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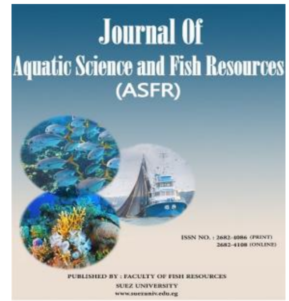

Control Measure of Fishborne Zoonotic Trematodes: A Review

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ABSTRACT

Fishborne zoonotic trematodes (FZT) were classified into small intestinal, lung, and liver flukes. Some species of the family Heterophyidae, Echinostomatidae, Clinostomidae, and Diplostomidae which considered to be fishborne zoonotic intestinal flukes infect the health of both fish and humans. Some species of the family Heterophyidae parasitize fish and cause diseases to humans when eating them. Symptoms are differed in patients with echinostomiasis, including anemia, stomach ache, headache, and loose stools. In heavy infections, there are other symptoms such as profuse watery diarrhea, easy fatigue, eosinophilia, edema, loss of body weight, and anorexia. Meanwhile, the important symptoms of Clinostomidae digenetic trematode are Yellow grub disease which is transmitted to humans via ingestion of raw or inappropriately cooked fish leading to Halazoun-like disease "laryngopharyngitis" that can cause death from asphyxiation. There are variable processing methods that can control in human disease by encysted metacercariae (EMC) such as grilling, frying, heating and cooking, boiling, freezing, and chilling. Grilling of some infected fish with EMC for 5-20 minutes at 60-80˚C and 90 °C for at least 5 min was sufficient to destroy the EMC. Frying for 10 minutes killed all EMC too. Heating for 15 min at 60 °C, 100 °C, and 180 °C can destroy all metacercariae. Cooking at 250°C for 15-20 min can decrease the viability of EMC. The complete destruction of EMC occurred after chilling at 4 °C for above 24 and over 48 hours. Freezing at -4C° to -5C° for 10 days will keep the fish completely safe for consumers.

1. Introduction

Trematoda (leaf-shaped) is a class of the Platyhelminthes (flat worms) phylum. The parasitic flatworms known as "flukes" with metacercariae as their infective stage make up the majority of it (**Bardhan, 2022).** Fishborne zoonotic trematodes (FZT) that infect humans were classified into small liver flukes (*Clonorchis sinensis*, *Opisthorchis* spp., *Fasciola* spp.), lung flukes (*Paragonimus* spp.) and intestinal flukes.

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Among 47 fishborne zoonotic intestinal flukes, species of 3 families were detected in humans and they were (Heterophyidae has 36 species; Echinostomatidae has 10 species, and Nanophyteidae has one species) (**Hung *et al.,* 2013).** Digenetic trematodes and their metacercariae were considered one of the most common parasites infecting fish causing low weight gain, high mortality, and unmarketability (**Hassan *et al.,* 2012)**. Encysted metacercariae can infect *Oreochromis niloticus* and *Clarias gariepinus* and cause black to orange nodules on the infected organs, respiratory disorder, loss of scales and excessive mucus (**Aly *et al.,* 2005)**. All digenetic trematodes have similar life cycles that involve a definitive host (human, dog, or cat), with snails and fish as intermediate hosts as shown in Fig. 1 59 species from over 100 trematode species which know to infect humans are stated as Fish-borne zoonotic trematode (FZT). FZT infections influence the health of above 50 million humans worldwide **(Fürst *et al.,* 2012).** Human infection with EMC are asymptomic or unknown. There are symptoms such as damage to the intestinal mucosa, bloody diarrhea, abdominal pains, and when eggs enter the lymph vascular system and migrate to the numerous organs, they cause granuloma and fibrosis (**Lobna *et al.,* 2010**).

