Experimental infection of *Anisakid sp.* Larvae, (Nematoda, Ascaridoidea, Anisakidae) in dogs

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ABSTRACT

A total of 900 fish belonging to *Atherina* sp. (common sand smelt) were examined for the presence of anisakid larvae. The detected larvae were collected from the fish and four puppies were infected experimentally. Puppies were sacrificed when began to shed eggs in their feces. The larvae, eggs and adult worms were described. Histopathological changes to the larval migration in different organs were studied.

INTRODUCTION

Anisakidae are of parasitic nematodes infecting a wide range of marine mammals, of fish species and some types of birds with a worldwide distribution ***(1-5).***

The most important, anisakid larvae are Anisakis, Contracaecum and Pseudoterranova which can infect or transmit to other frequently accidental hosts ***(6,7)***. Larval anisakid nematodes have been reported from many freshwater and marine fishes ***(2,4,8,9)***.

The third stage larvae are usually encapsulated in the viscera of fish. Larvae of the genus Contracaecum were found in four species of fish *(Acanthopagrus butcheri, Sillaginodes punctata, Mugil cephalus and Aldrichetta forsteri)* ***(10,11).*** The adults and fourth stage larvae of nematodes sp. of the genus Contracaecum, (Ascaridoidae, Anisakidae) are found in the digestive tracts of seals, some dolphins and piscivarous birds ***(10)***.

More species of Contracaecum were reported and differentiated to parasitize Argentinian birds; *Contracaecum microcephalum* (osculatum) ***(12)***, *Contracaecum travassosi* ***(13)***, *Contracaecum longicaecum* ***(14)***, *Contracaecum spheniscus* ***(15)*** and *Contracaecum philomultipapillatum* ***(16,17)***.

In this study, trials were done to inoculate *Anisakid sp.* larvae experimentally to dogs and completed life cycle. Also histopathological responses of migration of the larval stages in different organs were studied.

MATERIAL AND METHODS

A total of 900 fish specimens belonging to Atherina sp. (common sand smelt) (Fig. 1) were collected from Zagazig fish markets between December 2007 to May 2008. Fish specimens are transferred to the laboratory in plastic bags containing ice pieces for examination. The muscles and viscera of fishes were digested by artificial digestive fluid at a rate of 1 part to 20 part of fluid. The whole mixture was sieved after digestion and the supernatant fluid poured off and the sediment washed three times using saline solution. The sediment was put in Perti-dish and examined for the presence of anisakid larvae. Some of the detected larvae were cleared in Lactophenol and permanently mounted in polyvol then, left to dry in hot air oven at 40°C for 24 hours and examined microscopically for identification. Some viable larvae were used for experimental infection of puppies.

Four puppies of one month old free from internal parasites were proved to be experimentally infected by 10 ml saline solution containing viable anisakid larvae using a stomach tube ***(18)***. After one week of infection, daily fecal samples from each infected puppy were examined by direct and simple sedimentation technique.

Puppies which began to shed eggs in their feces were sacrificed and the small intestine (duodenum, jejunum and ileum) was opened each part in separate Petri-dish containing small amount of saline solution. The intestinal contents, its scrabing and intestinal wall were examined carefully for the presence of adult worms *(19)*. The worms were collected and washed several times with normal saline and left in the fridge at 4°C for 24 hours for completely relaxed. The collected nematode were cleared in lactophenol and permanently mounted in polyvol, then, left to dry in hot air oven at 40°C for 24 hours then, examined under low power microscope *(20)*.

Pieces under lower power microscope from the small intestine, liver lung, and kidney of infected puppies were fixed in 10% neutral buffered formalin solution and processed for histopathological examination. 5µ thickness paraffin sections were prepared and stained by H&E stain *(21)*.

RESULTS

One hundred and fifteen fish (12.7%) out of 900 examined common sand smelt (*Atherina sp.*) were infected by the third stage larvae of *Anisakid sp*.

I- The morphological descriptions

A-Third stage larva of *Anisakid sp*. (of *Contracaecum rudolphi*) (Fig. 2,3 and 4).

