to earthworms as intermediate host or reservoir host for parasites in Baghdad.

Materials and Methods

- **Sample collection:** 106 specimens of earthworms were obtained from different regions of Baghdad; Al – Tarmia, Al- Rashdia and Al. Shaab. All specimens were obtained during the months of April to November 2008.
- **Parasite Isolation and Identification:** Methods used for isolation and identification of parasites are currently recommended by (3). Briefly, each specimen was examined as follow:

A. External examination: The specimens of earthworms were killed by few drops of Formalin 10% and were examined directly under disecting microscope to identify the external parasites.

B. Internal examination: The specimens of earthworms were incised and all internal content were deposit to the following examination:

1. Direct smear, by 1-2 drop of saline; Helminthes eggs can be detected and usually identified under low power magnification (L.P.M.,10X) but high power magnification (H.P.M.,40X) may aid in identification.
2. Concentration methods: The specimens of earthworms were cut into small pieces 1-2 gm and divided into two parts: one part for floatation, second part for sedimentation methods.

- Floatation method: used saturated salt (Nacl) (3).
- Sedimentation method: Mix 1 part of earthworm with 2 parts of tap water into Wassermann tube. Centrifuge for 1 min. at 400 rpm. and allow the tube to come to rest without interference. After 1 to 2 mins. using a bacteriologic loop, transfer several loop – full of material from the surface film to a clean slide. Add 1 drop of carmine stain and mix, and cover with a cover slip. Examine under low power magnification (10x).

Results

Out of 106 specimens of earthworms examined, the rate of infection was 22 (20.7%) with one or more species of *Ascaridia* sp., *Toxacara* sp., *Heterodera* sp., *Monocystis* sp. were found in the body cavity. Table 1. Results show that common species among the four parasites is the nematode *Ascaridia* sp. since it is found in the 12 out of 22 infected worms; Brief definition of each parasite will be given below:

- *Ascaridia* sp.: Eggs are about 53 microns in length, 40 microns in width, ellipsoid, with smooth, thick, 3- layer shell. The middle layer is most developed. Unsegmented contents. They are not embryonated, colorless with carmine stain.
- *Toxocara* sp.:

A. Eggs are about 65microns in length, 75 microns in width spherical with thick rough, pitted shell (Fig.1,2) granular contents, unsegmented and occupying the whole of the shel.

B. Encysted larvae is about 900 microns in length, 45microns in width. (Fig.1) rounded anterior and pointed posterior, transparent, not colored.

- *Heterodera* sp.: Eggs are about 32microns in length, 14 microns in width. ovoidal, longer with rounded ends, larvae is embedded init, colorless with transparent shell, colorless with carmine stain (Fig.3).
- *Monocysitis* sp.: Oocysts are about 53 microns X 48 microns are semi – oval, with weak shell, central nucleus, pink colored with carmine stain (Fig.4).

Table (1) Percentage of infection of parasites species

Parasite species	No. of infected earthworms	% of total earthworms infection
***Ascaridia* sp.(egg)**	12	11.3
***Toxocara* sp. (egg)**	6	5.6
***Heterodera* sp. (larvae inside egg)**	2	1.8
***Monocystis* sp. (oocyst)**	2	1.8

Table (2) Results of methods identification of parasites

Methods	Results
External examination	-
Direct smear	-
Floatation method	-
Sedimentation method	+

Fig. (1) *Toxocara sp.* egg and larvae embedded in body cavity, 10x

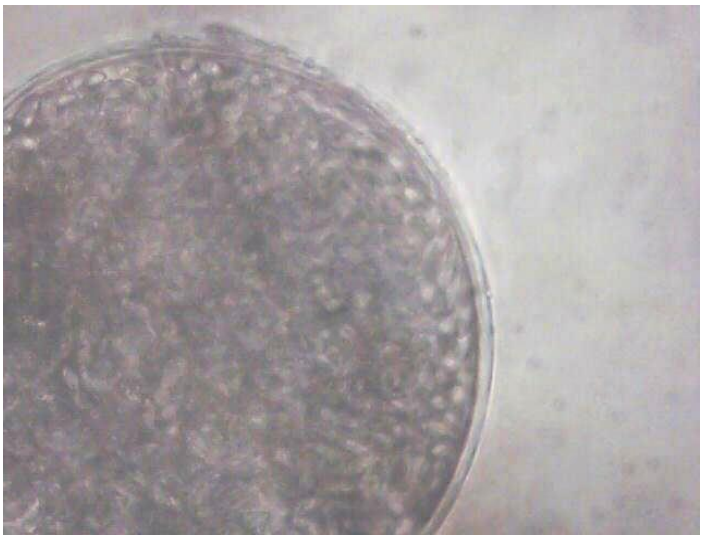

Fig. (2) Egg of *Toxocara sp.* border of membranes are cleared.40x

Fig. (3) Larvae inside egg of *Heterodera* sp. 40x

Fig. (4) Oocyst of *Monocystis* sp. 40x

Discussion

Many of previous authors are refered that earthworms are intermediate host or transmitted host or reservoir host for many species of parasites (4); (5) (6);. All of the four parasites are new records from earthworms in Iraq.

Eggs of *Ascaridia* sp. are found in earthworms cavity that mean *Ascaridia* sp. are in soil after contaminated with the feces of birds and earthworms play role in transmission of this parasites. This nematode is common distributed in Iraq especially in rural regions (as study region) at Laying chickens (7). So this *Ascaridia* is recorded the highest rate 11.3% among the four parasites.

Toxocara sp. are referred to as the cat, dog, fox and other wild carnivores nematodes. Eggs of *Toxocara* sp. may be *T. cati* because this species, relatively large roundworms, may infest their primary hosts, domestic cats, in urban settings through soil and feces contaminated with nematode ova, which ingestion by earthworms with the soil and then transmitted to another host (cat) who ingestion earthworms; that concordant with (4) which referred to *T. cati* transmitted by small rodents, beetles, earthworms but *T. leonina* and *T. canis* are transmitted by small rodents only (4).

The same is true if a cat eats an intermediate host such as earthworms which has encysted larvae (Fig.1), the migration of the larvae is similar to that of ingesting infective eggs. Larvae are released from the transfer host when it is eaten and digested.

The nematodes of plant *Heterodera* sp. Are much like the familiar Ascarid worms in vertebrate digestive tracts but they are much smaller.

In general, the female worm lays eggs which hatch either in the soil or in the host plant. If the host plants are not available, the eggs frequently will not hatch but will remain alive for a surprisingly a long time (5). It is probably true that earthworms ingest these eggs from soil.

The genus *Monocystis* sp. Is well known because various species are easily found in earthworms and in other Oligochaetes. In California (5) recorded *Monocystis lumbrici* from the seminal vesicle of the common earthworms. On the other hand, the sedimentation method is the best method to isolation the parasites and others are useless for identification that may be cleared the parasites (eggs and larvae) are embedding in body cavity of earthworms.

The spread of natural populations of parasites is a specialized aspect of biogeography of particular consequence for the parasitologist.

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