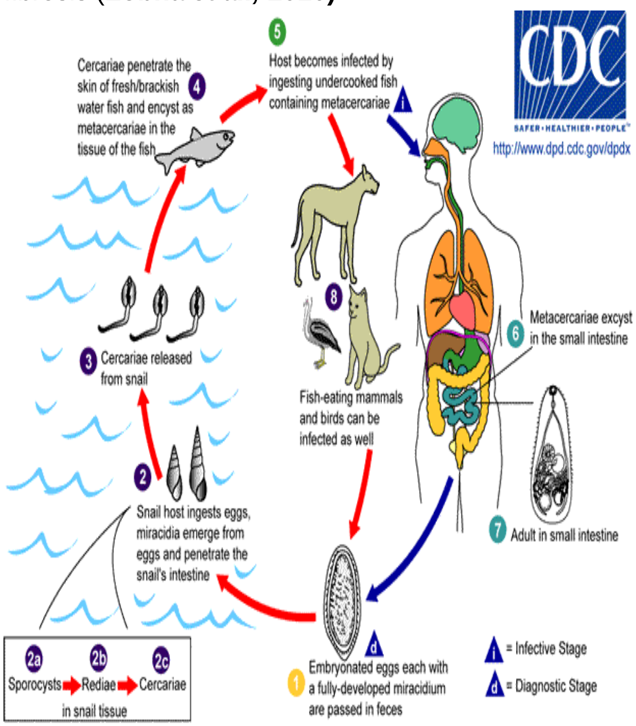

Fig. 1. Life cycle of digenetic trematodes **(CDC, 2019).**

2. Fish-Born Zoonotic Trematodes (FZT) of Family: Heterophyidae

The species of the family Heterophyidae have small sizes which less than 0.5 mm in length so they know to be minute intestinal flukes. Fish-eating birds, mammals and humans are the final host and adult worms are found in them **(Sohn and Chai, 2005).** The first intermediate host of heterophyid parasites is snails, the second intermediate host is several fish species which comprising the larval EMC, and the definitive host is fish-eating birds, mammals and humans which having the adult fluke causing heterophyiosis as shown in Fig. 2 **(Simões *et al.,* 2010).**

The most common species of family Heterophyidae in Egypt are *Centrocestus unequiorchalis* (**Saad, 1994),** *Heterophyes dispar* **(Murrell and Fried, 2007),** *Centrocestus cuspidatus* (**Bowman *et al.,* 2008),** *Heterophyes heterophyes*, *Heterophyes aequalis*, *Pygidiopsis genata*, *Haplorchis pumilio, Haplorchis taichui*, *Haplorchis yokogawi*, *Stictidora tridactyla, Phagicoal longicollis*, *Phagicoal ascolonga* and *Phagicoal italica* (**ElSheikha and El-Sahazly, 2008b).**

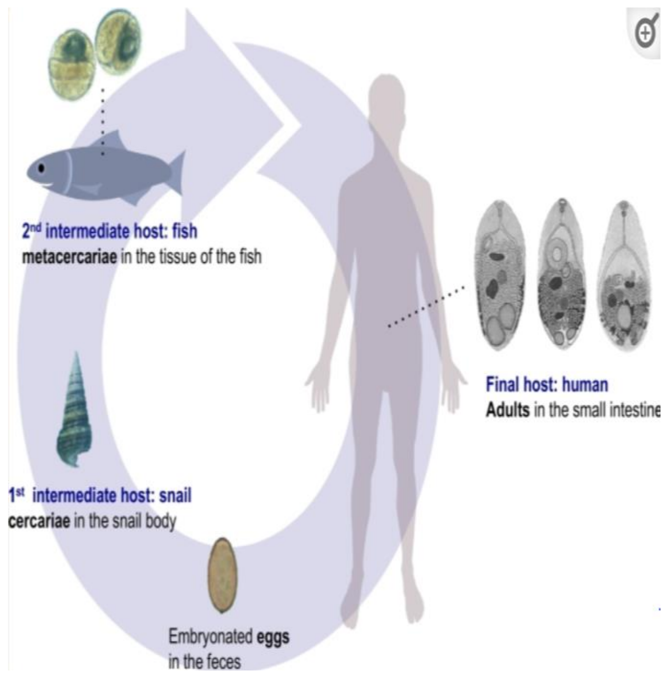

Fig. 2. life cycle of fishborne zoonotic heterophyid trematodes **(Chai and Jung 2017).**

The cercariae of *Heterophyes heterophyes* pass in the scales of brackish or freshwater fish such as tilapia, mullets*,* gobies and others fish, then encyst in the muscle of these fish host (**Paperna and Overstreet, 1981).** The cercariae of *H. nocens* pass in the scales of brackish water fish such as mullets and gobies (**Sohn, 2009**).

