STUDIES ON THE ROLE OF SHELLFISH AS A SOURCE FOR TRANSMITTING SOME PARASITES OF ZOONOTIC IMPORTANCE

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ABSTRACT

The present study was conducted to clarify the role of shellfish as a source for transmitting some parasites of zoonotic importance. For this purpose, a total of 2181 shellfish samples representing 1151 fresh water crayfish (Procambarus clarkii), 534 white shrimps (Penaeus seliferus) and 496 blue crabs (Callinectes sabidus) were collected from Sharkia, EL-Ismalia and Port Said Provinces and examined for the presence of trematode metacercariae and protozoal oocysts or cysts. The results revealed that the infection rates of encysted metacercariae in the examined shellfish were 79.93%, 52.06% and 38.7%, in crayfish, shrimps and crabs, respectively. The encysted metacercariae recovered from crayfish were belonging to four families of Heterophyidae, Microphallidae, Cyathocotylidae and Echinostomatidae. The infection rates of encysted metacercariae in shrimps in Sharkia, EL-Ismalia and Port Said Provinces were 48.76%, 54.04% and 52.87%, respectively. The obtained metacercariae were belonging to three families of Heterophyidae, Microphallidae and Cyathocotylidae. The rates of crabs infection with unidentified encysted metacercariae were 28.28%, 43.9% and 42.85% in Sharkia, EL-Ismalia and Port Said Provinces, respectively. Regarding the seasonal prevalence of encysted metacercariae in examined crayfish, the peak of infection (93.79%) was detected in summer followed by spring (75.6%), autumn (74.9%) and the lowest one was observed in winter (27.27%). One hundred and fifty six adult worms of ten trematode species were developed after experimental infection of sixteen puppies with different types of metacercariae. From metacercariae infecting crayfish, 82 adult trematodes were developed, where Heterophyes aequalis, Pygidiopsis summa, Centrocestus cuspidatus, Metagonimoides oregonensis, Microphallus minus, Prohemistomum vivax and Petasiger sharjahni, represented 23.1%, 20.7%, 15.8%, 7.3%, 9.7%, 12.2% and 10.9%, respectively. From the metacercariae infecting shrimps, 74 adult trema-

todes were developed. Of these trematodes; Heterophyes aequalis, Pygidiopsis genata, Centrocestus cuspidatus, Microphallus minus, Maritrema kitanesis and Prohemistomatid sp.; represented 24.3%, 18.9%, 21.6%, 21.6%, 4.05% and 9.4%, respectively. On the other hand, metacercariae infecting crabs were not able to develop adult trematodes in the experimentally infected puppies until 128 day postinfection (PI). Concerning the occurrence of protozoa oocysts or cysts in shellfish, the percentages of recovered Cryptosporidium parvum oocyst and Giardia sp. cyst were 9.29% and 4.77%, respectively, while, all examined shrimp and crab samples were free from infection. Experimental infections of five white albino rats with Cryptosporidium parvum oocysts of crayfish origin revealed that four (80%) rats were infected, of which, one shed oocysts at 3rd day PI, while, the remainder three infected rats shed the oocysts in their faeces at 5th day PI. On the other hand, two experimentally infected puppies with Giardia sp. cysts of crayfish origin shed the infective stage in their faeces at 7th day PI. Histopathological examination of intestinal sections of experimentally infected animals revealed histopathological reactions due to effect of trematodes and protozoa, togther with developmental stages of Cryptosporidium parvum and Giardia sp. It could be concluded that shellfish harbored many trematodes and protozoal agents transmissible to man. Zoonotic importance of recovered trematodes and protozoa was fully discussed.

INTRODUCTION

Shellfish is becoming to be an increasingly important seafood. About 70 species of intestinal flukes of fish and shellfish origin have been reported to infect people. These flukes belong to family Heterophyidae, Echinostomatidae, Microphallidae, Nanophyetidae, Gymnophallidae and Plagiorchiidae. People contract the infection through eating raw or improperly prepared fish and crustaceans infected with encysted metacercariae **(Hai and Mott, 1994)**. The public health and economic impact of fish and shellfish-borne parasitic zoonoses is considerable in terms of morbidity and even mortality in human as well as in losses due to condemnation of parasitized fish and shellfish. Accordingly to the **(WHO, 1995)**, fish or crustacean borne-trematodes (species of intestinal flukes, Clonorchis, Opisthorchis and Paragonimus) infect 39 million people and 550 million are at risk. Most of these infection occur in Asia, particularly the Far East and South East Asia.

Heterophyiasis is an intestinal infection, endemic in many localities in Egypt. Heavy infection can cause abdominal pain and diarrhea. The transmission of heterophyiasis in Egypt is continuous especially in lakes with brackish water **(Abou-Basha et al., 2000)**.

Echinostomiasis is a zoonotic disease, endemic in many countries especially those in the South East Asia and Far East, where the human infections are associated with common socio-cultural practices of eating raw or insufficiently cooked crustaceans, fish and mollusks (**Graczyk and Fried, 1998**).

Shellfish have the ability to carry and concentrate human waterborne pathogens such as Cryptosporidium and Giardia in their gills and tissues through absorption of protozoal agents from contaminated water with human and animal excreta (**Fayer et al., 2003**).

Since the introduction of fresh water crayfish, Procambarus clarkii in the early 1980s into the Egyptian fresh water systems for aquaculture from the United States of America (**Rawi, 1996**), it has been rapidly expanded in all aquatic ecosystems including streams, ponds and marshes with polluted or clean waters. P. clarkii becomes successfully adapted to the new habitats and become an important compound of the local aquatic fauna (**Ibrahim et al., 1995**). P. clarkii stands as an important food in many parts of the world, being a rich source of protein. In Egypt, it has been consumed in few areas, being cheaper than other crustacean. In Egypt, there are few studies carried out to verify the role of fresh water crayfish in transmission of parasites (**Fayek et al., 1999 and Raef et al., 2003**). Therefore, to clarify the role of shellfish in transmitting some parasites of zoonotic importance, this work was undertaken to investigate the occurrence of trematode metacercariae in shellfish and experimental infection of puppies with recovered metacercariae to determine the adult trematode species. Also the occurrence of protozoa in shellfish and experimental infection of puppies and rats with different isolated protozoal stages was carried out.

MATERIAL AND METHODS

A) Shellfish samples:

A total of 2181 shellfish were collected from Sharkia, EL-Ismailia and Port Said Provinces. These included 1151 fresh water crayfish, 534 white shrimps (Penaeus seliferus) and 496 blue crabs (Callinectes sabidus) and surveyed for encysted metacercariae and protozoa cysts or oocysts. Crayfish were collected from different canals of Zagazig, Abokebeer, Hehia and Menia EL-Kamah at Sharkia Province from the period extending from April 2004 till February 2005. The collected shrimp samples included 162 from Zagazig city at Sharkia, 198 from EL-Ismailia and 174 from Port Said, meanwhile, the collected crab samples included 152 crabs from Zagazig, 148 from EL-Ismailia and 196 from Port Said. Shrimps and crabs were collected from fish markets from the period extending from **December 2004** to **May 2005**. All samples of shellfish were packed in plastic bags containing ice and sent fresh to Zoonoses Department, Faculty of Veteri-

nary Medicine, Zagazig University for parasitological examinations.

