Journal of Animal and Veterinary Advances 18 (6): 175-186, 2019
ISSN: 1680-5593

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

[1]Hanaa Mohamed Fadel, [2] M.M Maather El-Lamie and [3] Nahla Hamed Sallam
[1]Department of Animal Hygiene and Zoonoses,
[2]Department of Fish Diseases and Management,
[3]Department of Parasitology, Faculty of Veterinary Medicine,
Suez Canal University, Ismailia, Egypt

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 30%) 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 haemorrages along the stomach and intestine. Experimental infection in pigeons and rats, revealed *Brachylaema migrans* from pigeons fed on *Penaeus japonicus* and (*Mesostephanus appendiculatus*, *Mesostephanus burmanicus*, *Procervum calderoni, Stictodora tanayensis, Apophallus donicus, Pygidiopsis genata*, *Maritrema orensense*, *Sphaeridiotrema globules*, *Prohemistomum vivax* and *Haplorchis pumilio*) 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: *Braunia cordiformis*, *Cyathocotyle prussica* and *Holostephanus dubinini*.

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 protozoses (Certad *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) (Serbina, 2014; Chantima *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

Corresponding Author: Hanaa Mohamed Fadel, Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

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 (Nouh *et al*., 2010). As they threat 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 can 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 food, decreasing in growth rates, emaciation, whitening of feces and ascites (CFSPH, 2018). *C. parvum* oocysts can survive in seawater for at least 1 year (Tamburrini 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 (*Thaiscarnifera*) and 200 bivalves (*Paphia andulata*) 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 Noga (2010).

Detection of protozoal infection

Preparation of smears: After sedimentation of human stool samples by formalin-ethyl acetate concentration method (Anh *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 concentred 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 metacercaiae grossly using a magnified hand lens (Mahdy *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 metacercareal 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 Directives86/609/EEC of the

24th November, 1986). A total of 20 one week old pigeon were used for infection of shrimps with digested fluid containing metacercaiae. They were reared in two groups (15 infected and 5 control), left 1 week under microscopical inspection of their feces. Also, ten ducks and 15 chickens were fed with EMC obtained from shrimp. In addition to, thirty white rats 3 weeks old that were divided equally in six groups, they were infected with digested fluid of cat fish, 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. Sacrification 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% ethyl alcohol for genotypic analysis and formalin 5% for staining with acetocarmine and morphologic identification (Yamaguti, 1995; 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 AGAGCGCAGCCAACTGTGTGA. AP102 R TGCCACGTCCTAGCATCAGCC. 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 prestained 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 sliminess with haemorrages 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 infested *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.

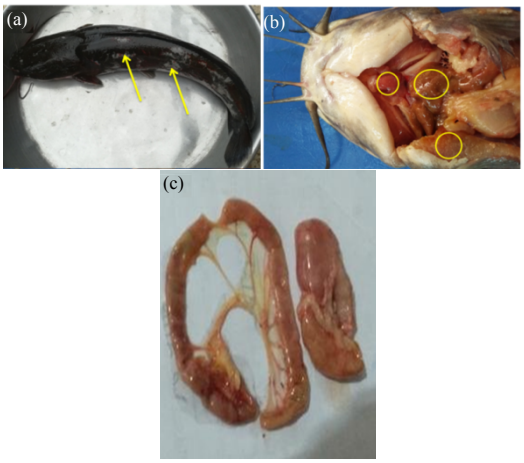

Fig. 1: Clarias gariepinus showing: (a) Abrasions and haemorrhages all over the body (arrows) (b) Minute white nodules embedded hardly in heart, liver and musculature tissues (circles) and (c) Haemorrhagic stomach and intestine

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 χ^2 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

	Examined	Metacercarial infection		Cryptosporidiosis	
Aquatic spp.	N	N	Percentage	N	Percentage
Cat fish (*Clarias gariepinus*)	100	74	74.0	24	24.0
Keeled mullet (*Liza Carinata*)	20	1	50.0	6	30.0
Total fin fish	120	75	62.5	30	25.0
Shrimp (*Penaeus japonicus*)	100	50	50.0	28	28.0
Gastropod) (Thais carnifera	100	20	20.0	30	30.0
Bivalve (*Paphia andulata*)	200	120	6 0.0	40	20.0
Total shellfish	400	190	47.5	98	24.5

$X^2 = 83.078$, $p<0.0001$; $X^2 = 4.809$, $p = 0.3057$

Table 2: Prevalence of FZTs and cryptosporidiosis infection in fishermen

Examined	FZTs infection		Cryptosporidiosis	
N	N	Percentage	N	Percentage
15	4	26.7	3	20

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. Testis 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 *Clarias 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 unconspicuous. 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).

