Surveillance of Parasitic Diseases of Zoonotic Importance in Fishermen, Some Fish and Shellfish Species

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Abstract: To study the epidemiologic status of zoonotic parasitic diseases in some fish and shellfish species and fishermen, a total of 120 freshly caught fish, 100 cat fish (Clarias gariepinus) collected from Nile tributaries in Ismailia city, 20 keeled mullet (Liza Carinata), 400 alive marine shellfish; 100 shrimps (Penaeus japonicus), 100 gastropods (Thais carnifera) and 200 bivalves (Paphia andulata) in addition to 15 fecal samples were collected from fishermen at the shores of lake Temsah in Ismailia city, Egypt during (fall) 2017 and (Spring) 2018. The parasitological study revealed that the eggs of Fishborne Zoonotic Trematodes (FZTs) and Cryptosporidium spp. oocysts were detected in 26.7 and 20% of the examined fishermen stool samples, respectively. The prevalence of Encysted Metacercarial infection (EMC) of FZTs and cryptosporidiosis among the examined fin fish and shellfish species were (74 and 24%) in cat fish, (5 and 50%) in keeled mullet, (50 and 28%) in shrimp, (20 and 30%) in gastropod and (60 and 20%) in bivalve. The main clinical picture of EMC affected fishes was slimness, haemorrhages all over the body with respiratory distress. Affected Penaeus japonicus were aggregated at the aerator sites while Thais carnifera and Paphia andulata showed no specific clinical picture. Cryptosporidiosis affected fishes showed emaciation, ascites and haemorrhages along the stomach and intestine. Experimental infection in pigeons and rats, revealed Brachylaema migrans from pigeons fed on Penaeus japonicus and (Mesostephanus appendiculatus, Mesostephanus bumanicus, Procervum calderoni, Stictodora tanayensis, Apophallus donicus, Pygidiposis genata, Maritrema orensense, Sphaeridioptrema globules, Prohemistomum vivax and Haplochis punilio) from albino rats fed on Clarias gariepinus. Some FZTs could not be identified by conventional morphologic typing, so, PCR targeting ITS2 (internal transcribed spacer) region was performed followed by bidirectional sequencing which revealed the following worms: Braurania cordiformis, Cyathoclyce prussica and Holostephanus dubini.

Key words: Fishbone zoonotic trematodes, metacercarial infection, Cryptosporidium, fish, shellfish, fishermen, experimental infection, sequencing

INTRODUCTION

Now a days, fish and aquaculture production attract a great attention of many researchers. Fish and shellfish are believed to be the healthiest form of protein, not only because their low contents of saturated fats but also their unique contents of essential nutrients including omega-3, fatty acids, vitamin D, calcium and iodine. Eating fish and shellfish can decrease the risks of heart diseases, obesity and hypertension (Reames, 2012). On the other side, they can be affected by many diseases and serve as a vehicle for some parasitic zoonoses including Fish borne Zoonotic Trematodes (FZTs) and some protozooses (Cerrad et al., 2015; Hung et al., 2015; Kirrella et al., 2018).

Fish borne Zoonotic Trematodes (FZTs) have been added to the list of emerging infectious diseases. There are 59 species of FZTs that can parasitize humans. They are divided into two groups, the first being the small liver flukes and the second the minute intestinal flukes. The intestinal trematodes in particular have a global distribution; more than 18 million people have been infected worldwide (WHO, 1995; Keiser and Utzinger, 2009). These adult digenetic trematodes ( subclass digenea) are endoparasites occurring in all vertebrates (Nouh et al., 2010). Their life cycle includes 1st intermediate host (aquatic snails and crustaceans) and a second one (fish, crustaceans and aquatic snails) (Serbins, 2014; Chantika et al., 2018). Humans and fish-eating mammals and birds can serve as final hosts depending on the trematode species involved (Nouh et al., 2010). People get infected with FZT by ingestion of raw or undercooked freshwater and sometimes brackish water fish, mollusks and shrimps containing infective

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175
metacercariae (Chai et al., 2009, Keiser and Utzinger, 2009; Chai, 2007). Most infections with intestinal flukes are asymptomatic. However, intense infections are accompanied by an eosinophilic gastroenteritis, mucus-reach diarrhea, ulceration and bleeding of the intestinal mucosa. Fatal pathology associated with infection is rare and is caused by lesions in the myocardium or the nervous system following the dissemination of fluke eggs in the lymph or circulatory system (Yu and Mott, 1994, WHO., 1995, ElSheikha, 2007). Metacercarial infestations (encysted forms) which are found in the second intermediate hosts affect aquaculture and fish industries (Noui et al., 2010). As they wear their cultivation and production (Yooyen et al., 2006), retard growth especially for young fishes and increase secondary infections possibilities by decreasing fish immunity and decrease the marketability of the affected fishes by their abnormal shape (ruptured corneas, hemorrhagic spots or encysted metacercarial spots) (Aly et al., 2005). Also, they decrease bivalves’ production by causing castration or production of histopathological changes in labial pulp tissue (Francisco et al., 2011). In crustaceans as penaeid shrimps, they are present in different tissues but do not cause overt disease except when found in large numbers by causing severe damage of major organs and tissues (Meyers and Burton, 2009).