It has a serrated cuticle which is clear at the posterior part of the body. It has three lips, one short dorsal and two large ventrolateral. A cuticular triangular boring tooth is present on the surface of the labium ventrolateral to the mouth. Excretory pore is situated just posterior to the boring tooth. The cuticle of the ventrolateral lips extends behind a bulb in anterolateral region forming triangular like projections. The esophagus is long and slender. Ventriculus extends posterior beside the caecum. Intestinal caecum is present and anteriorly directed. Genital organs are not developed.

Body length was 12.3 - 19.7mm. Width at ventriculus was 0.28 – 0.41mm. Length of the esophagus was 0.6 -0.8mm. Nerve ring was 0.2- 0.3mm from anterior. Length of ventricular appendix was 0.3 -0.5mm. Width of ventricular appendix was 0.06 – 0.09mm. Length of intestinal caecum was 1.6 -2.5mm. Width of intestinal caecum was 0.14 -0.27mm. Anal opening was 0.15 – 0.42mm from the posterior end.

B-Eggs; detected from feces of two puppies on 32 days post infection measured 40 -42µ and it had smooth and thin translucent shells (Fig 5).

C-Adult female of *(Contracaecum rudolphi)* was observed in the intestine of sacrificed puppies (Fig. 6&7). It had three hexagonal lips without dentigerous ridges. Dorsal lip has two ovate papillae. Two ventrolateral lips each with one lateral ovate papilla and one amphidial pore. Interlabia are well developed. Excretory pore opens below the ventral lip. Oesophagus consists of an anterior muscular part and a posterior glandular one and it has small globular ventriculus and ventricular appendix. Intestinal caecum directed anteriorly. Body length was 25 -48mm.and the width in the middle part of the body was 0.5 – 1.5mm. Length of the oesophagus was 2.5 – 5.5mm. Length of ventricular appendix was 0.7 – 1.6mm. Vulva was 9.5 -25.5mm from anterior end.

II-The histopathological findings

A- Liver showed migrating tracks filled with numerous esinophils, lymphocytes and macrophages and congestion in the portal blood veins and sinusoids (Fig. 8).

B- Kidney showed aggregation of esinophils, lymphocytes and macrophages among the degenerated renal tubules. Focal renal tissue was replaced by granulomatous nodules consist of necrosis and calcification encircled by fibrous connective tissue (Fig.9).

C- Intestine showed granulomatous reaction in the lamina propria. giant cells of foreign body type containing esinophilic fragments were observed (Fig. 10).

DISCUSSION

The present study trials were done to complete its life cycle in the experimental mammals and showing responses of migration of the larval stages in different organs. Even though much research has been done to elucidate the life cycles of aquatic ascaridoids, the life cycle of A*nisakid sp.* has not been previously carried out experimentally in mammals. The transmission of *Contracaecum rudolphi* in experimental infections to crustacean copepods and fish hosts was studied to determine the role of various potential hosts in life cycle of this seal parasite ***(10)***.

The third stage larvae were usually encapsulated and observed in the viscera of four species of fish (*Acanthopagrus butcheri, Sillaginodes punctata, Mugil cephalus and Aldrichetta forsteri*) ***(10,11).*** In this study, the third stage larvae of *Anisakid sp.* was detected in the musculature and intestine of the common sand smelt, *Atherina* sp.

The observation of the eggs in the feces of infected puppies 32 days post infection was an indication of the complete life cycle inside the final host of dog. This observation was also detected in final host of aquatic birds ***(10,17,22)***.

