PATHOGENS AND GLOBAL HEALTH
2020, VOL. 114, NO. 2, 91–98
https://doi.org/10.1080/20477724.2020.1727217

Taylor & Francis
Taylor & Francis Group

Fish-borne trematode infections in wild fishes in Bangladesh

Sharmin S. Labony[a], M. Abdul Alim[a], Muhammad Mehedi Hasan[b], Md. Shahadat Hossain[a], Ausraful Islam[c], Mohammad Zahangir Alam[a], Naotoshi Tsuji[d] and Anisuzzaman[a]

[a]Department of Parasitology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh; [b]Department of Fisheries Technology, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh; [c]Program for Emerging Infections, Infectious Diseases Division, icddr,b, Dhaka, Bangladesh; [d]Department of Parasitology and Tropical Medicine, Kitasato University School of Medicine, Sagamihara, Japan

ABSTRACT

Fish-borne liver and intestinal flukes are helminth pathogens that have a negative impact on public health worldwide. We herein investigated the status of infection by the metacercariae (MC) of fish-borne trematodes (FBTs) in randomly selected freshwater wild fishes. Five species of fishes were collected and digested artificially using digestion fluid to recover MC. All fish species, namely, ticto barb (*Puntius ticto*) (14/16, 87.5%), banded gourami (*Colisa fasciata)* (8/12, 66.7%), freshwater garfish (*Xenentodon cancila*) (9/14, 64.3%), flying barb (*Esomus danricus*) (5/12, 41.7%), and reba carp (*Cirrhinus reba*) (7/11, 63.7%), were infected with FBTs. The overall infection rate was 66.2% and the mean intensity was 748.3 ± 2947.5 MC/100 g of fishes. The loads of MC in ticto barb, reba carp, freshwater garfish, banded gourami, and flying barb per 100 g of fishes were 1978.8 ± 5053.7, 268.3 ± 440.7, 140 ± 105.4, 134.3 ± 109.2, and 117.6 ± 102.3, respectively. The infection rate was significantly higher ($P < 0.05$) in the body (55.4%) than in the head (40%) of fishes. Morphological and morphometrical analyzes identified the MC of *Clonorchis* spp., *Opisthorchis* spp., *Metorchis* spp., *Metagonimus* spp., and *Echinostoma* spp. Collectively, the present results suggest that wild freshwater fishes are important intermediate hosts for FBTs, and play a critical role in the transmission cycle of these parasites in Bangladesh. The results also indicate that people of the country are at risk of these infections.

KEYWORDS

Fish-borne trematodes; wild fishes; metacercariae; *Clonorchis*; *Opisthorchis*; *Metagonimus*; *Metorchis*; *Echinostoma*

1. Introduction

Infections by fish-borne trematodes (FBTs) affect human and animal health worldwide, particularly in Asian countries [1]. FBTs are an emerging and rapidly growing concern in developing and developed countries due to the expanding international trade in fishes and fish products, as well as human demographic diversity, especially mass refugee movement and global settlement, including in Bangladesh [2,3]. Recently, a total of 59 species of FBTs of public health importance have been identified. These trematodes have been divided into two groups: liver flukes (Opisthorchidae: 12 species) and intestinal flukes (Heterophyidae: 36 species, Echonostomatidae: 10 species and Nanophyetidae: 1 species) [4]. FBTs have a complex lifecycle, with a series of developmental changes in freshwater snails in which they produce cercariae, the freely swimming larval stage of trematodes. These cercariae emerge from snails into water and encyst in freshwater fishes, notably cyprinoid fishes, in which they develop into metacercariae (MC), the infective stage of trematodes for mammals and birds [5]. FBT infections are more common in wild fishes, which have a great public health concern [4]. Humans become infected through the consumption of raw or improperly cooked fishes containing viable MC [4,6].

More than 45 million people are affected globally only with opisthorchiasis, diseases caused by opisthorchid flukes. Of the opisthorchid flukes, *Clonorchis sinensis*, the most important liver fluke, affects 15 million people only in East Asia, including 13 million in China and 1 million in northern Vietnam [7,8]. Historically, although *C. sinensis* infection was highly endemic in Japan, only a few cases have recently been reported; however, approximately 1.2 million individuals are infected in South Korea [8]. On the other hand, 8.6 million people have been infected with *Opisthorchis viverrini* in Southeast Asian countries, approximately 6 million of whom were in Thailand [9]. Additionally, 1.6 million individuals are infected with *O. felineus* globally, including 1.5 million in the former Union of Soviet Socialist Republics [10]. According to Nguyen et al. [4], more than 750 million individuals are at risk to fish borne liver flukes throughout Asia. Furthermore, 40–50 million individuals have been affected with one or more species of fish-borne intestinal flukes and approximately half a billion individuals are at risk globally [11]. In a recent study, the disability-adjusted life years (DALYs) of FBTs had been reported to be