Cryptosporidium is a protozoan parasite that cause severe diarrhea in a wide range of vertebrates including humans. Recent studies have identified Cryptosporidium as one of the most important diarrheal pathogen affecting young children (Kotloff et al., 2013). In fish hosts, Cryptosporidium species are located either in the stomach or intestine (Ryan et al., 2014; Certad et al., 2015). They are often shed in feces even without clinical signs. Fish cryptosporidiosis causes off, decreasing in growth rates, emaciation, whitening of feces and ascites (CFSIP, 2018). C. parvum oocysts can survive in seawater for at least 1 year (Tamborrini and Pozio, 1999). Bivalve shell fish can filter out sea water, accumulate high numbers of oocysts in gills, digestive glands and intestinal tract (Willis et al., 2013) and constitute a risk to humans. The aim of this study is to throw light on parasitic infections in some fish and shellfish species and fishermen and to discuss their public health hazards.

MATERIALS AND METHODS

Sampling: A total of 120 fin fish, 100 catfish (Clarias gariepinus), 20 keeled mullet (Liza Carinata Valenciennes, 1836) and 400 marine shellfish that comprised 100 shrimps (Penaeus japonicus), 100 gastropods (Thais cornicula) and 200 bivalves (Paphia undulata) were obtained alive from local fishermen on the shores of lake Temsah and Nile tributaries in Ismailia (Geographic coordinates: 30°35'N 32°16'E) which is a city in North-Eastern Egypt. The study was carried out in September, 2017 (fall) and April, 2018 (Spring). In addition, fecal samples from 15 fishermen in Ismailia city were collected in tightly fitted cups and a full case history was recorded.

Fish samples were placed in an ice box containing aerated water to be immediately transported to the lab and then they were transferred to glass aquaria containing aerated water from the same site where the samples were collected from. Clinical signs of the collected samples were performed on live and freshly dead ones according to Nega (2010).

Detection of protozoal infection

Preparation of smears: After sedimentation of human stool samples by formalin-ethyl acetate concentration method (Aras et al., 2008, 2009), direct smears from sediment were made on duplicate slides. Also impression smears were taken from intestine of fish, soft tissue of shellfish. All smears were air dried and fixed in methanol for 3 min.

Detection of Cryptosporidium oocysts: Staining of smears using modified Ziehl Neelsen stain was performed. Microscopic examination and searching for acid fast Cryptosporidium oocyst was performed (Casemore, 1991; O’Donoghue, 1995).

Detection of FZTs eggs: Human stool samples were concentrated by formalin-ethyl acetate sedimentation method and examined under microscope.

Detection of metacercarial infection: Gills, musculature and internal organs of fish and shellfish were examined for the presence of the encysted metacercariae grossly using a magnified hand lens (Muhdy et al., 1995). Microscopically, small pieces from each examined part were compressed between two glass slides with addition of some drops of saline solution and then examined (Morishita et al., 1965, Schaperclaus, 1992).

Artificial Digestion method (AD): The infested fish and shellfish samples were individually ground in a stomacher. The technique involves the enzymatic digestion of musculature samples at 36°C for 2-3 h, followed by filtration procedures where the digested material was filtered with 1×1 mm of mesh and washed with 0.85% saline until the supernatant became clear. Finally, the sediment was carefully examined under a stereomicroscope. The collected metacercariae solutions were injected orally to the suspected laboratory animals as described by Garcia (2001).