Chai and Jung (2017) reported that *Heterophyes dispar* was detected in *Mugil spp., Tilapia spp., Sciaena aquilla, Lichia spp., Barbus canis,* and *Epinephelus enaeus*. *Heterophyes aequalis* was detected in *M. cephalus, M. auratus, M. capito, Tristramella simonis* and *Lichia glauca*). While *Pygidiopsis genata* was found in brackish water fish such as *Barbus canis, Tilapia spp*. and

Mugil capito meanwhile the *Stictodora fuscata* was found in redlip mullets, mullets, and gobies.

The metacercariae of *Pygidiopsis summa* were found in the muscles and gills of *Mugil cephalus* and *Liza menada*, redlip mullets, and *Acanthogobius flavimanus* (**Hung *et al.,* 2013).** While the metacercariae of *H. taichui* and *H. pumilio* found in *Tilapia ouria, Tilapia zilii, Tilapia galilae,* and *Tilapia nilotica* in Egypt while the *H. yokogawai* metacercariae were found in *Tilapia nilotica* and *Tilapia Zilli* from Dakhalia Governorate **(Chai, 2019).**

Heterophyid species are exclusively fish-borne and infect humans by eating raw or wrongly cooked fish **(Chai, 2014)**. There are 29 fishborne heterophyid species be a member of 13 genera worldwide. They are *M. yokogawai, M. miyatai, M. katsuradai,* and *M. minutus* from genus Metagonimus. *H. heterophyes, H. dispar, H. nocens,* and *H. aequalis* from genus Heterophyes. *H. taichui, H. yokogawai, H. pumilio,* and *H. vanissimus* from genus Haplorchis. *P. summa and P. genata* from genus Pygidiopsis. *H. continua* from genus Heterophyopsis. *S. falcatus* from genus Stellantchasmus. *C. kurokawai, C. formosanus, C. cuspidatus,* and *C. armatus* from genus Centrocestus. *S. fuscata and S. lari* from genus Stictodora. *P. varium* and *P. calderoni* from genus Procerovum. *A. felis* from genus Acanthotrema. *A. donicus, A. longa* and *C. lingua* from genus Apophallus, Ascocotyle and Cryptocotyle, respectively (**Chai and Jung, 2017).**

Heterophyid metacercariae infested muscles of *Oreochromis* spp. and showed fine streaks of black coloration (melanin pigment) **(El-Gohary and Samaha, 1997)**.

3. FZT of Family: Echinostomatidae

The Echinostomatidae has a circumoral collar armed with one or two ventrally interrupted crowns of spines which distinguish them. The position and number of suckers, sucker ratio, the arrangement of the reproductive organs, the form, excretory vesicle, the egg size, and the shape of the gut are measurements by which the *Echinostoma* spp. can be identified (**Prabha, 2022).**

The life cycle of the Echinostomatidae species has a three-host. The aquatic snails are the first intermediate hosts where a sporocyst, two generations of rediae, and cercariae develop. Several species of snails, clams, frogs, and even fish are the second intermediate host. The definitive host has infection after ingestion of the second intermediate host containing the EMC as shown in Fig. 3 (**Toledo *et al.,* 2009).**