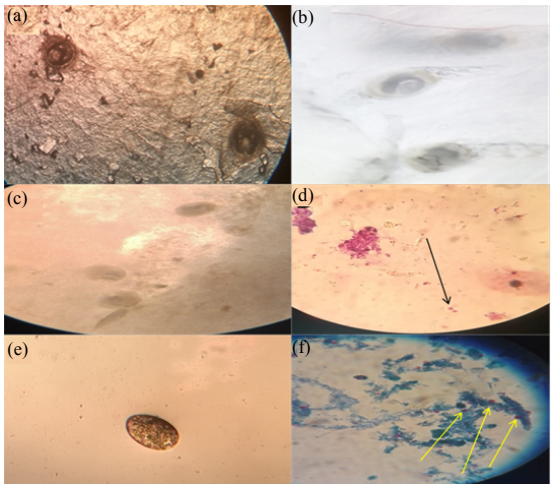

Fig. 2: Unidentified EMC: (a) *Clarias garipinius* musculature (X = 4), (b) *Penaeus japonicas* musculature (X = 10), (c) *Thais carnifera* (X = 100), (d) Acid fast *Cryptosporidium* sp. oocysts from*C. garipienius* intestinal smear (arrow) (X = 100). Human fecal smear with, (e) minute trematode egg (X = 10) and (f) Acid fast *Cryptosporidium* sp. oocysts (arrows) (X = 100)

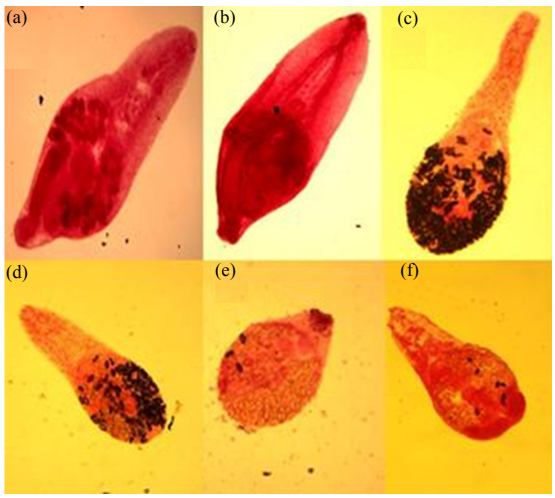

Fig. 3: (a) *Mesostephanus appendiculatus*, (b) *Mesostephanus burmanicus*, (c) *Procervum calderoni*, (d) *Stictodora tanayensis*, (e) *Apophallus donicus* and (f) *Pygidiopsis genata*

***Procervum calderoni*:** Encysted metacercariae were obtained from musculatures and body viscera of *Clarias gariepinus*, the adult worm was obtained experimentally from rats, its body is elongated and moderately expanded. Cuticle aspinosed, 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).

***Stictodora 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 intracacal, 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 testis. 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 ceca 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

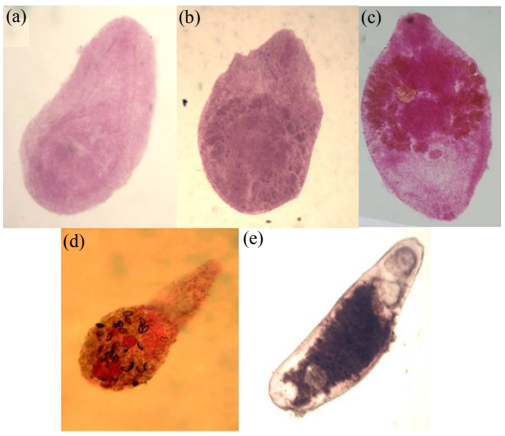

Fig. 4: (a) *Maritrema orensense*, (b) *Sphaeridiotrema globules*, (c) *Prohemistomum vivax* (d) *Haplorchis pumilio* and (e) *Brachylaema migrans*

adult worm was obtained experimentally from rats. Adult is compact oval or spherical with much larger ventral sucker than the oral one, located in the mid body. Pharynx is large and ovoid. Testes ovoid in transverse position one behind the other. Vitellaria are large follicle; obscure other organ, not extending to the posterior end (Fig. 4b).