Experimental infection: The experimental protocol in this study adhered to the standard ethical guidelines for laboratory animal use and care (The European community guideline, EEC Directives 86/609/EEC of the
24th November, 1986). A total of 20 one week old pigeons were used for infection of shrimps with digested fluid containing metacercariae. They were reared in two groups (15 infected and 5 control), left 1 week under microscopical inspection of their faeces. Also, ten ducks and 15 chickens were fed with EMC obtained from shrimps. In addition to, thirty white rats 3 weeks old that were divided equally in six groups, they were infected with digested fluid of catfish, keeled mullet, gastropod, bivalve and shrimp while 5 were kept as a negative control. These infected rats were kept two weeks under inspection and fecal samples from all groups were inspected daily for presence of eggs by direct examination and sedimentation techniques. Sacrifice occurred for positive ones, the small intestine was evacuated carefully from its content and examined under stereomicroscope for presence of any trematodes. The obtained trematodes were washed with normal saline, to be preserved in 80% ethanol alcohol for genotypic analysis and formalin 5% for staining with aceticarmine and morphologic identification (Yamaguti, 1955; Garcia, 2001).

PCR and sequencing of adult FZTs: Total genomic DNA was extracted and purified from adult trematodes by using the modified hot shot method as described by Meeker et al. (2018), Abouelhassan et al. (2019). About 5 μL of DNA solution were used per 50 μL PCR reaction. The PCR was conducted according to the guidelines of Arya et al. (2016): primers targeting ITS2 region were used: AP102 F AGAGGCGACCAAAGTGTGA, AP102 R GTCCACGTTCACTACGGCC. The PCR was run in a thermal cycler (Techne, UK). The conditions for amplification of 369 bp product of ITS2 region were: Initial denaturation for 5 min at 94°C, followed by 35 cycles (of denaturation for 30 sec at 94°C, primer annealing for 38 sec at 57°C and extension at 72°C for 42 sec) and a final extension at 72°C for 7 min. After amplification, 8 μL of PCR products were loaded on 1.5% agarose gel premixed with ethidium bromide, electrophoresed for 45 min and visualized by UV illumination.

Bidirectional sequencing was carried out and data were compared to the GenBank nucleotide database using the basic local alignment search tool to confirm the identity of suspect trematodes.

Statistical analysis: Comparison of the prevalence of infection in different spp. was done by using Chi square test and the p-value was set at ≤0.05 (SPSS Version 20).

RESULTS AND DISCUSSION

Clinical picture: EMC infested fin fishes showed slimness with haemorrhages and abrasions all over the body (Fig. 1a), gills and at the bases of fins. The positive case of Liza carinata showed scale detachment all over the body. Respiratory distress with fish tried to gasp air from water surface and aggregated at air pumps was recorded. Internal organs and musculature of C. gariepinus showed small black spots (1 mm) and/or 1-4 mm white nodules which were embedded hardly in the tissues (Fig. 1b). EMC infected Penaeus japonicus were aggregated at the aerator sites while Thais carnifera and Paphia andulata showed no specific clinical picture. Emaciation and ascites with haemorrhagic stomach and intestines were seen in some of fin fishes affected with cryptosporidiosis (Fig. 1c). Meanwhile, cryptosporidiosis affected shell fishes had no abnormal clinical picture.

In Table 1, Fig. 2a-c, it was clear that the prevalences of EMC infection of Fishborne Zoonotic Trematodes (FZTs) and cryptosporidiosis among the examined fin fish and shellfish species were (74 and 24%) in catfish, (5 and 30%) in keeled mullet, (50 and 28 %) in shrimp, (20 and 30%) in gastropod and (60 and 20%) in bivalve. The χ² values were 83.078, p<0.0001 (highly significant) and 4.809, p = 0.3057 (non-significant) for metacercarial infection and cryptosporidiosis, respectively.

From Table 2, Fig. 2e-f, we can find that the prevalences of eggs of FZTs and Cryptosporidium oocysts in fishermen were 26.7 and 20%, respectively.

Results of experimental infection

Mesostephanus appendiculatus: Encysted metacercariae were obtained from musculature and body viscera of Clarias gariepinus. The adult worm was obtained experimentally from rats. It has elongated body, broader at posterior with caudal appendage. It is covered with
Table 1: Prevalence of metacercarial infection and cryptosporidiosis in fin fish and shellfish spp