B) Parasitological examination:

Each shellfish was firstly examined macroscopically by naked eye and by the aid of magnifying hand lens after the removal of the carpace to detect any changes in the viscera, cephalothorax and musculature. The detected metacercariae were recovered from infected shellfish according to **Mahdy et al. (1995)**. Thereafter, the microscopic examinations were done as follows; the gills, heart, midgut glands, muscles and tissues which adhered to the inner surface of carpace were separately compressed between two slides and examined under dissecting microscope (**Sugiyama et al., 2004**), and also, the whole muscles, gills and tissue of each examined shellfish were digested in the artificial digestive juice. After digestion, the sediment was examined under microscope and encysted metacercariae were collected and counted per each shellfish. A part of the obtained encysted metacercariae was used for experimental infection of puppies and also a microphoto was done for each type of encysted metacercariae (**Garcia, 2001**). The number of recovered metacercariae from crayfish in each season was also recorded. The presence of protozoa oocysts or cysts was detected in homogenized soft tissue, viscera and cephalothorax of each shellfish by using cover slip floatation with Sheather's sugar solution (**Levine, 1985**).

C) Experimental infections:

1. **Experimental infection of puppies with encysted metacercariae to develop adult trematodes.**

Nineteen puppies, of 4 weeks old, reared on milk and bread, were used. Twelve were experimentally infected with encysted metacercariae obtained from crayfish, while, four puppies were used for experimental infection with metacercariae obtained from shrimps. Also three puppies were experimentally infected with metacercariae obtained from crabs. Faecal samples were examined twice weekly for two weeks before infection to exclude any possible infection with intestinal parasites by simple and sedimentation techniques (**Happich and Boray, 1969**). Also a prophylactic dose of anthelmintic drug, Praziquantel, 50mg/10kg B.WT., was given one week before experimental infection and the puppies were kept under suitable hygienic measures. Puppies infection was done by administrating 10ml saline solution containing viable encysted metacercariae which were obtained from infected shellfish by using a stomach tube (**Shibahara** and **Nishida, 1986**). After one week of infection, daily faecal examination was done by direct and simple sedimentation techniques till demonstration of eggs. The experimentally infected puppies which began to shed eggs, were scarified and the small intestine was carefully examined for the presence of adult flukes (**Reid, 1962**). In puppies in which no eggs were detected in the faeces, the

lungs, livers, diaphragm and body cavity were examined for immature trematodes. The worms were collected, fixed, stained and mounted according to **Bowser et al. (1984)** and **Tantawy (1993)**. Identification of the recovered trematodes was carried out depending on some characteristic morphological criteria according to **Yamaguti (1958)**, **Shalaby (1982)**, **Raef (1994)** and **Saba (2004)**.

2. Experimental infection of white albino rats with Cryptosporidium parvum oocysts:

Eight weaning white albino rats were obtained from Faculty of Veterinary Medicine, Zagazig University and tested to ensure their freedom from protozoal infection. The isolated C. parvum oocysts were collected, suspended in 2.5% Potassium dichromate solution, sieved and then sporulated by aeration for one week at room temperature. The sporulated C. parvum oocysts were concentrated by Sheather's sugar solution, and washed in de-ionized water by centrifugation. Five rats were inoculated orally by using small stomach tube with 0.2ml of the concentrated Cryptosporidium suspension recovered from crayfish, while, the remaining three rats were left as a control (**Reese et al., 1982**). Daily examinations of faecal pellets of all rats for one week before experimental infection and for 1-18 days postinfection (PI) for experimentally infected rats by coverslip floatation using Sheather's sugar solution. Also rats-faecal smears were prepared, air dried then stained using the modified Ziehl-Neelsen technique (**Henriksen and Pohlenz, 1981**), and examined microscopically to detect C. parvum oocysts.

3. Experimental infection of puppies with Giardia sp. cysts.

Three puppies of 4 week old were used. Giardia sp. cysts obtained from naturally infected crayfish were concentrated, and 0.2ml of Giardia cysts suspension were orally inoculated to each of two puppies, but the remaining one puppy was left as a control (**Siam et al., 1994**). Daily examination of faecal material was carried out to determine the prepatent period.

D) Histopathological examinations:

Small portions of small intestines of infected puppies with encysted metacercariae obtained from crayfish and shrimps, small portion of duodenum of infected puppy with Giardia sp. cysts recovered from crayfish and small parts of ileum of infected rat with C. parvum oocyst recovered from crayfish, were fixed in 10% buffered formalin then embedded in paraffin wax blocks, thereafter, sectioned at 5µ and stained with Hematoxylin and Eosin. These sections were examined microscopically to study the pathological changes due to the effect of adult worms and develop-

mental stages of protozoa on their hosts according to **Reese et al. (1982), Ibrahim et al. (1989) and Fayer et al. (1997)**.

DISCUSSION

The incidence of fish or shellfish-borne parasitic disease of zoonotic importance varies greatly between areas depending on food habits of peoples, and micro and macroclimate of the environment. Many species of trematodes inhabiting fish or shellfish as larval stage are capable of causing infections and diseases in human beings **(WHO, 1995)**. Moreover, fish and shellfish might be carry in their body some protozoal agents transmissible to the human consumers.

Table (1) shows the occurrence and intensity of infection with encysted metacercariae in shellfish at Sharkia, El-Ismailia and Port-Said Provinces. The infection rate of encysted metacercariae in examined crayfish at Sharkia Province was 79.9%. Nearly similar percentages of infection of trematodes metacercariae in River Nile fresh water fishes were previously reported by **EL-Dally (1988) and Saba (2004)**. Lower incidences were also cited by **Shalaby (1982)**, **EL-Aroussi (1984)** and **Tantawy (1993)**. On the other hand, higher percentage (100%) was recorded in Mugil sp. in Egypt by **Rifaat et al. (1980)**. Previous studies revealed that the rate of metacercariae infections in M. cephalus, T. zilli and C. Lazera were 72%, 86% and 88%, respectively as reported by **EL-Dally (1988)**. Whereas, **EL-Gohary** and **Samaha (1997)** recorded that the infection rates of encysted metacercariae in Oreochromis sp. and C. Lazera were 72.9% and 68%. It was evident from the results recorded in table (1) that the total number of metacercariae was 5911 metacercariae, and ranged from 1-30 with an average of 6.4 per infected crayfish. These metacercariae were belonged to families Heterophyidae, Echinostomatidae, Cyathocotylidae and Microphallidae, table (1) and figures (1-5). **Fahmy et al. (1976)** reported that the incidence of encysted metacercariae of Prohemistomum vivax and Haplorchis yokogawi in River-Nile fishes was ranged from 60 to 90%. **Shalaby (1985)** observed an infection rate of 72.85% of Heterophyidae and Prohemistominae metacercariae in catfish, C. Lazera collected from River Nile at Giza and Cairo Provinces.

The occurrence and intensity of infection with metacercariae in examined shrimps and crabs at different Provinces are shown in table (1). It was found that the overall infection rate of encysted metacercariae in the examined shrimps was 52.06%. Nearly similar results was obtained in marine fish by **Abdel-Maksoud (1992)**. However, lower figure was previously recorded in grass shrimp, Palmonete vulgaris by **Pung et al. (2002)**. It is clearly obvious from the results recovered in table (1) that the total number of metacercariae was 2514, and ranged from 1-25 with an average of 9.04 per infected shrimp. The infection rate of encysted metacercariae in shrimps col-

lected from Zagazig fish markets was 48.76%, (table1). These metacercariae were belonging to family Heterophyidae after identification of metacercariae as well as adult worms recovered from experimental infection. Meantime, the rate of metacercarial infection in shrimps collected from EL-Ismailia Province was 54.04%. These metacercariae were belonging to families Heterophyidae, Microphallidae and Cyathocotylidae. At Port Said Province, the infection rate was 52.87%, table (1), and the types of recovered metacercariae were belonging to families Heterophyidae and Microphallidae. The degree of salinity of water in each locality may be affect on the infection rates of white shrimps with metacercariae as was previously confirmed by **Pung et al. (2002)** who stated that the salinity preference of the parasite's snail host have an impact or effect on the infection rate with encysted metacercariae.