The Histopatholgical changes as granulomatous nodules detected in the liver, kidney and intestine may be due to the migration of the larvae in these organs before reaching to the intestine to become adults. Similar findings were observed in previous several studies ***(6,7,23).***

The morphological characters of the larvae, adults and eggs of *Anisakid sp.* (*Contracaecum rudolphi*) were distinguished with references to several investigators ***(10,23-26).***

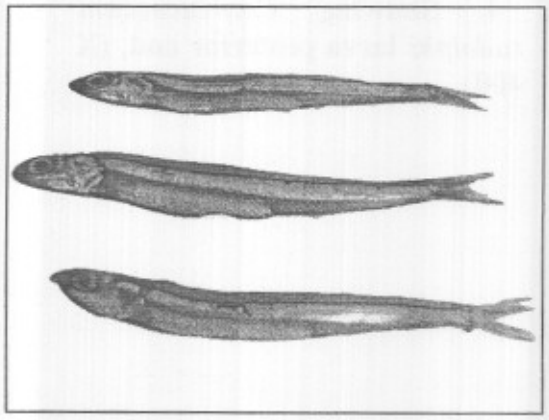

Fig. 1. Showing Sand smelt fish (*Atherina sp.*)

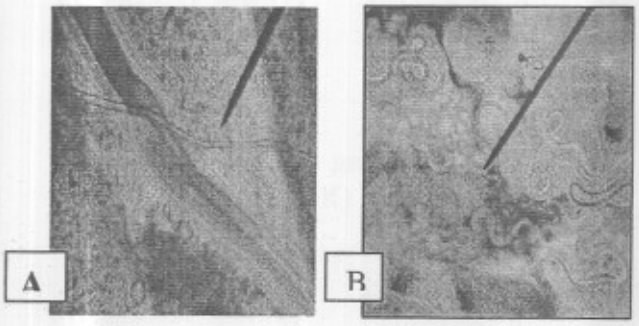

Fig. 2 A&B. Showing *Anisakid sp.* larvae (X 400).

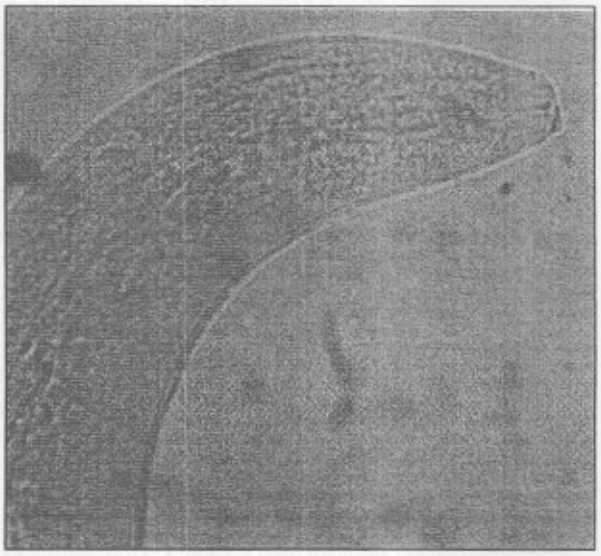

Fig. 3. Showing *Conreacaecum rudolphi* larva anterior end, (X 400) (X 400).

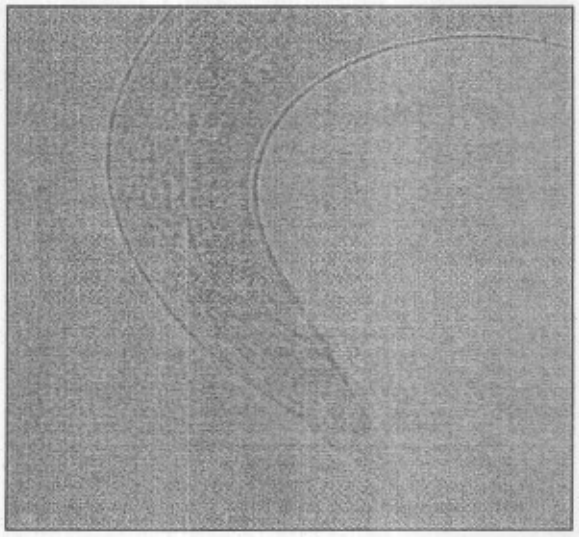

Fig. 4. Showing *Conreacaecum rudolphi* larva posterior end, (X 400)