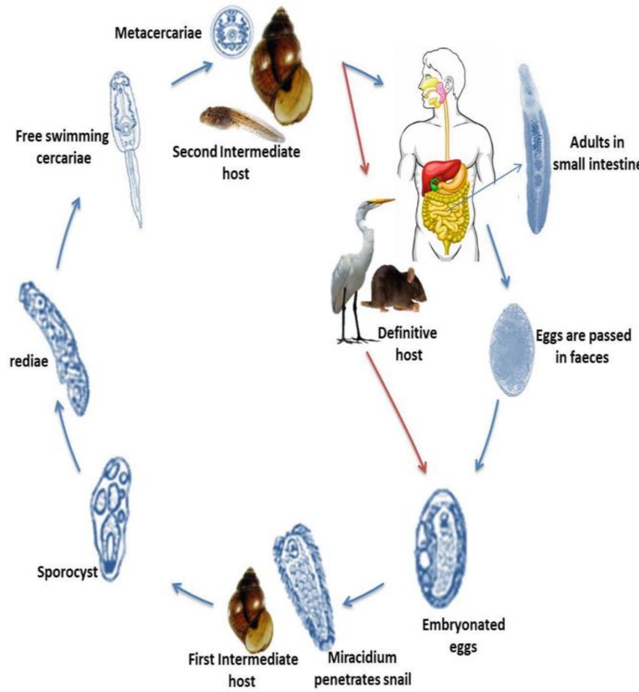

Fig. 3. Life cycle of *Echinostoma* spp. **(Prabha, 2022).**

The *Acanthoparyphium, Echinochasmus, Echinoparyphium, Artyfechinostomum, Episthmium, Echinostoma, Himasthla, Hypoderaeum,* and *Isthmiophora* are genera of the family Echinostomatidae and containing 20 species which considered to be zoonotic importance. The *Echinochasmus japonicus* and *Echinostoma hortense* are echinostomes that can infect humans by eating raw fish (**Toledo *et al.,* 2012)**.

The *Oncomelania* spp., *Allocinma longicornis, Gyraulus* spp., *Pila* spp., and *Parafossarulus manchouricus* are the first intermediate hosts of *E. japonicus.* Marsh clams, *Pseudorasbora parva*, *Hypomesus olidus*, *Gnathopogon strigatus* and *Macropodus opercularis* are the second intermediate hosts. Meanwhile, the final hosts are ducks, chickens, egrets, dogs, cats, rats, and humans.

4. FZT of Family: Clinostomidae

Clinostomum spp. found in freshwater and estuarine systems worldwide and have a complex life cycle. the definitive hosts of *Clinostomum* spp.

are fish-eating birds where the adult stage is commonly found. While the first intermediate hosts are snails harboring sporocysts. Meanwhile, the second intermediate hosts are fish, reptiles, and amphibians having the EMC. When fish-eating birds ingest infected second intermediate hosts, the life cycle have been completed. The parasite migrates from deeper tissues of the hosts before becoming adults in the anterior part of the digestive system of fish-eating birds **(Tavares-Dias *et al.,* 2023).** *C. attenuatum*, *C. cutaneum, C. marginatum, C. complanatum* and *C. phalacrocoracis* are considered species of genous *Clinostomum* **(Caffara *et al.,* 2014).**

5. FZT of Family: Diplostomidae

Diplostomidae have a three-host life cycle. The first intermediate host are snails (gastropod) where fork-tailed cercariae are produced in sporocysts. The second intermediate hosts are fishes, annelids, mollusks, and amphibians where the cercariae emerge from the snails and penetrate and form metacercariae. Definitive hosts become infected by the ingestion of the second intermediate host or the paratenic host harboring metacercariae. Eggs typically hatch and penetrate the first intermediate host as shown in Fig. 4 Only *Neodiplostomum seoulense* and *Fibricola cratera* can parasitize humans **(Toledo *et al.,* 2014).**