Concerning the occurrence and intensity of metacercarial infection in crabs at different Provinces, table (1) illustrates that the overall infection rate of crabs was 38.7%. Nearly similar percentage was obtained by **Moyou et al. (1983)** who found that the infection rate of crabs with metacercariae of Paragonimus sp. was 45.4%. Lower percentages were previously obtained by **Raef et al. (1999)** and **Sugiyama et al. (2004)**. It was evident from the results recorded in table (1) that the total number of metacercariae was 431, and ranged from 1-7 with an average of 2.24 per infected crab, (figure 6). In one study carried out in Sharkia Province, Egypt, **Raef et al. (1999)** found that the number of metacercariae per infected crab was ranged from 1-45 with an average 13.8 per crab. However, in another study carried out in Japan, **Sugiyama et al. (2004)** found that the number of metacercariae per infected crab was ranged from 1-190 with an average 13.1. Table (1) clarifies that the infection rate of encysted metacercariae in crabs collected from Zagazig was 28.28%. Meantime, at EL-Ismailia Province, the rate of metacercarial infection in crabs was 43.9%. However, the infection rate of metacercariae was 42.85% in crabs collected from Port Said, (table1).

From the results recorded in the present study, one could be easily deduce that the highest infection rate was in crayfish followed by that in shrimps and lastly in crabs. The variations in the percentages of infection may be attributed to the water habitats either fresh or marine and to the difference in localities of collection of shellfish samples. Fresh water may be polluted with sewage which may contain different eggs of trematodes. In addition, crayfish, Procambarus clarkii is a voracious snail predator and it compete with snails by consuming aquatic plants used by snails as refuge, oviposition and food. Moreover, fresh water crayfish possibly eat the fry and the young fish, so, crayfish may acquire the infection and act as a paratenic or transport host. This explanation was also confirmed by **Raef (1994)** who reported that the metacercarial infection in marine fishes was lower than that in fresh water fishes, this is attributed to higher water pollution with human and animal excreta in fresh water than that in marine water. On the other

hand, the lowest infection rates detected in shrimps and crabs may be attributed to the industrial or chemical pollution of marine water, having an effect on the intermediate host snail or free living stages of parasites.

Regarding the seasonal prevalence of encysted metacercariae in examined crayfish, table (2) clarifies that the peak of infection (93.79%) was in summer followed by spring (75.6%), autumn (74.9%) and lowest one was observed in winter (27.27%). These results are nearly similar to the results of previous work of **Tantawy (1993)** and **Saba (2004)** who recorded higher incidences of metacercariae in summer season in fresh water fishes. However, a lower percentage of infection was recorded by **Raef (1994)**. Seasonal prevalence of encysted metacercariae in crayfish depend mainly on seasons and activities of the first intermediate host which disseminates infection to crayfish. Warm environment increases the activity, growth and reproduction of snails and crayfish, and maintenance and liberation of cercariae. This interpretation was previously mentioned by **Shalaby (1982)**.

Table (3) and figures (2-5) indicate that the metacercariae infecting fresh water crayfish and white shrimps were able to develop into their adult stages. From the metacercariae infecting fresh water crayfish, 82 adult trematodes were developed, where Heterophyes aequalis (23.1%), Pygidiopsis summa (20.7%), Centrocestus cuspidatus (15.8%), Metagonimoides oreganesis (7.3%), Microphallus minus (9.7%), Prohemistomum vivax (12.2%) and Petasigar skrjabini (10.9%) were identified. From the metacercariae infecting white shrimps, 74 adult trematodes were developed. Of these trematodes, H. aequalis (24.3%), P. genata (18.9%), Centrocestus cuspidatus (21.6%), Microphallus minus (21.6%), Maritrema kitanesis (4.05%) and Prohemistomatid sp.(9.4%) were collected (tables 3). In the present study, all developed trematodes recovered from puppies experimentally infected with metacercariae were belonged to 4 families, Heterophyidae (H. aequalis, P. summa, P. genata, Centrocestus cuspidatus and Metagonimoides oreganesis), family Microphallidae (Microphallus minus and Maritrema kitanesis), family Cyathocotylidae (Prohemistomum vivax and Prohemistomatid sp.) and family Echinostomatidae (Petasigar skrjabini). **Fahmy et al. (1976)** identified P. vivax and Haplorchis yokogawi from puppies and kittens fed on metacercariae infecting fishes. **Rifaat et al. (1980)** recovered H. heterophyes, P. vivax and Haplorchis yokogawi from puppies fed on fishes carrying metacercariae. **Massoud et al. (1981)** found that the infection rates with heterophyid worms were 2.56%, 0%, 33.3% and 14.28% in stray dogs, cats, red foxes and golden jackles, respectively. **Shalaby (1982)** obtained P. vivax and Centrocestus sp. after feeding of dogs on heavily infected fishes with metacercariae. **EL-Aroussi (1984)** recovered H. heterophyes, P. genata and Mesostephanus appendiculatus after feeding dogs on metacercariae infecting C. Lazera. **Shalaby (1985)** detected four trematodes (Haplorchis pumilio, M. appendiculatus, P. vivax and Cyndiplostomum azimi) in intestine of dogs af-

ter experimental infection with metacercariae in catfish. **EL-Dally (1988)** obtained 5 flukes of zoonotic importance after experimental infection of dogs with metacercariae infecting fishes, and worms were identified into Haplorchis pumilio, P. genata M. appendiculatus, P. vivax and Centrocestus armatus in small intestine of birds after experimental infection with metacercariae recovered from Tilapia sp., C. Lazera and B. bayad. **Tantawy (1993)** identified P. genata, P. vivax, Procerovum caderoni, Haplorchis pumilio and M. appendiculatus from experimentally infected kittens, rats and pigeons with metacercariae infecting fresh water fishes. **Raef (1994)** obtained P. vivax, M. appendiculatus and M. burmanicus after experimental infection of puppies, chickens, ducks and albino rats with metacercariae infecting marine fishes. **Fayek et al. (1997)** isolated Microphallus minus and Maritrema kitanesis after feeding ducklings on metacercariae infecting white shrimp. **Saba (2004)** obtained H. heterophyes, H. aequalis, P. vivax and other different types of trematodes after orally administrating the metacercariae in fresh water fishes to puppies, chickens and ducklings.

It is evident from the obtained results that the experimentally infected puppies with metacercariae obtained from crabs did not develop into adult worms, table (3). However, **Raef et al. (1999)** detected egg of Paragonimus kellicotti in feces of puppies as well as immature worm in abdominal cavity after experimental infection.