CONTACT Naotoshi Tsuji tsujin@med.kitasato-u.ac.jp Department of Parasitology and Tropical Medicine, Kitasato University School of Medicine, 1-15-1 Kitasato, Minami, Sagamihara, Kanagawa 252-0374, Japan; Anisuzzaman zaman.a.bau@gmail.com Department of Parasitology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

1.8 million, and estimated to have increased by 8.5% within 2007–2017 [12]. FBTs, particularly liver flukes, have been implicated in biliary tract obstruction, bile flux block, and icterus [13]. Infections by intestinal flukes cause fatigue and mild gastrointestinal symptoms, such as epigastric pain, anorexia, and diarrhea, however, in severe infections they cause abdominal cramps, malabsorption, and weight loss [14].

Despite advances in the development of aquaculture in Bangladesh in recent years, small wild freshwater fishes are in high demand and they are used as a delicacy from villages to large cities, and even among people living abroad. These wild fishes generally live in natural water bodies such as rivers and low lying marshy lands, which are inhabited by many intermediate host snails. Reservoir hosts of FBTs, such as street dogs, cats, foxes, and jackals, also inhabit areas alongside these natural water bodies. About half a century ago, FBTs, such as *C. sinensis* and *O. felineus*, were detected in street dogs and wild carnivores in Bangladesh [15]. Moreover, *Metorchis orientalis*, a zoonotic liver fluke affecting humans, carnivores, and aquatic birds, was also found in the livers of ducks [16], indicating that wild fishes in Bangladesh are infected with the infective stage of FBTs. However, despite being of enormous importance, these parasites have not yet been investigated in fishes in Bangladesh. Therefore, we herein isolated and identified the MC of different FBTs in common wild freshwater fishes marketed in Bangladesh for human consumption.

2. Materials and methods

2.1. Study area

Two districts, Mymensingh (24°45′22.90″N and 90° 24′23.26″E) and Kishoreganj (24°45′83″N and 90° 88′33″E) (Figure 1), were selected because they are rich in natural water bodies and are regarded as the hub of indigenous fishes in Bangladesh.

2.2. Sampling of fishes

Varieties of small wild freshwater fishes were collected from local markets in the study areas. A total of 14 kg of fishes, belonging to five species, were collected and examined. Fishes were identified as described previously [17,18], and the identified species were ticto