***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).

***Haplorchis 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, taprering anteriorly and broad posteriorly. Long prepharynx and esophagus intestinal bifurcation at the beginning of middle

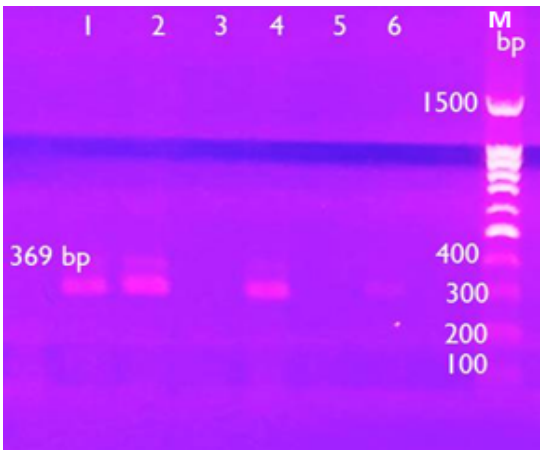

Fig. 5: PCR amplification of 369 bp of ITS2 region. Lanes 1, 2, 4, 6 Positive samples. M 100 bp DNA ladder

third. Intestinal caeca end posterior to middle, one testis at posterior half and the ovary is anterior to it (Fig. 4d).

***Brachylaema migrans*:** Encysted metacercariae were obtained from the musculature of shrimps and the adult was obtained from pigeons experimentally, it is more or less elongate, rounded anteriorly and tapers posteriorly with smooth body. The ventral sucker lies within the anterior third of the body. The testes are irregularly rounded; the posterior one is median and the anterior one lies to the left of the mid-line while the ovary lies between them. The vitellaria consist of fine follicles extending in the lateral fields forwards from the level of the posterior testis (Fig. 4e).

Results of protozoal infestation

***Cryptospordium* sp.:** Oocysts of *Cryptospordium* sp. are acid fast, spherical with a diameter of 4-6 µm (Fig. 2d, f).

Results of PCR and sequencing: PCR targeting ITS2 (internal transcribed spacer) region of doubtful trematodes was performed (Fig. 5). Bidirectional sequencing revealed the following worms: *Braunia cordiformis*, *Cyathocotyle prussica*, *Holostephanus dubinini*.

Fish borne zoonotic trematodes reside mainly in the liver or intestine of vertebrates including humans (Chai *et al*., 2009; Hung *et al*., 2015). People get infected with FZTs if they eat raw or undercooked freshwater, brackish water fish, mussels or shrimps containing viable metacercariae (Yu and Mott, 1994; Keiser and Utzinger 2009; Lobna *et al*., 2010; Yang and Peng, 2012).

In this study, the clinical picture of EMC infested fin fishes included slimeness with haemorrages and abrasions 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. gariepinius*; *Oreochromis niloticus*, *Bagrus bajad* and *Cyprinius carpio* fihses, 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 affection 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. garipienius* and *Cyprinus carpio*, respectively.

Regarding, the metacercaiae 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 metacercaial cysts attached with antennae and appendages of *Penaeus indicus* and *Penaeus semisculcatus* 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-Seify *et al*., 2005; Lobna *et al*., 2010; Ammar and Arafa, 2013; El Sayad *et al*., 2014; Hamouda, 2018; Kirrella *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 bayad* 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, Alexanderia); (42 in *Bagrus bajad* from several localities in