Shellfish can recover and concentrate environmentally derived water pathogens and can be used for the sanitary assessment of water quality by the detection of Cryptosporidium and Giardia in their tissues **(Graczyk et al., 2001)**. Table (4) and figure (7A,B) show the occurrence of protozoa oocysts or cysts in examined crayfish at Sharkia Province. The infection rates of crayfish with Cryptosporidium parvum oocyst and Giardia sp. cyst were 9.29% and 4.77%, respectively. Lower results were obtained by **Raef et al. (2003)** who reported that the infection rate of crayfish with C. parvum oocyst was 6%. Also, these results were higher than that found by **Fayer et al. (2003)** who detected that the infection rate of oysters and clams with Cryptosporidium oocyst was 3.7%. Moreover, **Slam et al. (1994)** found an infection rate of 20% of Cryptosporidium oocyst in Nile crocodile. Giardia sp. cysts were previously detected from different shellfish types by **Graczyk et al. (1999)** and **Graczyk et al. (2003)**.

In the present study, all examined shrimps and crabs were negative for Cryptosporidium oocyst and Giardia cyst. This may be attributed to the extent of pollution of marine water (habitat of crabs and shrimps) with animal or human sewage less than that in river water (habitat of crayfish). Moreover, the slow movement of fresh water in river tributaries, where crayfish collected, helps in settling of Giardia sp. cyst in the sediment of different canals. Since, the behaviour of crayfish is the burrowing in the sediment, so, these crayfish may take the infection with protozoal agents. This explanation was supported by **Graczyk et al. (1999)**.

It is precede to investigate the possibility of experimental infection of warm-blooded animals with Cryptosporidium and Giardia isolates recovered from fresh water crayfish to verify its zoonotic importance. In the present study, experimental infection of five white albino rats with C. parvum oocyst reveals that four rats (80%) were infected with Cryptosporidium. One rat shed the oocyst in their faecal pellets at 3rd day postinfection (PI), meanwhile, the remainder three rats descend C. parvum oocyst in their faecal pellets at 5th day PI, (figure 7C). Infected rats shed Cryptosporidium earlier than that observed by **Raef et al. (2003)** who found that experimentally infected rats shed C. parvum oocyst in their faecal pellets at 6th day PI. On the other hand, **Amin et al. (1993)** detected that experimentally infected catfish with Cryptosporidium oocysts of human origin shed the infective agent in its faces at 3rd day PI.

Regarding to the experimental infection of two puppies with Giardia sp. cyst, it was noticed that all experimentally infected puppies shed Giardia sp. cyst in their feces at 7th day PI. **Hewlett et al. (1982)** experimentally infected mongrel dogs with cysts or trophozoite of G. Lamblia. Moreover, **Amin et al. (1993)** established an experimental infection of catfish with G. Lamblia cyst of human origin, the authors found that catfish shed G. Lamblia cyst at 5th day PI.

In the present study, histopathological sections were taken from small intestine of experimentally infected puppies with metacercariae to determine the pathological effect of adult trematodes. Figure (8, A to E) show adult heterophyid trematodes in histopathological sections of small intestine of experimentally infected puppies with metacercariae obtained from crayfish and shrimps. The adult heterophyid flukes, with increased numbers of goblet cells and desquamated epithelial cells together with inflammatory cell infiltration in the lamina propria of mucosa, were seen. These results were in agreement with the findings of **Shalaby (1993)**. The effect of Cryptosporidium parvum on rats and Giardia sp. on puppies were assessed by histopathological examinations. Figure (9-A) reveals the developmental stages of C. parvum in histopathological section of ileum of experimentally infected rat with C. parvum oocyst obtained from crayfish. The developmental stages of C. parvum were seen in the brush border of the corrugated intestinal villi, these histopathological finding agrees with that results obtained by **Reese et al. (1982) and Fayer et al. (1997)**. Figure (9-B) shows Giardia trophozoite in histopathological section in duodenum of experimentally infected puppy with Giardia sp. cyst from crayfish. This result was in agreement with the finding of **Amin et al. (1993)**.

It could be concluded that, shellfish (fresh water crayfish, white shrimps and blue crabs) were harbouring different types of trematode metacercariae. These metacercariae were developed after experimental infection of puppies into adult worms of ten trematodes species belonging to four families of Heterophyidae, Microphallidae, Cyathocotylidae and Echinostomatidae. All of these trematodes have a zoonotic importance. Crayfish showed higher occurrence of metacercariae

than shrimps or crabs. The peak of infection in crayfish was in summer season. The high level of trematode metacercarial infection detected in the present study, indicating the significance role of these crustaceans as a potential reservoir for such zoonotic trematodes. In addition the higher infection rates of crayfish with trematode metacercariae recorded in this study, reflecting the role of crayfish as a new host responsible for dissemination of such zoonotic parasites. Moreover, Cryptosporidium parvum oocysts and Giardia sp. cysts were detected in the examined crayfish. This would indicate the role which may be played by this type of crustacean as a source of these protozoal agents for shellfish consumers and handlers. On the other hand, isolates of Cryptosporidium and Giardia recovered from crayfish had established infections in rats and puppies, suggesting the possibility of human infection with these isolates. Accordingly, periodical parasitological assays of edible shellfish to clarify their sanitary condition, avoid collection of shellfish from areas with high incidence of infection, adequate cooking and efficient hygiene practice during the preparation and evisceration of the shellfish, control of snails with molluscicides where feasible and avoid contamination of aquatic environment with raw human and animals sewage are the recommended preventive measures for the control of shellfish-borne parasitic zoonoses.

RESULTS

Table (1) : Occurrence and intensity of infection with metacercariae in examined shellfish samples collected from Sharkia, El-Ismalia and Port-Said Provinces.

Type of shellfish	Locality	No. of examined	No. of infected	% of infected	Total no.of metacer-cariae	Total no. of metacer-cariae per crayfish	
						Range	Average
Crayfish	Sharkia*	1153	920	79.9	5911[+]	1-30	6.4
White shrimps	Sharkia	162	79	48.76	684[++]	1-16	8.6
	EL-Ismalia	198	107	54.04	856[+++]	1-24	8
	Port Said	174	92	52.87	974[++++]	1-25	10.59
	Total	534	278	52.06	2514	1-25	9.04
Blue crabs	Sharkia	152	43	28.28	108	1-4	2.5
	EL-Ismalia	148	65	43.9	154	1-5	2.36
	Port Said	196	84	42.85	169	1-7	2.01
	Total	496	192	38.7	431	1-7	2.24

* Crayfish were collected from different canals of Zagazig, Abokebeer, Hehia and Menia EL-Kamah at Sharkia Province

(+): Metacercariae belonged to families Heterophyidae, Echinostomatidae, Microphallidae and Cyathocotylidae.

(++): Metacercariae belonged to family Heterophyidae.

(+++): Metacercariae belonged to families Heterophyidae, Microphallidae and Cyathocotylidae

(++++): Metacercariae belonged to families Heterophyidae and Microphallidae.

Table (2): Seasonal prevalence of the encysted metacercariae in examined crayfish at Sharkia Province.

Seasons	No of examined	No. of infected	% of infected
Spring	373	282	75.6
Summer	403	378	93.79
Autum	331	248	74.9
Winter	44	12	27.27
Total	1151	920	79.93

Table (3). Differentiation of adult trematode species developed in puppies post-infection with metacercariae obtained from shellfish*.