<table>
<thead>
<tr>
<th>Aquatic spp.</th>
<th>Examined</th>
<th>Metacercarial infection</th>
<th>Cryptosporidiosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>N</td>
<td>Percentage</td>
</tr>
<tr>
<td>Cat fish</td>
<td>100</td>
<td>74</td>
<td>74.0</td>
</tr>
<tr>
<td>(Clarion gariepinus)</td>
<td>20</td>
<td>1</td>
<td>50.0</td>
</tr>
<tr>
<td>Keeled mullet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Liza carinata)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total fin fish</td>
<td>120</td>
<td>75</td>
<td>62.5</td>
</tr>
<tr>
<td>Shrimp</td>
<td>100</td>
<td>50</td>
<td>50.0</td>
</tr>
<tr>
<td>(Penaeus japonicus)</td>
<td>100</td>
<td>20</td>
<td>20.0</td>
</tr>
<tr>
<td>Gastropod</td>
<td>100</td>
<td>20</td>
<td>20.0</td>
</tr>
<tr>
<td>(Thais carnifera)</td>
<td>100</td>
<td>20</td>
<td>20.0</td>
</tr>
<tr>
<td>Bivalve</td>
<td>100</td>
<td>20</td>
<td>20.0</td>
</tr>
<tr>
<td>(Paphia anulata)</td>
<td>100</td>
<td>20</td>
<td>20.0</td>
</tr>
<tr>
<td>Total shellfish</td>
<td>100</td>
<td>20</td>
<td>20.0</td>
</tr>
</tbody>
</table>

$X^2 = 83.078, p < 0.0001; X^2 = 4.809, p = 0.0357$

Table 2: Prevalence of FZTs and cryptosporidiosis infection in fishermons

<table>
<thead>
<tr>
<th>Examined</th>
<th>FZTs infection</th>
<th>Cryptosporidiosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N</td>
<td>Percentage</td>
</tr>
<tr>
<td>15</td>
<td>4</td>
<td>26.7</td>
</tr>
</tbody>
</table>

FZTs: Fish borne Zoonotic Trematodes

spines which decreased posteriorly. Oral sucker is subterminal. Prepharynx is absent and the pharynx is well developed and muscular. Esophagus is short and intestinal bifurcation away from the ventral sucker. The intestinal caeca are simple and hidden by the vitelline follicles. The tribocytic organ is ill developed. Testes are irregular triangular in shape, tandem in position, they are medially situated which contain vaginal sphincter. Uterine eggs have thin shell are yellow in color and few in number (Fig. 3a).

Mesostephanus burmanicus: Encysted metacercariae were obtained from musculatures and body viscera of Clarion gariepinus, the adult worm was obtained experimentally from rats. Its body is elongated, broader at posterior with caudal appendage. It is covered with spines which decreased posteriorly. Oral sucker is subterminal while the ventral sucker is un conspicuous. Prepharynx is absent and the pharynx is well developed, muscular. Esophagus is short and the intestinal bifurcation away from the ventral sucker. The intestinal caeca are simple. Testes are ovoid, smooth and slightly oblique in position and located in the posterior half of the body. Cirrus pouch is well developed, claviform and contains protruding cirrus. Ovary is small, round in shape, lays lateral to the anterior testis, ovoid in shape. Uterus is well developed contain few numbers of eggs and ending in genital pore near the end of the appendages. Uterine eggs are few in number, oval in shape, operculated and yellow in color. Vitelline follicles are fairly large and forming two lateral curves around the gonads (Fig. 3b).

Procervum calderoni: Encysted metacercariae were obtained from musculatures and body viscera of Clarion gariepinus, the adult worm was obtained experimentally from rats, its body is elongated and moderately expanded. Cuticle aspinous, fading posteriorly. Oral sucker globular, prepharynx is long. Intestinal bifurcation is about at the anterior third of the body, caeca reached to
posterior end. Excretory vesicle bipartite. Testis median in position, seminal vesicle is sausage shaped. Vitellaria coarse follicles expand anterior to the testis to the posterior end of the body (Fig. 3c).

**Sictodora tanayensis**: Encysted metacercariae were obtained from musculatures and body viscera of *Clarias gariepinus*, the adult worm was obtained experimentally from rats. It has elongated body, cuticle with fine spines gradually fading toward posterior end. Intestinal bifurcation is at about anterior third of the body. Caeca terminating some distance from posterior end of the body, testes are oblique at the middle. Seminal vesicle is thin walled, elongate, bipartite. Vitellaria follicular intracaval, post testicular. Uterus is with ascending and descending coils filling almost all available space in hind body (Fig. 3d).

**Apophallus donicus**: Encysted metacercariae were obtained from musculatures and body viscera of *Clarias gariepinus*, the adult worm was obtained experimentally from rats. Its body is pear shaped, prepharynx is absent. Testes are oval, smooth, oblique and located at the junction of the middle with the posterior third of the body. Ovary is rounded in shape and lies between the seminal vesicle and the posterior testes. Vitellaria start at the level of the posterior testis (Fig. 3e).