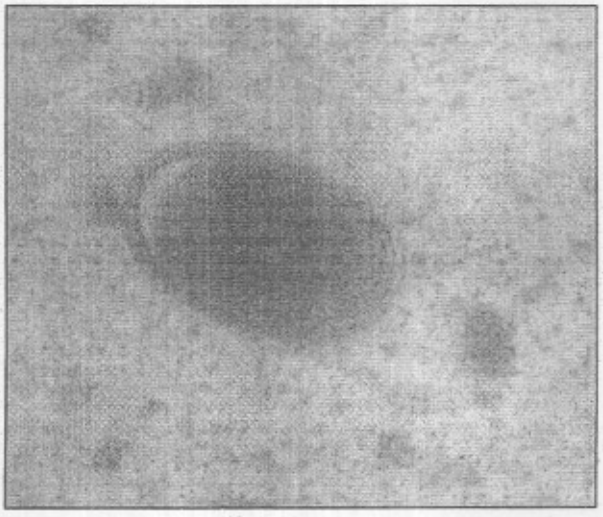

Fig. 5. Showing *Contracaecum rudolphi* egg, (X 400).

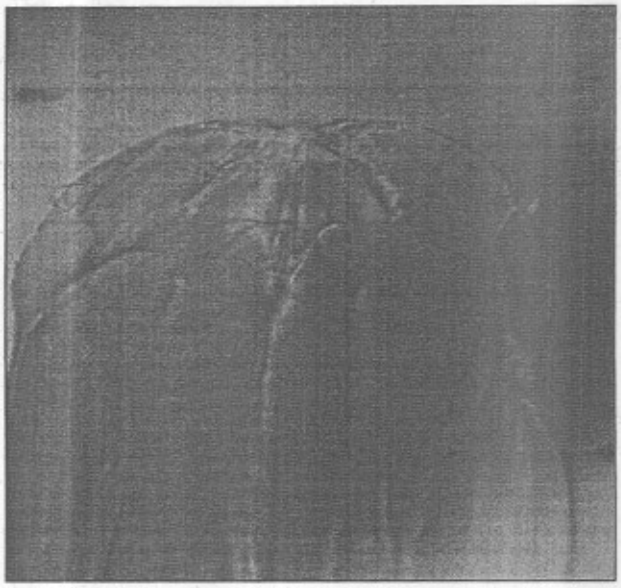

Fig. 6. Showing *Contracaecum rudolphi* adult female, anterior end, (X 400)

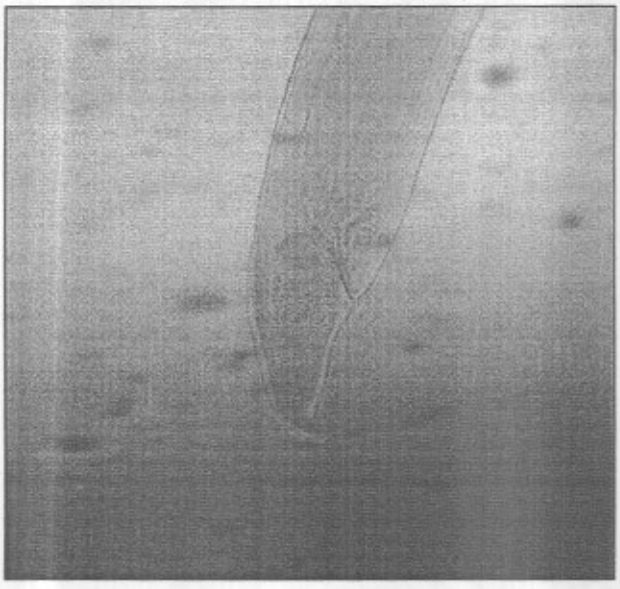

Fig. 7. Showing *Contracaecum rudolphi* adult female, posterior end, (X400).