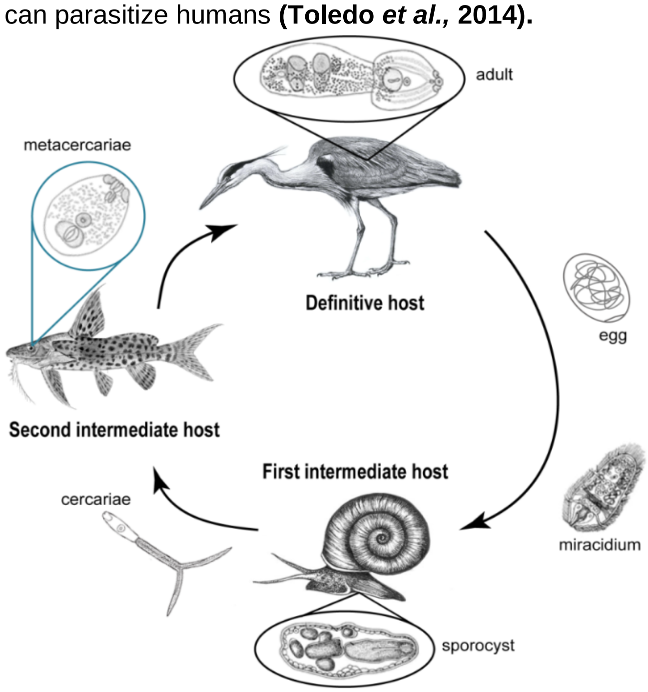

Fig. 4. Life cycle of *Diplostomum* spp. **(Robb, 2020).**

6. Clinical manifestations of FZT:

Infections with FZT can affect people of any age range. The flukes of heterophyid species occasionally infiltrate the mucosa, depositing eggs on tissues. Granulomas may develop around such eggs, resulting in convulsions or neurologic impairments **(Clausen *et al.,* 2015)**. While humans with low-grade of heterophyid infections are of no clinical consequence, while in heavy infections there is diarrhea, anorexia, mucus-rich feces, abdominal pain, dyspepsia, nausea, and vomiting. Worm eggs of heterophyid may enter the circulatory system of humans and cause emboli which may be fatal (**Toledo *et al.,* 2011; 2012).** The adult flukes of Heterophyid species inhabit the mucosa of the middle part of the small intestine in human and eggs released by the worms may penetrate the gut and travel to vital organs via the circulatory/ lymphatic system **(Hung *et al.,* 2013).**

The patient with *Echinostoma hortense* infection complained of lower abdominal pain, diarrhea and tenesmus, easy fatigability, urinary incontinence, severe ulcerative lesions and bleeding in the duodenum **(Chai *et al.,* 1994)**. In light to moderate infections caused by Echinostomes, patients have been observed to have anemia, headache, dizziness, stomach ache, gastric pain, and loose stools. Other symptoms such as eosinophilia, abdominal pain, profuse watery diarrhea, anemia, edema, easy fatigue, loss of body weight, and anorexia are associated with heavy infections (**Hung *et al.,* 2013).**

Human beings are liable to get intoxicated through ingestion of Clinostomidae digenetic trematode metacercariae such as *Clinostomum complanatum* is an important zoonotic agent of human infection that causes Yellow grub disease which is transmitted to humans via ingestion of raw or improperly cooked fresh-water fish leading to Halazoun- like disease "laryngyopharyngitis" that can cause death from asphyxiation. In accidental human infections, *C. complanatum* attaches to the mucous membrane of the throat and generally causes acute pharyngitis and laryngitis. An unusual case of eye infection caused by *Clinostomum* sp. **(Hefnawy *et al.,* 2019 and Menconi *et al.,* 2020)**. Human infected with *Neodiplostomum seoulense* was found to be suffering from severe enteritis with abdominal pain, fever, diarrhea, fullness, and anorexia **(Toledo *et al.,* 2011).**

7. Encysted metacercariae which were found in some fresh water fish in Egypt

Prohemistomatid and *Haplorchid* EMC were found in *Oreochrmis* spp. and *Clarias lazera* (**El-Gohary and Samaha, 1997 & Aly *et al.,* 2005)**, *Diplostomatid* EMC were found in both *Oreochromis niloticus* and *Clarias gariepinus* (**Aly *et al.,* 2005)**. While *Diplostomatid*, *Heterophyid* and *Clinostomatid* EMC were found in *Oreochrmis* spp. **(El-Gohary and Samaha, 1997)** especially in *Oreochromis niloticus* **(Zaki and Hussien, 2004 & Aly *et al.,* 2005).** *Cyathocotylide* EMC was also found in *O. niloticus* **(Goda *et al.,* 2024).** Species of the family Clinostomatid detected in *O. niloticus* were *Clinostomum tilapiae* **(Abou-Eisha *et al.,* 2008)** and *Clinostomum phalacrocoracis* **(Ammar and Arafa, 2013).** While from family heterophyid, the species detected in *O. niloticus* were *Heterophyes heterophyes, H. aequlis, Pygidiopsis genata* (**Elsheikha and Elshazly, 2008a)** and *Haplorchis species* (**Hefnawy *et al.,* 2019)** such as *Haplorchis yokogawai* **(Satour *et al.,* 2019).** Other species were found in *O. niloticus* such as *Euclinostomum ardeolae* **(Mahmoud *et al.,* 2018)**, *Prohemistomum vivax, and Euclinostomum heterostomum* **(Satour *et al.,* 2019).** *Euclinostomum* spp., *Pygidiopsis* spp., *Phagicola* spp., and *Stictodora* spp. were detected in *Tilapia Zilli* **(Hassan *et al.,* 2012)**. EMC detected in *Clarias gariepinus* were from the family Cyanodiplostomatid **(ElKamel *et al.,* 2014 and Attia *et al.,* 2021**), *Prohemistomatid* (**ElKamel *et al.,* 2014)** and *cyathocotylide* (**Saad *et al.,* 2019)** while *P. vivax* was found in *C. lazera* **(Hefnawy *et al.,* 2019).** While Prohemistomatid (Caythocotylidae), Heterophyid spp. and Diplostomatidae were observed in *Mugil capito* and *Mugil cephalus* **(Kotb *et al.,* 2014).** Species of Heterophyid EMC which detected in *M. capito* were *H. heterophyes, H. aequalis, P. genata* and *Stictodora* spp., **(Elsheikha and Elshazly, 2008a)** meanwhile *Heterophyes* spp were observed in *M. cephalus* **(Mahdy *et al.,* 2020).**