**Pygidiopsis genata**: Encysted metacercariae were obtained from musculatures and body viscera of *Clarias gariepinus*, the adult worm was obtained experimentally from rats. It is pyriform, its oral sucker is minute and subterminal and the ventral one ill developed and nearly equal to the oral one, located just behind the middle. Esophagus is long intestinal caeca end just posterior to the middle. Testes are oval, horizontal and lie at the posterior, ovary is globular and pretesticular. Vitelline follicles appear as large mass beside the testes (Fig. 3f).

**Maritrema orensense**: Encysted metacercariae were obtained from musculatures of *Clarias gariepinus*, the adult worm was obtained experimentally from rats. Adult is narrowly oval, covered with spines. Oral sucker is subterminal. Prepharynx is almost as long as esophagus, muscular pharynx and two short and thick intestinal caeca extending laterally terminating before ventral sucker. Ovary middle-dextral with anterior border overlapping ventral sucker. Testes oval, posterior and lateral, to ovary. Vitellaria are oval follicles in an inverted U-shaped incomplete ring between testes and ovary. Uterus extends from anterior end of testes to posterior end of body (Fig. 4a).

**Sphaeridiotrema globulus**: Encysted metacercariae were obtained from musculatures of *Clarias gariepinus*, the adult worm was obtained experimentally from rats. It is pyriform in shape, attenuated anteriorly and posteriorly and wide at the middle part. A deep ventral pouch in fresh specimens is noticed nearly at the middle of the worm. Oral sucker is subterminal and leads directly to well developed muscular pharynx. The short esophagus is bifurcated away of acetabulum into intestinal caeca. Acetabulum is ill developed and spherical in shape. Testes are tandem in position, the anterior one is smooth and ovoid in shape and the posterior one is quadrilateral. Ovary is nearly pyramidal in shape, situated between two testes. Cirrus pouch is well developed and located lateral in the left side in the caudal region. Vitelline follicles are fairly large confined in horse shoe manner postacetabular around the gonads. Uterus is slightly large and extended from the middle of the fluke till the common genital pore and contains large operculated oval egg, yellow in color and thin shelled (Fig. 4c).

**Prohemistomum vivax**: Encysted metacercariae were obtained from musculatures and body viscera of *Clarias gariepinus*, the adult worm was obtained experimentally from rats. It is pyriform in shape, attenuated anteriorly and posteriorly and wide at the middle part. A deep ventral pouch in fresh specimens is noticed nearly at the middle of the worm. Oral sucker is subterminal and leads directly to well developed muscular pharynx. The short esophagus is bifurcated away of acetabulum into intestinal caeca. Acetabulum is ill developed and spherical in shape. Testes are tandem in position, the anterior one is smooth and ovoid in shape and the posterior one is quadrilateral. Ovary is nearly pyramidal in shape, situated between two testes. Cirrus pouch is well developed and located lateral in the left side in the caudal region. Vitelline follicles are fairly large confined in horse shoe manner postacetabular around the gonads. Uterus is slightly large and extended from the middle of the fluke till the common genital pore and contains large operculated oval egg, yellow in color and thin shelled (Fig. 4c).

**Haplochis pumilio**: Encysted metacercariae were obtained from musculatures and body viscera of *Clarias gariepinus*, the adult worm was obtained experimentally from rats. It has small elongate body, tapering anteriorly and broad posteriorly. Long prepharynx and esophagus intestinal bifurcation at the beginning of middle...
all over the body, gills and at the bases of fins. These were in agreement with that obtained by (Goulding et al., 2004; Aly et al., 2005; Reda et al., 2010; Hamouda, 2018; Sumuduni et al., 2018) but from infested Fundulus heteroclitus, C. gariepinus, Oreochromis niloticus, Bagrus bajad and Cyprinus carpio fishes, respectively. Scales were detached in the positive case of Liza carinata, similar to what was recorded by Aly et al. (2005) and Younis et al. (2009) from infested O.niloticus. This may be attributed to rubbing the body against the hard objects due to irritation. The recorded respiratory distress matched with (Younis et al., 2009; Sumuduni et al., 2018). Metacercarial affections of gills leads to severe gill damage and hypoxia (Sumuduni et al., 2018). Internal organs and musculature of C. gariepinus showed small black spots (1 mm) and/or 1-4 mm white nodules which were embedded hardly in the tissues. The same finding was recorded by Aly et al. (2005) and Sumuduni et al. (2018) in the infested C. gariepinus and Cyprinus carpio, respectively.

Regarding, the metacercariae infested Thais carnifera and Paphia andulata; they showed no specific clinical picture in contrast to that obtained by Carella et al. (2013) who recorded pearl-like whitish formations in mantel, labial pulps and gills of infested Donax trunculus clam in Italy. Also, Seppala et al. (2013) found that trematode species exploit their mollusc hosts and induce gigantism by diverting host resources towards growth instead of reproduction.