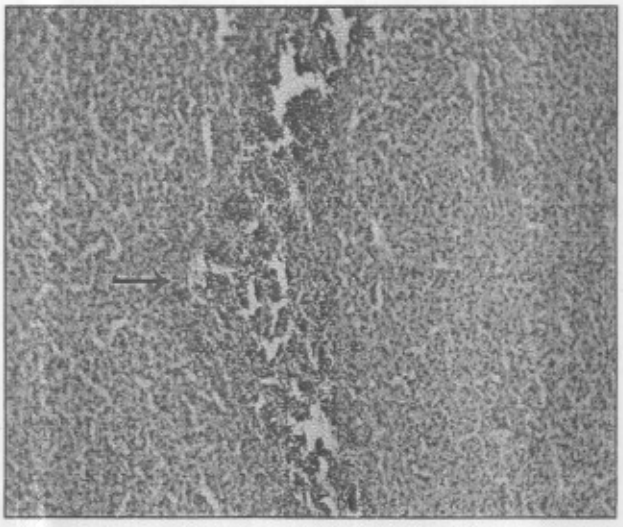

Fig. 8. C.S. in Liver showing migrating tracks filled with numerous esinophils, (H&E X 300).

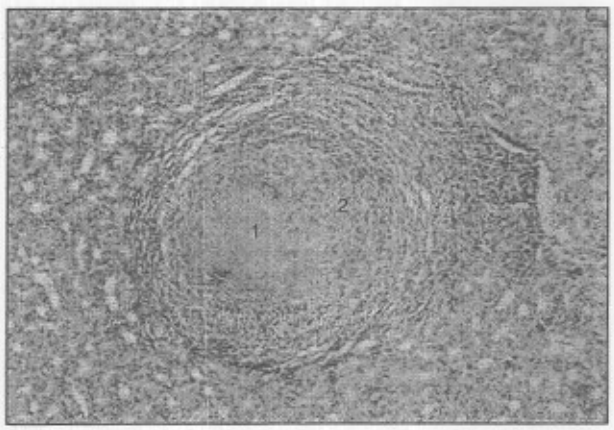

Fig. 9. C.S in Kidney showing focal replacement of the renal tissue with granulomatous nodules. 1-caseous necrosis, 2-leucocetic infiltration, 3-fibrous connective tissue (H&E X300).

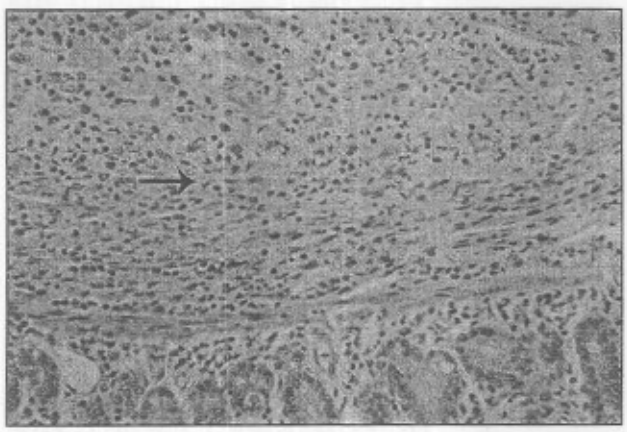

Fig. 10. C.S. in Intestine showing granulomatous reaction in the lamina propria (H&E X 1200)

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الملخص العربى

عدوي تجريبية بيرقات الأنساكيد في الكلاب

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أجريت هذه الدراسة علي عدد ٤ جراء حيث أجريت لهم العدوي التجريبية بنوع من ببرقات الأنساكيد وهي من الديدان الاسطوانية التي تصيب الثدييات المائية والطيور التي تتغذى علي الأسماك وقد يتعرض لها الانسان عن طريق المصادفة أثناء تغذيته علي الأسماك (اثرينا) وهى من أرخص أنواع الأسماك التى يتغذى عليها الأنسان فى مصر –ومن العدوى التجريبية للكلاب حصلنا على الطور اليافع فى الأمعاء- تم عمل دراسة هستوباثولوجية لتأثير هذه اليرقات على الأعضاء المختلفة مثل الكبد والكلية والأمعاء فى الكلاب