8. Adult trematodes were detected in some experimental animals via eating infected fish with EMC in Egypt:

Prohemistomum vivax, *Mesostephanus appendiculatus* and *Centrocetus armatus* were obtained from the small intestine of rats and/or pigeons after feeding on infected *Oreochromis niloticus* with *Diplostomatidae, Cyathocotylide* and *Heterophyidea* EMC and infected *Clarias gariepenus* with *Cyanodiplostomatidae* and *Cyathocotylide* at (**Saad *et al.,* 2019).** *Heterophyes heterophye, H. equalis, Pygidiopsis genata, Haplorchis yokogawai* and *P. vivax* were collected from the small intestines of albino rats (*Rattus norveguis*) which feeding on infected *O. niloticus* with EMC at 7-14 days post infection **(Satour *et al.,* 2019).**

P. vivax, *Mesostephanus sp.* and *Cynodiplostomum azimi* were collected from the intestine of albino rats after experimentally fed on infected *Clarias lazera* with EMC at 7 days PI **(Youssef *et al.,* 2020).** *Heterophyes heterophyes*, *H. dispar*, *Haplorchis pumilio*, *Haplorchis taichui*, *Pygidiopsis genata*, *Procerum varium*, *Centrocestus cuspidatus*, and *P. vivax* were detected from the small intestines of albino rats (*Rattus norveguis*) after feeding on infected *O. niloticus* with EMC at 10–15 days PI **(El-Seify *et al.,* 2021).** *P. vivax* and *Mesostephanus* spp., were detected in the small intestines of albino mice that fed on infected *Oreochromis niloticus* with Cyathocotylidae EMC (**Abd-ELrahman *et al.,* 2023).**

Clinostomum phalacrocoracis was found in the small intestine of albino mice after feeding on infected *Tilapia nilotica*, catfish, bajad, and carp fish with EMC (**Ahmed *et al.,* 2023).** *H. heterophyes*, *H. aequalis*, *P. genata*, *H. yokogawi*, *P. vivax*, *Phagicola ascolonga*, and *Stictodora tridactyla* were obtained from the small intestines of puppies that feeding on infected *Mugil cephalus*, *Mugil capito*, *Mugil auratus*, *Tilapia nilotica*, and *Tilapia zilli* with EMC at 21 days PI (**Elsheikha and Elshazly, 2008b).**

H. heterophyes, *H. aequalis*, *P. genata*, *Procerovum varium*, *Haplorchis pumilio*, *Haplorchis taichui*, *Stictodora tanayensis*, *Prohemistomum vivax*, *Gelanocotyle milvi*, *Mesostephanus* *appendiculatus*, *Mesostephanus milvi* and *Mesostephanus fajardensis* were detedcted in the duodenum and jejunum of puppies after feeding on infected *O. niloticus* and *C. gariepinus* with EMC **(Nouh *et al.,* 2010).** *H. heterophyes, H. nocens, H. pumilio, Metagonimus yokogawi, P. genata, Stictodora tanayensis* from family Heterophyidae*. P. vivax, M. appendiculatus, M. burmanicus, M. milvi* from family Cyathocotylidae*.*

Cyanodiplostomum azimi, Neodiplostomum spathula from family Diplostomatidae and *Echinochasmus liliputans* from family Echinostomatidae were detected from the small intestine of white rats and chickens after feeding on the infected *O. niloticus*, *C. gariepinus*, *Chrysichthys auratus*, *Bagrus bayad*, *Ctenopharyngodon Idella* and *Barbus binny* with EMC (**Saleh *et al.,* 2009).** *Prohemistomum vivax*, *Haplorchis pumilio* and *Pygidiopsis genata* were found in intestine of pigeons after feeding on infected *O. niloticus* with *Prohemistomum* spp., *Haplorchis* spp. and *Pygidiopsis* sp. of EMC at the seventh and tenth days PI (**Mahdy *et al.,* 2021a).**