Concerning the infested Penaeus japonicus with encysted metacercariae, it showed only aggregations near the aerator sites in contrast to that obtained by Rao and Soni (1988) who recorded small oval-shaped metacercarial cysts attached with antennae and appendages of Peneaus indicus and Penaeus semisulcatus in India.

Regarding the total prevalence of EMC in fish, it was 62.5%, Clarias gariepinus (74%) and Liza carinata (5%). These results are nearly similar to previous studies in different areas in Egypt; El-Siefy et al. (2005) and Saad (2007) who recorded a prevalence of (62.01% in Clarias lazera collected from the River Nile) and (62% and 63.3%) from (O. niloticus and T. zillii) in Aswan Governorate, respectively.

While in Egypt (El-Siefy et al., 2005; Lobna et al., 2010; Ammar and Arafa, 2013; El Sayed et al., 2014; Hamouda, 2018; Kirella et al., 2018) recorded lower percentages than that obtained in this study as they recorded (50.19 in O. niloticus and 50 in Synodontis schall, 35.16 in Bagrus bajad and 11.94 in Mugil cephalus), (22 for brackish and 42 freshwater Tilapia fishes), (57.5 in wild and cultured O. niloticus collected in Assiut city); (11-23 in Tilapia nilotica and Mugil cephalus from El-Max Bay, Alexandria); (42 in Bagrus bajad from several localities in

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Fig. 5: PCR amplification of 369 bp of ITS2 region. Lanes 1, 2, 4, 6 Positive samples. M 100 bp DNA ladder.
Aswan at Lake Nasser) and (24.0 and 30.0 in *O. niloticus* and *C. gariepinus* in Kafrelsheikh), respectively.

These differences may be attributed to different fish species, different localities and different seasons at which each study was performed, variation in sanitation degree, water pollution and availability for live snails. Also, the total prevalence is higher than that obtained by Chi et al. (2008); Cho et al. (2014); Wiriya et al. (2013) and Hung et al. (2015) who recorded FZTs metacercariae with percentages of (44.6 in *O. niloticus* and six carp species in Northern Vietnam); (17.3 in mud carp and common carp farm pond fishes in Vietnam); (26.7 and 50.3) for the zoonotic *Centrocestus armatus* and *Metagonimus* spp. metacercariae from freshwater fish in Korea); (53.3 in wild-caught fish in Thailand); (56.1 in cultured and wild freshwater fishes in Vietnam), respectively.

Wiriya et al. (2013), Ammar and Arafa (2013) and Khalil et al. (2014) recorded much lower levels of metacercarial infestation rates as 2.5 and 10% in cage cultured tilapias and pond culture fish in Thailand, 26.7% in cultured *O. niloticus* in Assiut city, Egypt. 27.2% from two freshwater fish species and five marine fish species in Saudi Arabia, respectively. These may be attributed to the different study areas and different fish species.

Our result is lower than that obtained by El-Ezz et al. (2000), El-Seify et al. (2005), Taher (2009), Reda et al. (2010) and Ammar and Arafa (2013) who recorded the total prevalence of EMC as 74.33% in fresh water fishes (*O. niloticus*, *Mugil cephalus* and fingerlings of *Ctenopharyngodon idella*). 100% in *Sclite mysus* and *Mormyrus niloticus* from the River Nile. 78.25% from *O. niloticus* in Egypt, 80.30% among *O. niloticus* in Sharkeya andAbbasa, respectively and 88.3% in wild *O. niloticus* fish.

This may be due to the high percentage of the intermediate host in the River Nile and its tributaries (Abdel-Latif, 2007).

The prevalence of EMC among the examined shell fish was 47.5%; *Penaeus japonicus* (50%), *Thais carinata* (20%) and *Paphia andulata* (60%). Chai and Lee (2002) reported that brackish water bivalves are the source of infection with *Acanthocephalus tysonense*, metacercariae of *Gymnophalloides seoi* were observed in oysters and the natural definitive hosts are in most cases, mammals (rats, cats and dogs). Lassalle et al. (2007) and Carella et al. (2013) recorded that 39% of the examined *Ruditapes philippinarum* clam and 95% of *Cerastoderma edule* cockle from France were affected with EMC and 75-100% of the examined *Donax trunculus* clam in Italy were affected by *Postmonorchis* sp. metacercariae, respectively. Serbina (2014) confirmed the role of bithyniid snails (Gastropoda: *Bithyniidae*) as the first and second intermediate hosts of trematodes in lake-river systems of the steppe zone of the West Siberia Plain, Russia. Twelve species of metacercariae (6 families) were found in bithyniid snails. The most prevalent metacercariae were *Echinoperophyllum acomatum* and *E. recurvatum* and *Cotylocoyle bistriata*. *Anchophora* *Holostephanus* sp. and five original species of trematode metacercariae were also discovered.