Shellfish species	Localities	Total No. of trematode sp.	*Heterophyes aequalis*		*Pygidiopsis summa*		*Pygidiopsis genata*		*Centrocestus cuspidatus*		*Metagonimoides oregonensis*		*Microphallus minus*		*Maritrema khanesis*		*Prohemistomum vivax*		*Prohemistomum sp.*		*Petasiger skrjabini*	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Crayfish	Sharkia	82	19	23.1	17	20.7	0	0	13	15.8	6	7.3	8	9.7	0	0	10	12.2	0	0	9	10.9
White shrimp	Sharkia	18	6	33.3	0	0	5	27.8	7	38.9	0	0	0	0	0	0	0	0	0	0	0	0
	EL-Ismalia	22	4	18.1	0	0	6	27.2	0	0	0	0	7	31.8	3	13.6	0	0	2	9.09	0	0
	Port-Said	34	8	23.5	0	0	3	8.8	9	26.4	0	0	9	26.4	0	0	0	0	5	14.7	0	0
	Total	74	18	24.3	0	0	14	18.9	16	21.6	0	0	16	21.6	3	4.05	0	0	7	9.4	0	0

*No adult trematodes were developed from metacercariae infecting blue crabs.

Table (4): Occurrence of protozoa oocysts or cysts in fresh water crayfish samples at Sharkia Province.

Type of shellfish	No of examined	*Cryptosporidium parvum* oocysts		*Giardia sp.* cysts		Total	
		No. of infected	%	No. of infected	%	No. of infected	%
Crayfish*	1151	107	9.29	55	4.77	162	14.07

* Crayfish were collected from different canals of Zagazig, Abokebeer, Hehia and Menia EL-Kamah at Sharkia.

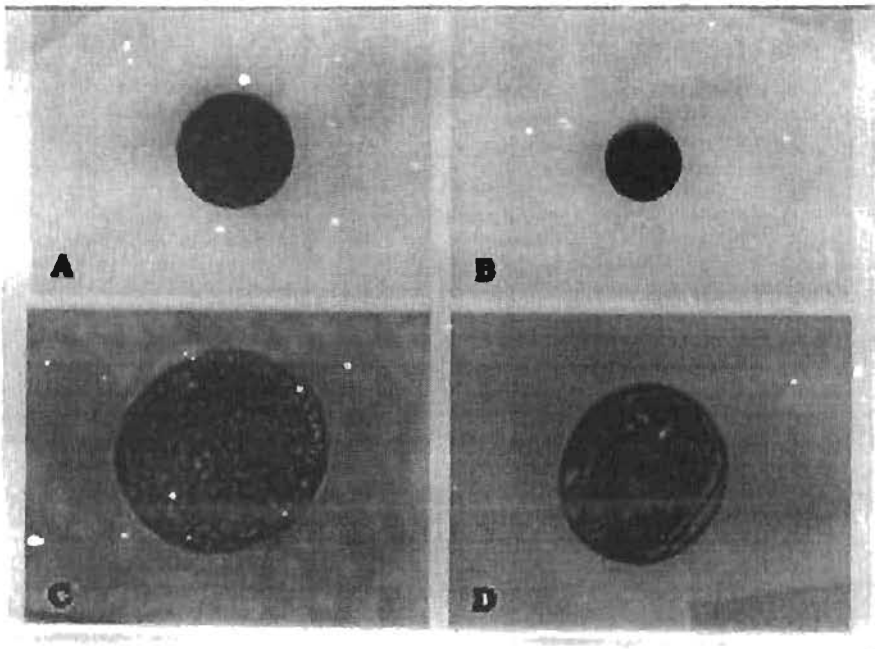

Fig. (1) : Heterophid metacercariae from crayfish and shrimps. A, B, D; (X100). C; (X400).

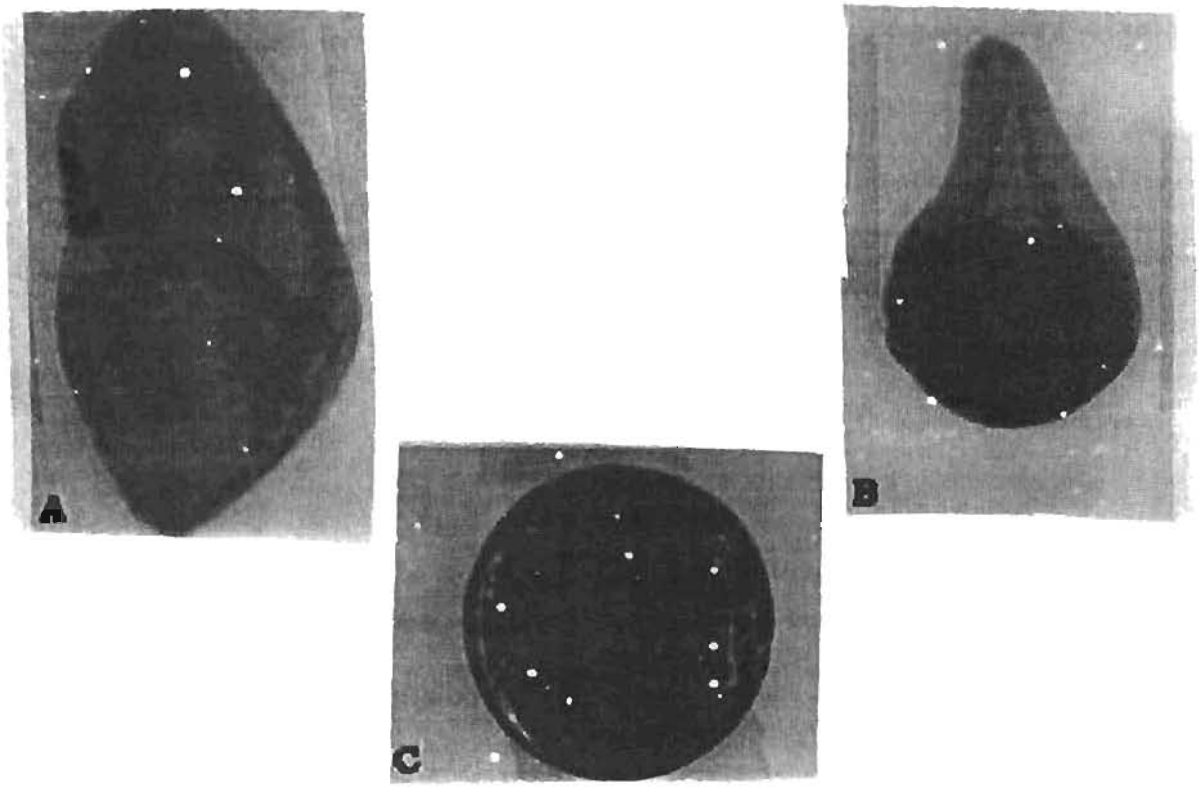

Fig (2): (A); Prohemistomum vivax, adult, X100. **(B);** Prohemistomid sp., adult, X100.

(C); Cyathocotylid metacercariae from crayfish and shrimps, X400.