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 farmed 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 *Schilbe mystus* and *Mormyrus niloticus* from the River Nile, 78.25% from *O. niloticus* in Egypt, 80.30% among *O. niloticus* in Sharkeya and Abbasa, 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 carnifera* (20%) and *Paphia andulata* (60%). Chai and Lee (2002) reported that brackish water bivalves are the source of infection with *Acanthoparyphium tyosenense*, 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 *Echinoparyphium aconiatum* and *E. recurvatum* and *Cyathocotyle bithyniae*. Anew cercariae *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 *Brachylaema migrans* from pigeons fed on shrimps and (*Mesostephanus appendiculatus*, *Mesostephanus burmanicus*, *Procervum calderoni, Stictodora tanayensis, Apophallus donicus, Pygidiopsis genata*, *Maritrema orensense*, *Sphaeridiotrema globules*, *Prohemistomum vivax* and *Haplorchis pumilio*)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) infested by freshwater fish metacercariae, respectively.

Recovering 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 metacercaiae from *O. niloticus* and *Clarias lazera*) and (puppies fed on *O. niloticus*).

Detection of *Haplorchis pearsoni* 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 (*Columba livia domestica*) and heron (*Ardea ibis* ibis), (puppies) and dogs, respectively after experimental infection.

The recovered *Prohemistomum 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 garipinius, (Oreochromis niloticus and Clarias garipinius*), brackish water fish *oreochromis niloticus* and cat fish, respectively. Sphaeridiotrema 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 (Sulgostowska, 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*. Sulgostowska (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 semilunaris*) 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 heterophyiasis 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 heterophyes infection was (9.6%) and (13.3%), respectively. The trematodes infection was correlated with the habit of eating raw fish (mullet and *O. noliticus*) particularly in fishermen and housewives. Also, Sohn *et al.* recorded that the positive rate for Small Trematode Eggs (STE) which may include *H. taichui* 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 *Pygidiopsis summa*, *Stictodora fuscata* and *S. lari* among the examined humans. Dung *et al.* (2007) reported that *H. pumilio* was the most common trematode among human in Vietnam. They also reported *H. pumilio*, *H. taichui*, *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, ascites and haemorrhagic stomach and intestines in fishes with cryptosporidiosis were observed as what previously recorded by Ryan (2010), Certad *et al.*, 2015; CFSPH, 2018).

The total prevalence of cryptosporidiosis in fin fish was 25%; *C. gariepinius* (24%) and *L. carinata* (30%). These results are lower than that obtained by Barugahare *et al.* (2011) who found 95% of *Maccullochella peelii* peelii to be affected with *Cryptosporidium molnari*, also Certad *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 Arafa (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 (Tamburrini and Pozio, 1999; Miller *et al.*, 2005). Certad *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 Heidarnegadi *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

Abdel-Latif, A.M., 2007. Gill parasitic diseases of some freshwater fishes. Ph.D Thesis, Benha University, Banha, Egypt.

Abou-Basha, L.M., M. Abdel-Fattah, P. Orecchia, D.D. Cave and A. Zaki, 2000. Epidemiological study of heterophyiasis among humans in an area of Egypt. Eastern Mediterranean Health J., 6: 932-938.

Alda, P., N. Bonel, R.F. Hechinger and S.R. Martorelli, 2013. Maritrema orensense and Maritrema bonaerense (Digenea: Microphallidae): descriptions, life cycles and comparative morphometric analyses. J. Parasitol., 99: 218-228.

Alghabban, A.J.M., 2014. Fish Farms as a source for parasites transport: Parasitological and developmental studies of *Prohemistomum vivax* with the ameliorating role of *Moringa oleifera* in the treatment. J. Am. Sci., 10: 6-14.

Aly, S., E. Ismail, B. Ahmed, M. Elamie and B. Hussein, 2005. Pathological studies on encysted metacercariae infections among some freshwater fish in egyptian aquaculture. Duetscher Tropentag, 5: 11-13.

Ammar, M.A.M. and M.I. Arafa, 2013. Cryptosporidium and other zoonotic parasites in *Oreochromis niloticus* in Assiut governorate. Assiut Vet. Med. J., 59: 142-151.

Anh, N.T.L., N.T. Phuong, G.H. Ha, L.T. Thu and M.V. Johansen *et al.*, 2008. Evaluation of techniques for detection of small Trematode eggs in faeces of domestic animals. Vet. Parasitol., 156: 346-349.