The wide range between infestation rates may be attributed to the difference in shellfish species, detected EMC species and the study areas.

By experimental infection, we recovered *Brachylaena migrans* from pigeons fed on shrimps and (*Mesostephanus appendiculatus*, *Mesostephanus burmanicus*, *Procerovum calderoni*, *Sictodora tanayensis*, *Appottalis donicus*, *Pygidiopsis genata*, *Maritremia orensense*, *Sphaeridiotrema globules*, *Prohemistomon vivax* and *Haplorchis pumilum*) from rats fed on *Clarias gariepinus*.

In contrast, chickens and ducks didn’t reveal any infection. The recovery of certain worms in rats and pigeons and failure of their recovery in ducks and chickens may be related to host specificity as described by Taraschewski (1985).

Isolation of *Mesostephanus appendiculatus* is in agreement with that obtained by Tantawy (1993), Aly et al. (2005) and El-Seify et al. (2005) from (rats, kittens and pigeons), (Chickens) and (rats and mice) infected by freshwater fish metacercariae, respectively.

Recovery of *Mesostephanus burmanicus* is similar to that detected by Azza (1994), El-Seify et al. (2005) and Lobna et al. (2010) but from other hosts (puppies, chickens, ducks and albino rats) infected with marine fish metacercariae, (rats and mice fed metacercariae from *O. niloticus* and *Clarias lazera*) and (puppies fed on *O. niloticus*).

Detection of *Haplorchis pumilus* is in agreement with Elsheikha and Elshazly (2008), Taher (2009) and Lobna et al. (2010) who obtained the same trematode genus but from puppies infected by brackish water fish metacercariae, dogs infected by *O. niloticus* and puppies fed on freshwater fishes, respectively.

Recording of *Procerovum calderoni* is in agreement with that obtained by Tantawy (1993) from experimentally infected rats, kittens and pigeons with fresh water fish metacercariae.

Recording of *Pygidiopsis genata* is the same as that recorded by Tantawy (1993), El-Ezz et al. (2000), Elsheikha and Elshazly (2008) and Lobna et al. (2010) from (rats, kittens and pigeons), (pigeon (*Columbia livia domestica*) and heron (*Ardea ibis ibis*), (puppies) and dogs, respectively after experimental infection.

The recovered *Prohemistomon vivax* is in agreement with that detected by Tantawy (1993), Azza (1994), Saba (2004), Aly et al. (2005), El-Seify et al. (2005), ElSheikha (2007), Taher (2009) and Alghabban
(2014) from (kittens, rats, pigeons), (puppies, chickens, ducks and albino rats), (puppies, chickens and ducklings), (chickens), (rats and mice), (puppies), (puppies) and (albino rats) fed on freshwater fish, marinewater fish, freshwater fish, Clarias gariepinus, (Oreochromis niloticus and Clarias gariepinus), brackish water fish oreochromis niloticus and cat fish, respectively. Sphaenidiotrema globulus agreed in its description with that given by McLaughlin et al. (1993). Maritrema orensense agreed in its description with that given by Alda et al. (2013).

The digenean trematode Cyathocotyle prussica is an intestinal parasite of wild aquatic birds such as the mallard, Eurasian coot, common pochard, ferruginous pochard and common moorhen (Sulcogostowska, 2007). Adults are frequent intestinal parasites of ducks while faucet snails (Bithynia tentaculata L., 1758) are known to serve as the first intermediate host of at least two species of Cyathocotyle including C. prussica. Sulcogostowska (2007) revealed 2 species belonging to the family Cyathocotylidae (Cyathocotyle prussica, Holostephanus dubinini) in the examined birds. Kvach et al. (2016) reported the presence of metacercariae of Cyathocotyle prussica in both muscle tissue and the peritoneal cavity of western tubenose goby (Proterorhinus semilimaris) from the River Dyje (Czech Republic).

**Prevalence of FZTs infection among the examined fishermen:** The public health importance of fish borne intestinal flukes is being increasingly noticed. Their wide geographic distribution, high morbidity rates have been underscored through studies from East to West; from Egypt (Abou-Basha et al., 2000; Lobna et al., 2010), from Korea (Chai and Lee, 1990), from Vietnam (Dung et al., 2007) and from Japan (Kino et al., 2002).