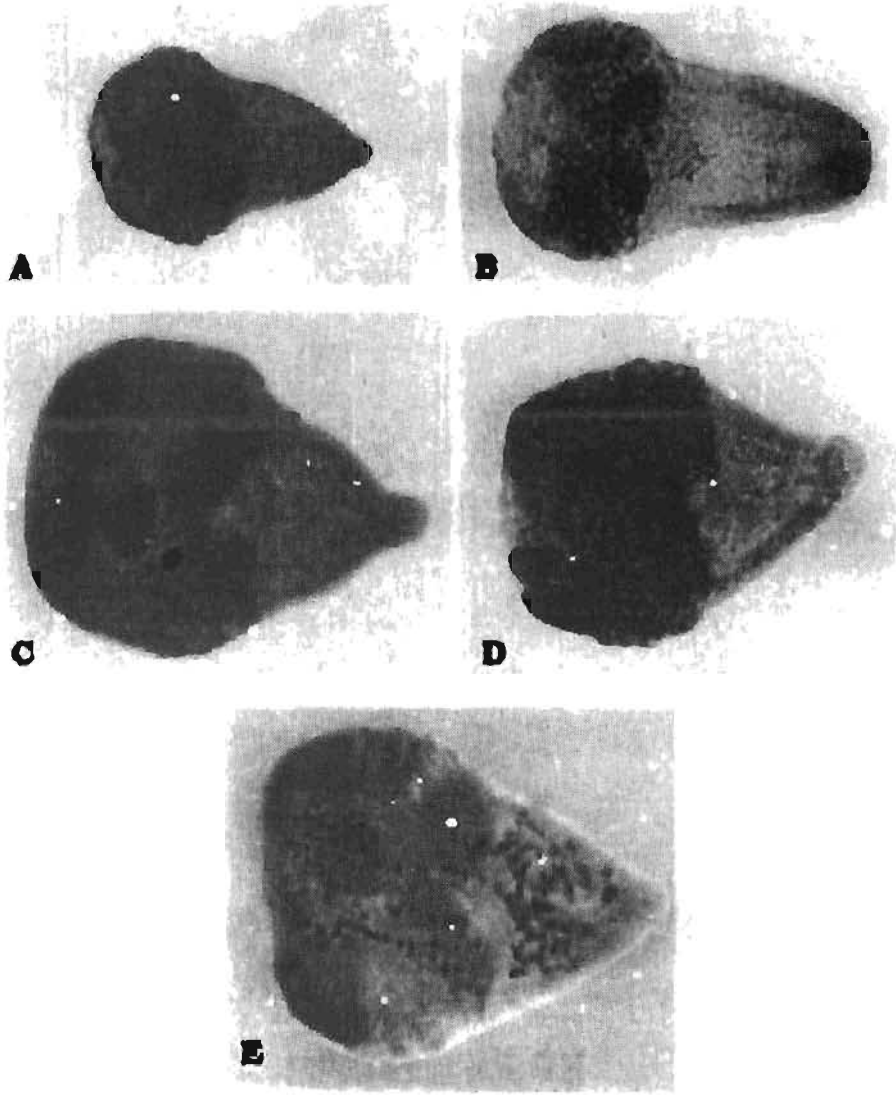

Fig. (3) : Adult heterophyid trematodes obtained from small intestine of experimentally infected puppies. **(A)**; Heterophyes aequalis, X200. **(B)**; Pygidiopsis summa, X200. **(C)**; Pygidiopsis genata, X400. **(D)**; Metagonomoides oreganesis, X200. **(E)**; Centrocestus cuspidatus, X400.

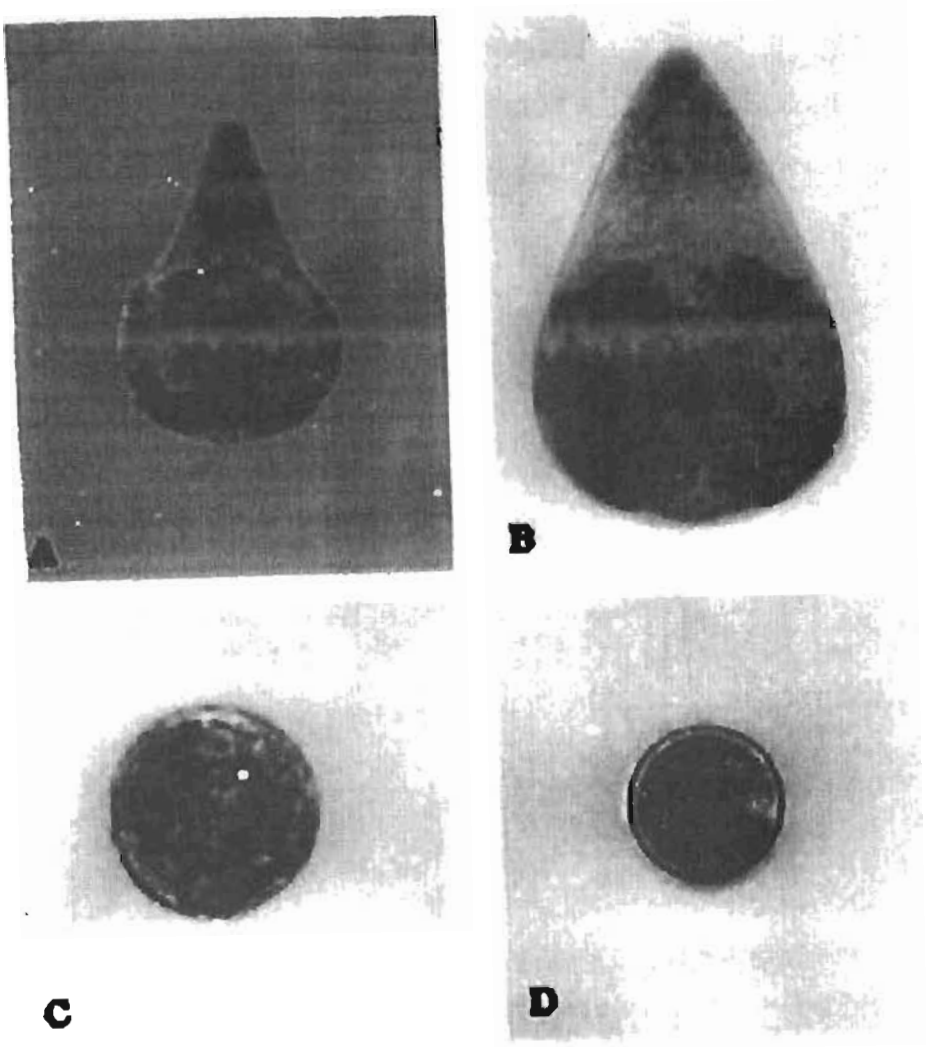

Fig. (4): (A); Microphallus minus, adult, X100. (B); Maritrema kilanensis, adult, X400. (C&D); Microphallid metacercariae, X100.

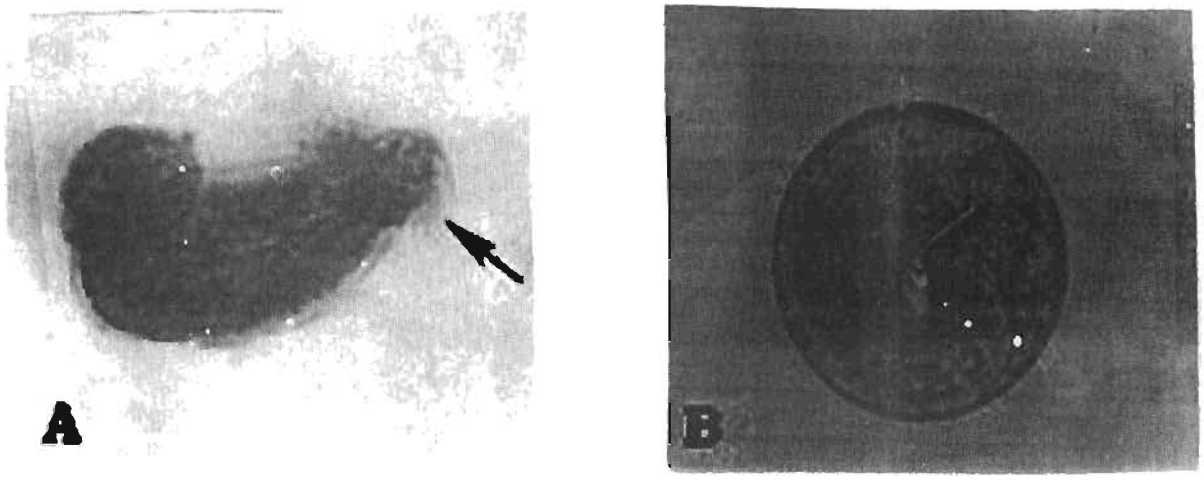

Fig. (5A); Petasigar skrjabini, adult, X100. **(B)**; Echinostomatid metacercariae, X100.