Anh, N.T.L., N.T. Phuong, M.V. Johansen, K.D. Murrell and P.T. Van *et al.*, 2009. Prevalence and risks for fishborne zoonotic Trematode infections in domestic animals in a highly endemic area of North Vietnam. Acta Trop., 112: 198-203.

Arya, L.K., S.R. Rathinam, P. Lalitha, U.R. Kim and S. Ghatani *et al.*, 2016. Trematode fluke Procerovum varium as cause of ocular inflammation in children, South India. Emerging Infect. Dis., 22: 192-200.

Azza, M.R., 1994. Role of marine fish in transmission of some parasites to animals and birds. Ph.D Thesis, Zagazig University, Zagazig, Egypt.

Barugahare, R., M.M. Dennis, J.A. Becker and J. Slapeta, 2011. Detection of Cryptosporidium molnari oocysts from fish by fluorescent-antibody staining assays for Cryptosporidium spp. affecting humans. Appl. Environ. Microbiol., 77: 1878-1880.

CFSPH., 2018. Cryptosporidiosis. Center for Food Security and Public Health, Iowa State University, Ames, Iowa. http://www.cfsph.iastate.edu/Factsheets/pdfs/cryptosporidiosis.pdf

Carella, F., J. Culurgioni, S. Aceto, G. Fichi and T. Pretto *et al.*, 2013. *Postmonorchis* sp. inq (Digenea: Monorchiidae) metacercariae infecting natural beds of wedge clam *Donax trunculus* in Italy. Dis. Aquat. Org., 106: 163-172.

Casemore, D.P., 1991. ACP broadsheet 128: June 1991 laboratory methods for diagnosing cryptosporidiosis. J. Clin. Pathol., 44: 445-451.

Certad, G., J. Dupouy-Camet, N. Gantois, O. Hammouma-Ghelboun and M. Pottier *et al.*, 2015. Identification of Cryptosporidium species in fish from Lake Geneva (Lac Leman) in France. PloS One, 10: 1-13.

Chai, C.Y., 2007. Intestinal Flukes. In: Food-Borne Parasitic Zoonoses: Fish and Plant-Borne Parasites, Murrell, K.D. and B. Fried (Eds.). Springer, New York, USA., ISBN-13:978-0-387-71357-1, pp: 53-115.

Chai, J.Y. and S.H. Lee, 1990. Intestinal trematodes of humans in Korea: Metagonimus, heterophyids and echinostomes. Korean J. Parasitol., 28: 103-122.

Chai, J.Y. and S.H. Lee, 2002. Food-borne intestinal trematode infections in the republic of Korea. Parasitol. Int., 51: 129-154.

Chai, J.Y., E.H. Shin, S.H. Lee and H.J. Rim, 2009. Foodborne intestinal flukes in Southeast Asia. Korean J. Parasitol., 47: S69-S102.

Chantima, K., K. Suk-Ueng and M. Kampan, 2018. Freshwater snail diversity in Mae Lao agricultural basin (Chiang Rai, Thailand) with a focus on larval trematode infections. Korean J. Parasitol., 56: 247-257.

Chi, T.T.K., A. Dalsgaard, J.F. Turnbull, P.A. Tuan and K.D. Murrell, 2008. Prevalence of zoonotic trematodes in fish from a Vietnamese fish-farming community. J. Parasitol., 94: 423-428.

Cho, S.H., W.J. Lee, T.S. Kim, W.S. Seok and T. Lee *et al.*, 2014. Prevalence of zoonotic Trematode metacercariae in freshwater fish from Gangwon-do, Korea. Korean J. Parasitol., 52: 399-412.

Dung, D.T., N.V. De, J. Waikagul, A. Dalsgaard and J.Y. Chai *et al*., 2007. Fishborne zoonotic intestinal trematodes, Vietnam. Emerging Infect. Dis., 13: 1828-1833.

El Sayad, M.H., S.A.A. Holw, O.G. Yassine and H.A. El-Taweel, 2014. Heterophyid metacercariae in free living and farmed fish of El-Max Bay, West of Alexandria, Egypt. Parasitologists U. J., 7: 110-115.