9. Control Treatments to Avoid Foodborne Zoonosis

Grilling of the infected *Oreochromis niloticus* with EMC for 15-20 minutes at 60-80˚C was sufficient to destroy the EMC but grilling for 5 and 10 minutes was not sufficient to destroy all EMC in fish muscles **(Abou-Eisha *et al.,* 2008)**. While grilling of infected *Tilapia zillii* was sufficient to kill EMC after 10 minutes; however, five minutes was sufficient only to kill EMC in *Clarias gariepinus* **(Abdallah *et al.,* 2009).**

Boiling of the infected *T. zillii*, *C. gariepinus*, *Bagrus bajad* and *Chlrysichthys auratus* was sufficient to kill the EMC, frying of this fish for five minutes was quite sufficient to inhibit the viability of EMC, but frying for 10 minutes killed all EMC **(Abdallah *et al.,* 2009).** Boiling the infected cyprinid fish with *Opisthorchis viverrini* (OV) at 90 °C for at least 5 min could kill OV metacercariae **(Sripan *et al.,* 2017).** Heating of the infected *Mugil liza* with *Ascocotyle (Phagicola) longa* for 15 min at 60 °C, 100 °C and 180 °C was sufficient to kill all metecercariae **(Borges *et al.,* 2018).**

Storing the infected Cyprinoid fish with *Haplorchis taichui* at -20°C in a commercial freezing (ice-cream) cabinet for at least 72 hours to completely eliminate the metacercaria **(Kaenjampa *et al.,* 2017)**. Chilling the infected *T. nilotica* with *Haplorchis pumilio* and *Prohemistomum vivax* at 4 °C detected that the EMC were viable for 24 hours only and complete destruction occurred after 24 hours of chilling storage but in *Clarias lazera,* the EMC were viable for 48 hours and complete destruction occurred after 48 hours of chilling storage **(Youssef *et al.,* 2016).**

Freezing of infected *T. nilotica* and infected *Mugil cephalus* with metacercariae at −15°C for 4, 7, or 14 days detected the percentages of worms recovered decreased from 36% to 20, 5, and 0% after freezing for 4, 7, and 14 days, respectively (**El-Sayed *et al.,* 2014).** Storing of infected Nile Tilapia with *Euclinostomum ardeolae* and *Clinostomum* spp. under freezing conditions below -4°C to -7°C for about 7 days, declared that all treated samples were lysed and lost their viability (100%) **(Mahmoud *et al.,* 2018)**. Freezing of infected *O. niloticus* muscles with EMC at −10°C for 3, 7, 14 days and at -30°C for 24 hrs revealed that all EMC were killed at -10°C/14 days and at -30°C / 24 hrs **(Satour *et al.,* 2019)**. Freezing of infected muscle of *Tilapia* spp. and *Mugil* spp. with EMC at (-4C° to -5C°) for 10 days will keep the fish completely safe for consumers **(Elghayaty and Tadros, 2020)**.

Cooking of infected muscles of *O. niloticus* and *C. gariepinus* with Cyathocotylidae EMC using an electric oven at 250°C for 15-20 min was sufficient only to destroy the EMC of Cyathocotylidae in *O. niloticus* muscles but wasn't sufficient to destroy EMC in *C. gariepinus* **(Goda *et al.,* 2024).**

10. CONCLUSION

Fishborne zoonotic intestinal trematodes can infect both fish and humans. When encysted metacercariae (EMC) infected fish, some or many clinical signs were observed in fish bodies and organs. Also, when adult trematodes entered the intestines of humans, acute and severe symptoms were detected in human beings according to the type of infestation. There are numerous processing methods which can reduce or kill EMC in fish such as cooking, heating, microwaving, freezing and chilling.

11. RECOMMENDATION

1. Educating the public about the risks of fishborne zoonotic trematodes.
2. Avoiding consumption of raw or undercooked fish is very important to avoid the probability of human infection with encysted metacercariae.
3. Improving sanitation and hygiene practices.
4. Controlling of aquatic and migratory birds (final hosts of digenetic trematodes) by different methods is recommended to prevent the spreading of the disease through fish and then to humans.
5. Be attention and monitor the water sourse which introduced to fish ground to ensure it is free from snails that carry parasites.

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