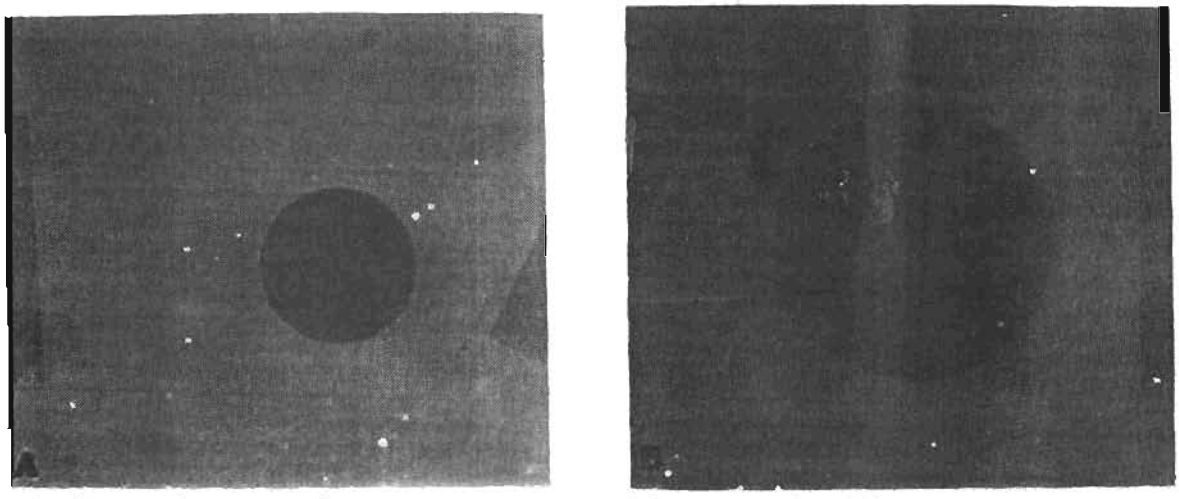

Fig. (6A & B); Encysted metacercariae obtained from the gills of blue crabs X100.

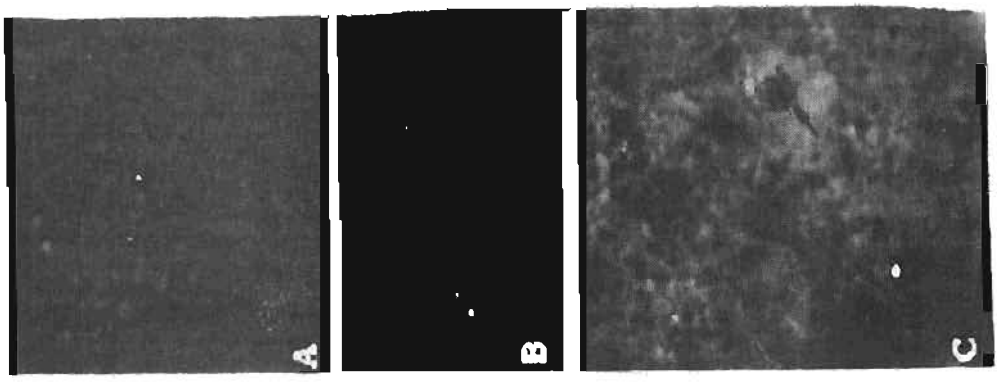

Fig. (7): (A); Giardia sp. cyst from crayfish (unstained), X400. **(B)**; Cryptosporidium parvum oocyst from crayfish (unstained), X1000. **(C)**; Cryptosporidium parvum oocyst detected in rat faecal pellets (stained by modified Ziehl-Neelsen technique), X1000.

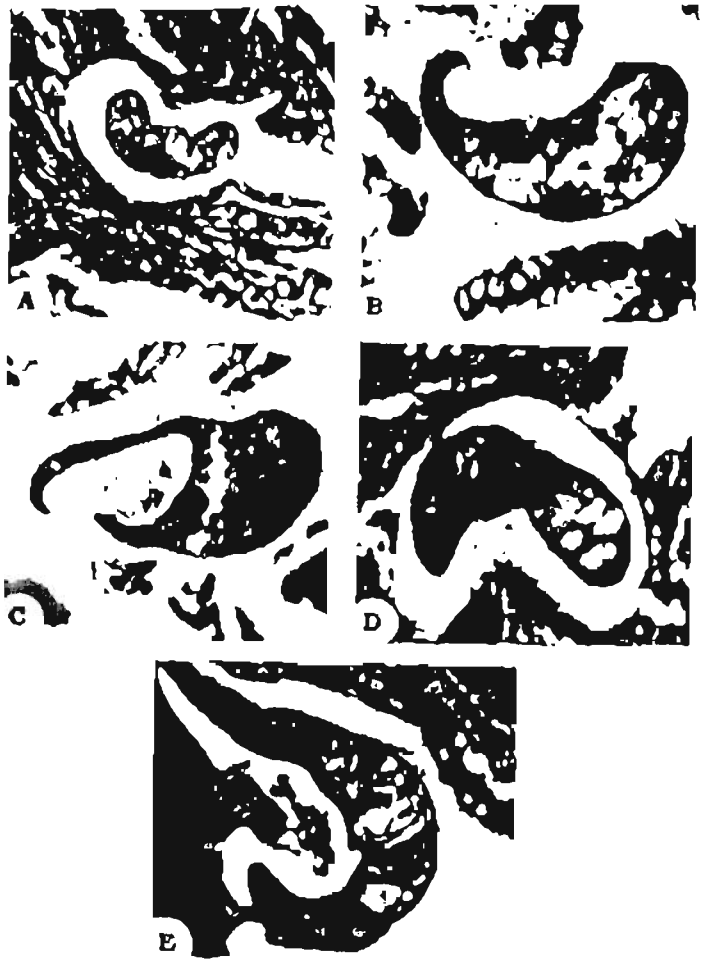

Fig. (8): Adult heterophyid trematodes in histopathological sections of small intestines of experimentally infected puppies with metacercariae obtained from crayfish and shrimp (H&E). X400.