El-Ezz, N.M.T.A., E.A. Tantawy, O.A. Mahdy and A.A. El-Massry, 2000. Studies on heterophyid infections among some fishes in Egypt. Egypt. J. Vet. Sci., 34: 11-29.

El-Seify, M.A., A.R.Y. Desouky and E.K. Bazh, 2005. Epidemiological studies on some fish-borne parasites. Egypt. Vet. Med. Soc. Parasitol. J., 2: 570-592.

ElSheikha, H.M., 2007. Heterophyosis: Risk of ectopic infection. Vet. Parasitol., 147: 341-342.

Elsheikha, H.M. and A.M. Elshazly, 2008. Preliminary observations on infection of brackish and fresh water fish by heterophyid encysted metacercariae in Egypt. Parasitol. Res., 103: 971-977.

Francisco, C.J., A. Almeida, A.M. Castro, S. Pina and F. Russell-Pinto *et al*., 2011. Morphological and molecular analysis of metacercariae of Diphtherostomum brusinae (Stossich, 1888) Stossich, 1903 from a new bivalve host *Mytilus galloprovincialis*. J. Helminthol., 85: 179-184.

Garcia, L.S., 2001. Diagnostic Medical Parasitology. 4th Edn., American Society for Microbiology, Washington, DC.

Goulding, D.R., T.L. Blankenship-Paris, G.A. Lewbart, P.H. Myers and T.K. Demianenko *et al*., 2004. Gill trematodes (flukes) in wild-caught killifish (*Fundulus heteroclitus*). J. Am. Assoc. Lab. Anim. Sci., 43: 32-34.

Hamouda, A.H., 2018. Epizootiological studies on some parasitic infections in *Bagrus bajad* from Lake Nasser, Egypt. Alexandria J. Vet. Sci., 58: 40-47.

Heidarnegadi, S.M., M. Mohebali, S. Maraghi, Z. Babaei and S. Farnia*et al*., 2012. *Cryptosporidium* spp. infection in human and domestic animals. Iran. J. Parasitol., 7: 53-58.

Hung, N.M., D.T. Dung, N.T.L. Anh, P.T. Van and B.N. Thanh *et al*., 2015. Current status of fish-borne zoonotic Trematode infections in Gia Vien district, Ninh Binh province, Vietnam. Parasites Vectors, 8: 1-10.

Keiser, J. and J. Utzinger, 2009. Food-borne trematodiases. Clin. Microbiol. Rev., 22: 466-483.

Khalil, M.I., I.S. El-Shahawy and H.S. Abdelkader, 2014. Studies on some fish parasites of public health importance in the Southern area of Saudi Arabia. Rev. Bras. Parasitol. Vet., 23: 435-442.

Kino, H., H. Oishi, Y. Ohno and M. Ishiguro, 2002. An endemic human infection with *Heterophyes nocens* Onjiet Nishio 1916 at Mikkabi-cho, Shizuoka, Japan. Jpn. J. Trop. Med. Hyg., 30: 301-304.

Kirrella, G.A.K., N.M. Elhawary, E.K. Bazh, S.S.G. Sorour and K.M. El-Dakhly, 2018. *Oreochromis niloticus* and *Clarias gariepinus* fish infected with digenean metacercariae: Experimental infection of puppies and some chemical alterations in fish. Asian J. Anim. Vet. Adv., 13: 352-359.

Koinari, M., S. Karl, J. Ng-Hublin, A.J. Lymbery and U.M. Ryan, 2013. Identification of novel and zoonotic Cryptosporidium species in fish from Papua New Guinea. Vet. Parasitol., 198: 1-9.

Kotloff, K.L., J.P. Nataro, W.C. Blackwelder, D. Nasrin and T.H. Farag *et al*., 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (The Global Enteric Multicenter Study, GEMS): A prospective, case-control study. Lancet, 382: 209-222.

Kvach, Y., M. Ondrackova and P. Jurajda, 2016. First report of metacercariae of Cyathocotyle prussica parasitising a fish host in the Czech Republic, Central Europe. Helminthologia, 53: 257-261.

Lassalle, G., X.D. Montaudouin, P. Soudant and C. Paillard, 2007. Parasite co-infection of two sympatric bivalves, the Manila clam (Ruditapes philippinarum) and the cockle (*Cerastoderma edule*) along a latitudinal gradient. Aquat. Living Resour., 20: 33-42.