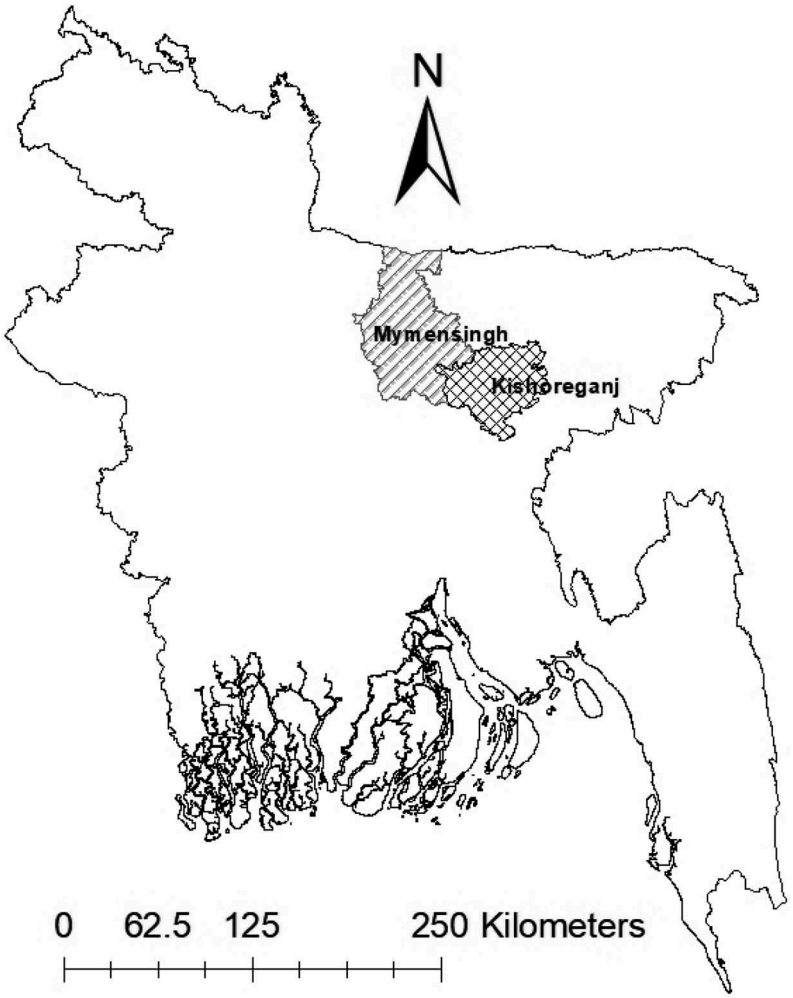

Figure 1. Map of Bangladesh showing study areas. The locations selected were two of the most important areas that produce wild fishes.

barb (*Puntius ticto*), flying barb (*Esomus danricus*), banded gourami (*Colisa fasciata*), freshwater garfish (*Xenentodon cancila*), and reba carp (*Cirrhinus reba*). Detail is in the Table 1. During collection, the sources of fishes were confirmed by fishermen. After collection, samples were preserved in ice and transported to the laboratory.

2.3. Fish processing and preparation

Different species of fishes were weighed and used immediately for the detection of MC or kept separately in polythene bags at −20 °C until examined within one week. Pooled samples were used, with each sample consisting of 150–200 g of fishes of the same species. The visceral organs of each fish were carefully removed. The head and body of fishes were separated, cut into small pieces, and blended in a blender to which artificial gastric juice containing 0.3% Pepsin (LOBA Chemie Pvt. Ltd., Mumbai, India) and 1% conc. HCl (Merck, Germany) were added.

2.4. Artificial digestion and recovery of MC

Processed samples were digested according to the procedures described by Sohn [19] with slight modifications. Briefly, processed samples were incubated at 37 °C overnight in artificial gastric juice under vigorous stirring conditions. The next morning, larger particles (scales, fins, bones, and undigested materials) were removed by filtering through a sieve (1 × 1-mm mesh). The filtrate was washed extensively by adding normal saline (2 L), stirring vigorously, and leaving to stand for 30 minutes for sedimentation. The supernatant was then discarded and washing with normal saline was repeated until the filtrate became clear. The sediment was centrifuged at 3000 rpm for 3 min, the supernatant was discarded, and the pellet was examined.

2.5. Detection of MC

The pellet was re-suspended with PBS to a total volume of 15 ml and mixed thoroughly. A 0.15-ml aliquot of the suspension was added to a clean glass slide and examined under a microscope (Labomed, USA) using the 10X objective. MC were identified to the genus level following previously reported keys and descriptions [19–24]. Each sample was examined at least in triplicate and averaged. The average number of MC was multiplied by 100 to assess the total number of MC present in 15 ml of the suspension, and MC per 100 g of fishes was calculated. Microphotographs of MC were taken using an inverted microscope to which a digital camera (Labomed, USA) had been fit.

2.6. MC size measurements

The length and width or diameter of MC were estimated using a pre-calibrated eye piece.

2.7. Statistical analyses

Data were analyzed using SPSS software. The infection rate of fishes was analyzed using the Z-test and the loads of MC in different species were compared using the chi-squared (χ^2) test. $P < 0.05$ was considered to be significant.

3. Results

3.1. The rate of FBT infections is pretty high in small wild freshwater fishes

A total of five species of fishes marketed for human consumption were purchased from local markets. After collection, fishes were transported to the laboratory, identified, and processed for the isolation of the MC of FBTs. All five species of fishes: ticto barb, flying barb, banded gourami, freshwater garfish, and reba carp, were infected with MC (Figure 2). We examined 65 pooled samples and of them 43 (66.2%) were positive for the MC of FBTs, suggesting that the selected small wild fishes play important roles as the second intermediate hosts. The highest infection rate was in ticto barb (87.5%) and the lowest in flying barb (41.7%), and this difference was significant ($p < 0.05$). Furthermore, the mean load of MC per 100 g of fishes was the

Table 1. Size, weight and amount of different species of fishes used.