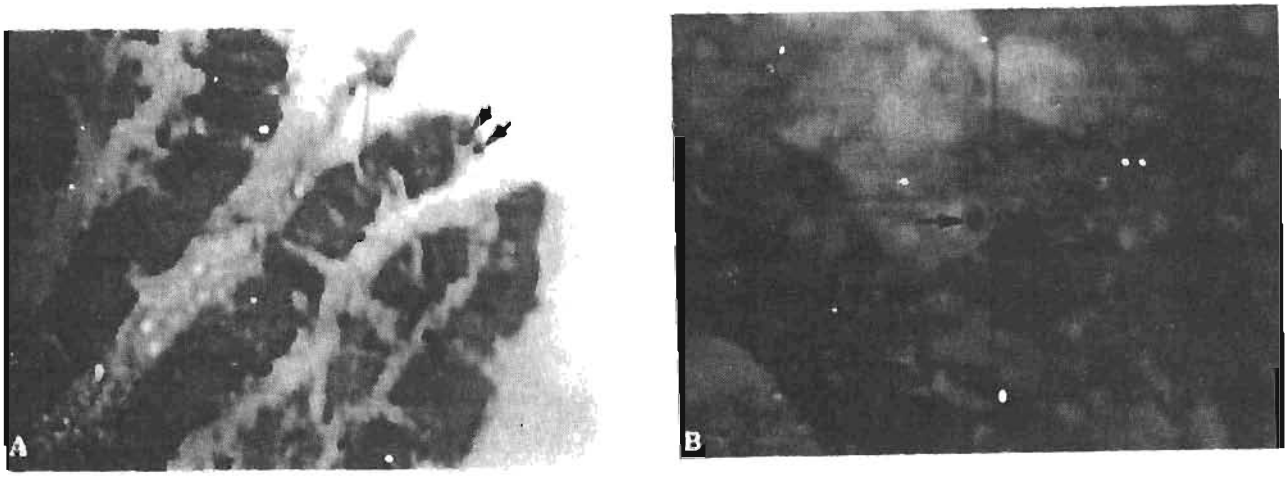

Fig. (9): (A); Developmental stages of Cryptosporidium parvum in histopathological section in ileum of experimentally infected rat with Cryptosporidium parvum oocyst obtained from crayfish (H&E), X400. (B);Giardia trophozoite in histopathological section in duodenum of experimentally infected puppy with Giardia sp. cyst recovered from crayfish (H&E), X400.

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دراسات عن دور الأسماك القشرية كمصدر لنقل بعض الطفيليات التى لها أهمية من وجهة الأمراض المشتركة

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عمر حسن عامر عبدالله محمد أمين

أجريت هذه الدراسة لترضيح دور الأسماك القشرية فى نقل بعض الطفيليات التى لها أهمية من وجهة الأمراض المشتركة، من أجل هذا الغرض، قد تم جمع ٢١٨١ عينة من الأسماك القشرية والتى إشتملت على ١١٥١ استاكوزا المياه العذبة، ٥٣٤ جمبرى أبيض و٤٩٦ كابوريا زرقاء من أماكن مختلفة بمحافظات الشرقية، الاسماعلية وبورسعيد، وقد تم فحص كل العينات لتواجد الأطوار اليرقية المتحوصلة لديدان التريماتودا وأيضاً تم فحصها لتواجد حويصلات أو أكياس الأوليات، أظهرت النتائج أن معدل إصابة استاكوزا المياه العذبة، الجمبرى والكابوريا بالميتاسركاريا هى ٩٣ر٧٩٪، ٠٦ر٥٢٪ و ٧ر٣٨٪ على التوالى، وكانت الميتاسركاريا المتحوصلة فى الاستاكوزا تتبع أربع عائلات من هيتروفيدى، ثياثوكوتيليدى، ميكروفاليدى واكينوستوماتيدى، أما بالنسبة لمعدلات إصابة الجمبرى بالميتاسركاريا كانت ٦٧ر٤٨٪، ٠٤ر٥٤٪ و ٨٧ر٥٢٪ فى محافظات الشرقية، الاسماعلية وبورسعيد علي التوالى واتضح أن الميتاسركاريا المعزولة تابعة لثلاث عائلات من هيتروفيدى، ميكروفاليدى وثياثوكوتيليدى، ومن الناحية الأخرى، وجد أن نسبة إصابة الكابوريا بالميتاسركاريا الغير المصنفة كانت ٢٨ر٢٨٪، ٩ر٤٣٪ و٨٥ر٤٢٪ فى محافظات الشرقية، الاسماعيلية وبورسعيد على التوالى، وبالإشارة إلى نسبة إصابة الاستاكوزا بالميتاسركاريا لى مدار فصول السنة المختلفة، وجد أن أعلى نسبة إصابة كانت فى فصل الصيف (٧٩ر٩٣٪) ثم فى الربيع (٦ر٧٥٪) يليها فى الخريف (٩ر٧٤٪) بينما أقل نسبة إصابة (٢٧ر٢٧٪) قد لوحظت فى فصل الشتاء.

تم الحصول علي ١٥٦ دودة يافعة من التريماتودا بعد إجراء العدوى التجريبية لعدد ١٦ جروا، حيث تم الحصول على ٨٢ دودة ناشئة من الميتاسركاريا المتحوصلة فى الاستاكوزا وكانت نسبتها كالتالى : ١ر٢٣٪ هيتروفيس أكواليز، ٧ر٢٠٪ هيجيدوسيس سيوما، ٨ر١٥٪ سنتروسيستس سيسبيداتس، ٣ر٧٪ ميتاجونوموس أورجانيسيس، ٧ر٩٪ ميكروفاليس مينس، ٢ر١٢٪ بروهيموستومم فايفكس و٩ر١٠٪ هيتاسيجر سكر جابنى، بينما تم الحصول على ٧٤ دودة ناشئة من الميتاسركاريا المتحوصلة فى الجمبرى وكانت نسبتها كالآتى : ٢٤.٣٪ هيتروفيس أكواليز، ٩ر١٨٪ هيجيدوسيس جيناتا، ٦ر٢١٪ سنتروسيستس سيسبيداتس، ٦ر٢١٪ ميكروفالبس مينس، ٠٥ر٤٪ مارتريا كيتانينسيس و٤ر٩٪ من نوع بروهيموستوماتيد، ومن ناحية أخرى كانت الميتاسركاريا المتحوصلة فى عينات الكابوريا غير قادرة إلي الوصول إلى الأطوار اليافعة حتى يوم ١٢٨ بعد العدوى للجروان.

أما بالنسبة لمدى تواجد أكياس أو حويصلات الأوليات في الأسماك القشرية، وجد أن نسبة إصاب الاستاكوزا

بحويصلات الكريتوسبورديم بارقم وأكياس من نوع الجياردبا هى ٢٩ر٩٪ و٧٧ر٤٪ على التوالي، فى حين أن كانت كل عينات الجمبرى والكابروريا خالية من العدوى، وباجراء العدوى التجريبية لعدد ٥ فئران بيضاء لعدد ٥ فئران بيضاء بحويصلات الكريتوسبورديم بارقم المعزولة من الاستاكوزا وجد أن العدوى قد تمت فى أربعة فئران (٨٠٪) حيث أن (٨٠٪)، حيث أن فأر واحد قد زرف الحويصلات فى اليوم الثالث من العدوى، بينما باقي الفئران الثلاث قد رزفوا الحويصلات فى اليوم الخامس من العدوى، وباجراء العدوى التجريبية لجروين بأكياس من نوع الجياردبا المعزولة من الاستاكوزا وجد أن الجروين قد رزفوا هذه الأكياس فى البراز فى اليوم السابع من العدوى، أظهر الفحص الهستوباثولوچى للحيوانات المعدية تجريبياً، كثيراً من التغيرات الباثولوچية الناتجة عن تأثير ديدان التريماتودا والأوليات وكذلك الأطوار المختلفة لها، ويستخلص من الدراسة أن الأسماك القشرية تحتوى على كثيراً من ديدان التريماتودا والأوليات التى ممكن أن تنتقل إلي الإنسان وقد تم مناقشة الأهمية الصحية لجميع الطفيليات المعزولة من وجهة الأمراض المشتركة.