Lobna, S.M.A., Y.F. Metawea and H.M. Elsheikha, 2010. Prevalence of heterophyiosis in Tilapia fish and humans in Northern Egypt. Parasitol. Res., 107: 1029-1034.

Mahdy, O.A., M.A. Essa and M. El-Easa, 1995. Parasitological and pathological studies on the heterophyid infection in Tilapia species from Manzala Lake, Egypt. Egypt J. Comp. Pathol. Clin. Pathol., 8: 131-145.

McLaughlin, J.D., M.E. Scott and J.E. Huffman, 1993. *Sphaeridiotrema globules* (Rudolphi, 1814) (Digenea): Evidence for two species known under a single name and a description of *Sphaeridiotrema pseudoglobulus* n.sp. Can. J. Zool., 71: 700-707.

Meyers, T. and T. Burton, 2009. Diseases of Wild and Cultured Shell Fish in Alaska. Alaska Department of Fish and Game, Juneau, Alaska, USA., Pages: 136.

Miller, W.A., E.R. Atwill, I.A. Gardner, M.A. Miller and H.M. Fritz*et al*., 2005. Clams (*Corbicula fluminea*) as bioindicators of fecal contamination with Cryptosporidium and Giardia sppi. in freshwater ecosystems in California. Intl. J. Parasitol., 35: 673-684.

Mirzaei, M., 2007. Prevalence of *Cryptosporidium* sp. infection in diarrheic and non-diarrheic humans in Iran. Korean J. Parasitol., 45: 133-137.

Monib, M.E.M.M., A.A.A.E. Hassan, R.A.E.H. Attia and M.M. Khalifa, 2016. Prevalence of intestinal parasites among children attending Assiut University Children's Hospital, Assiut, Egypt. J. Adv. Parasitol., 3: 125-131.

Morishita, K., Y. Komiya and H. Matsubayashi, 1965. Progress of Medical Parasitology in Japan. Vol. 2, Meguro Parasitological Museum, Tokyo, Japan,.

Noga, E.J., 2010. Fish Disease Diagnosis and Treatment. 2nd Edn., Blackwell Publicationi, USA.

Nouh, W.G., S.M. Aly, K. Abdel-Rahman and O.H. Amer, 2010. Histopathological, parasitological and molecular biological studies on metacercariae from *Oreochromis niloticus* and *Clarias gariepinus* cultured in Egypt. Zagazig Vet. J., 38: 92-105.

O'Donoghue, P.J., 1995. Cryptosporidium and cryptosporidiosis in man and animals. Intl. J. Parasitol., 25: 139-195.

Rao, P.V. and S.C. Soni, 1988. Diseases and parasites of penaeid prawns of India: A short review. J. Indian Fish. Assoc., 18: 289-298.

Reames, E., 2012. Nutritional benefits of seafood. Southern Regional Aquaculture Center, Stoneville, Mississippi. https://agrilifecdn.tamu.edu/fisheries/files/2013/09/SRAC-Publication-No.-7300-–-Nutritional-Benefits-of-Seafood.pdf

Reda, R.M., G.A. El-Nobi, M.E. Hassanin, M.A. El-Hady and Z.M. El-Bouhy, 2010. Study on some encysted metacercaria digenetic trematodes affecting gills of *Oreochromus niloticus*. Proceedings of the 10th International Conference on Scientific Veterinary Medical, February 10-13, 2010, Zagazig University, Zagazig, Egypt, pp: 518-525.

Ryan, U., R. Fayer and L. Xiao, 2014. *Cryptosporidium* species in humans and animals: Current understanding and research needs. Parasitology, 141: 1667-1685.

Ryan, U.M., 2010. *Cryptosporidium* in birds, fish and amphibians. Exp. Parasitol., 124: 113-120.

Saad, A.I., 2007. A comparative study of different metacercarial infections in two freshwater fishes from Lake Nasser, Nile river and hatcheries at Aswan-Egypt. J. Egypt. Ger. Soc. Zool., 51: 107-125.