In the present study, the eggs of FZTs were detected in 26.7% of the examined fishermen’s fecal samples. Positive cases had a history of consumption of slightly salted fish.

Nearly similar result was reported by Abou-Basha et al. (2000) where the prevalence of heterophyasis was (33.8%) among inhabitants of El-Meaddeya, Egypt with the highest intensity of infection detected among fishermen. Lower results were recorded by Kino et al. (2002) in Shizuoka Prefecture, Japan and Lobna et al. (2010) in Northern Egypt who confirmed that the total prevalence of human heterophyasis infection was (9.6%) and (13.3%), respectively. The trematodes infection was correlated with the habit of eating raw fish (mullet and O. niloticus) particularly in fishermen and housewives. Also, Sohn et al. recorded that the positive rate for Small Trematode Eggs (STE) which may include H. taihu and other heterophyids, Opisthorchis viverrini and lecithodendriids was 15.2% in Lao. Chai and Lee (2002) in the Republic of Korea reported 12 species of the heterophyidae that included Pygidioptis summa, Stictodoras fuscata and S. lari among the examined humans. Ding et al. (2007) reported that H. pumilio was the most common trematode among human in Vietnam. They also reported H. pumilio, H. taihu, H. yokogawai and S. falcatus.

**Prevalence of cryptosporidiosis:** Cryptosporidium is one of the most common causes of diarrhea in animals and man (Monib et al., 2016; Ryan et al., 2014). In this study, emaciation, asites and haemorrhagic stomach and intestines in fishes with cryptosporidiosis were observed as what previously recorded by Ryan (2010), Certal et al., 2015, CFSPH, 2018.

The total prevalence of cryptosporidiosis in fin fish was 25%; C. gariepinus (24%) and L. carinata (30%). These results are lower than that obtained by Baraghare et al. (2011) who found 95% of Maculichanna pelleti pelleti to be affected with Cryptosporidium molnari, also Certal et al. (2015) found that the rate of infection with Cryptosporidium spp. among the examined fishes from Lake Geneva, France was 37%; they were distributed as (87%) C. parvum, (7%) C. molnari and (7%) mixed infection (C. parvum and C. molnari). This may be attributed to the difference of fish hosts and study areas.

Our result is higher than that recorded by Koinari et al. (2013) who investigated the prevalence of Cryptosporidium species in cultured freshwater (n = 132), wild freshwater (n = 206) and wild marine (n = 276) fish in Papua New Guinea (PNG) by PCR screening. They identified a total of seven fish (2 cultured freshwater, 1 wild freshwater and 4 wild marine fish) as positive for Cryptosporidium giving an overall prevalence of 1.14%. Of the seven positive isolates, five were identified as C. parvum and two were a novel piscine genotype. Also, Ammar and Araf (2013) recorded that the prevalence of Cryptosporidium in wild and cultured O. niloticus was 15.0 and 23.3%, respectively.

The prevalence of cryptosporidiosis among the examined shellfish was 24.5%; Penaeus japonicus (28%), Thais carnifera (30%) and Paphia andulata (20%). Mollusks are a potential source of Cryptosporidium parvum infection for humans (Tamburini and Pozio, 1999; Miller et al., 2005). Certal et al. (2015) found a little data for identification of Cryptosporidium species in wild aquatic environments. Also we found a shortage in the data about the prevalence of shellfish cryptosporidiosis in the world.

**Prevalence of cryptosporidiosis among the examined fishermen:** Cryptosporidium was detected in 20% of examined fishermen stool samples. Two studies in Iran carried out by Mirzaei (2007) and Heidarnejadi et al. (2012) showed that the overall prevalence of
Cryptosporidium spp. infection in humans was 10.8 and 14.5%, respectively. Youssef et al. (2008) reviewed nineteen studies that examined immunocompetent individuals with diarrhea presenting to inpatient or outpatient clinics in Egypt with a cryptosporidiosis prevalence ranging from 0-47%. Monib et al. (2016) detected Cryptosporidium spp. in 2.3% of children attending Assiut University Children’s Hospital, Egypt.

CONCLUSION

The present study revealed that FZTs and cryptosporidiosis are prevalent among the examined fishermen, fish and shellfish species. So, more efforts to control infection should be implemented that include sanitary disposal of human stool, health education of individuals (avoid defecation in water courses, abandon eating raw, slightly salted or insufficiently cooked fish and shellfish) and control of stray dogs and cats.

REFERENCES


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