Saba, S.E.R., 2004. Some studies on parasites encystations of fresh water fishes. Ph.D Thesis, Zagazig University, Zagazig, Egypt.

Schaperclaus, W., 1992. Fish Diseases. 1thEdn.,/Vol. 2, CRC Press, Boca Raton, Florida, USA., ISBN-13:978-9061919520, Pages: 164.

Seppala, O., A. Karvonen, M. Kuosa, M. Haataja and J. Jokela, 2013. Are sick individuals weak competitors? Competitive ability of snails parasitized by a gigantism-inducing Trematode. PloSOne, 8: 1-6.

Serbina, E.A., 2014. Larval trematodes in bithyniid snails (Gastropoda: Bithyniidae) in the lake-rivers systems from the steppe zone (The West Siberian Plain, Russia). Helminthologia, 51: 293-300.

Sulgostowska, T., 2007. Intestinal digeneans of birds (superfamily Diplostomoidea) of the Masurian Lakes. Wiadomosci Parazytologiczne, 53: 117-128.

Sumuduni, B.G.D., D.H.N. Munasinghe and A. Arulkanthan, 2018. Chronological analysis of the damages caused by the metacercariae of *Centrocestus formosanus* in the gills of *Cyprinus carpio* and lesions caused by the adult flukes in *Ardeola ralloides* : An experimental study. Intl. J. Vet. Sci. Med., 6: 165-171.

Taher, G.A., 2009. Some studies on metacercarial infection in *Oreochromis niloticus* in Assiut Governorate and their role in transmission of some trematodes to dogs. Assiut Univ. Bull. Environ. Res., 12: 63-79.

Tamburrini, A. and E. Pozio, 1999. Long-term survival of *Cryptosporidium parvum* oocysts in seawater and in experimentally infected mussels (*Mytilus galloprovincialis*). Intl. J. Parasitol., 29: 711-715.

Tantawy , E.A., 1993. Muscular parasites in market fishes. MVSc Thesis, Cairo University, Giza, Egypt.

Taraschewski, H., 1985. Transmission experiments on the host specificity of Heterophyes species in 16 potential definitive hosts. Z. Parasitenkunde, 71: 505-518.

WHO., 1995. Control of Food Borne Trematode Infections: Report of a WHO Study Group. Vol. 849, World Health Organization, Geneva, Switzerland, ISBN:9789241208499, Pages: 157.

Willis, J.E., J.T. McClure, J. Davidson, C. McClure and S.J. Greenwood, 2013. Global occurrence of Cryptosporidium and Giardia in shellfish: Should Canada take a closer look?. Food Res. Intl., 52: 119-135.

Wiriya, B., J.H. Clausen, T. Inpankaew, U. Thaenkham and S. Jittapalapong *et al*., 2013. Fish-borne trematodes in cultured Nile tilapia (*Oreochromis niloticus*) and wild-caught fish from Thailand. Vet. Parasitol., 198: 230-234.

Yamaguti, S., 1995. Systema Helminthum: The Digenetic Trematodes of Vertebrates. Vol. 1, Interscience Publishers Inc., New York, USA., Pages: 979.

Yang, Q. and L. Peng, 2012. A laryngeal fluke. N. Engl. J. Med., 367: 1-1.

Yooyen, T., C. Wongsawad, K. Kumchoo and M. Chaiyapo, 2006. A new record of *Clinostomum philippinensis* (Valasquez, 1959) in *Trichogaster microlepis* (Gunther, 1861) from Bung Borapet, Nakhon Sawan, Thailand. Southeast Asian J. Trop. Med. Public Health, 37: 99-103.

Younis, A.A., A.E.T.F. Gharib and E.A.A. Tantawy, 2009. Studies on some prevailing parasites affecting *Oreochromis niloticus* fingerlings with a trial of treatment. Egypt J. Aquat. Biol. Fish., 13: 135-148.

Youssef, F.G., I. Adib, M. Riddle and C. Schlett, 2008. A review of cryptosporidiosis in Egypt. J. Egypt. Soc. Parasitol., 38: 9-28.

Yu, S.H. and K.E. Mott, 1994. Epidemiology and morbidity of food-borne intestinal Trematode infections. Trop. Dis. Bull., 91: R125-R152.