	Length (cm)		Weight (g)		
Common name/species of fishes	Ranges	Mean±SD	Ranges	Mean±SD	Amount used (g)
Ticto barb (*Puntius ticto*)	2.20–9.4	6.63 ± 1.41	1.54–11.66	4.61 ± 2.99	3,415
Banded gourami (*Colisa fasciata*)	2.10–9.3	7.95 ± 0.68	5.22–15.15	9.71 ± 2.34	2,037
Freshwater garfish (*Xenentodon cancila*)	6.55–22.0	17.59 ± 2.64	5.18–17.75	10.51 ± 3.91	2,576
Flying barb (*Esomus danricus*)	1.53–5.6	3.88 ± 0.61	0.24–1.5	0.47 ± 0.28	3,078
Reba carp (*Cirrhinus reba*)	2.4–14.3	9.72 ± 4.21	3.25–20.98	12.79 ± 5.95	2,894
Total weight					**14,000**

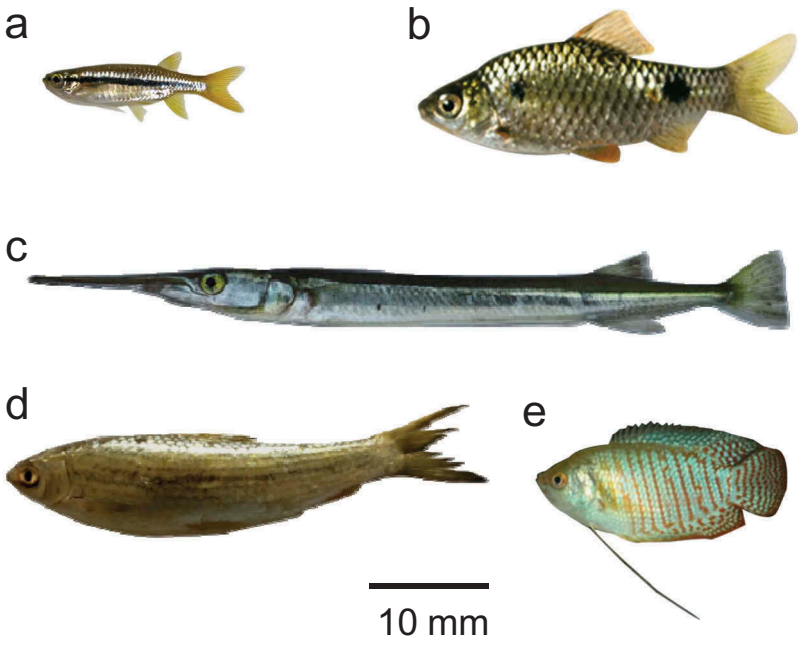

Figure 2. Small wild fishes infected with MC of FBTs. Five species of fishes, (a) flying barb (*Esomus danricus*), (b) ticto barb (*Puntius ticto*), (c) freshwater garfish (Xenentodon cancila), (d) reba carp (*Cirrhinus reba*), and (e) banded gourami (*Colisa fasciata)* were collected and identified. The selected fishes are commonly used for human consumption in Bangladesh.

highest in ticto barb (1978.8 ± 5053.7) and the lowest in flying barb (117.6 ± 102.3) (Table 2).

3.2. Morphological characteristics of different MC in freshwater wild fishes

Based on morphology and morphometry, the MC of *Clonorchis* spp. were elliptical and measured 0.17 ± 0.01 × 0.14 ± 0.01 mm in size. A detailed morphological study revealed oral and ventral suckers of similar sizes and an O-shaped excretory bladder. Morphologically, the internal structures of MC of *Opisthorchis* spp. were similar to those of *Clonorchis* spp., except the size of the cyst was a bit bigger (0.19 ± 0.01 × 0.14 ± 0.01 mm). The size of the MC of *Metorchis* spp. was 0.17 ± 0.06 × 0.17 ± 0.05 mm and was characterized by the presence of a clearly visible double-layered cyst wall. The MC of *Metagonimus* spp. were distinct both in size and shape. The MC of *Metagonimus* spp. were subglobular or disc-shaped, measuring 0.15–0.14 mm in diameter, and were distinguished by the presence of yellow-brownish pigments and a V-shaped excretory bladder. On the other hand, echinostome-MC were elliptical in shape with an average size of 0.15–0.16 × 0.11–0.13 mm, and were typically characterized by the presence of collar spines (Figure 3).

3.3. MC of FBTs in different fishes

The MC of FBTs, such as *Clonorchis* spp., *Opisthorchis* spp., *Metorchis* spp., *Metagonimus* spp., and *Echinostoma* spp., were identified. In most samples, infections with more than one type of FBT were detected. *Clonorchis/Opisthorchis* were isolated from all species of fishes examined. *Metorchis*-MC were found in four species of fishes, namely, ticto barb,

Table 2. Metacercariae detected in small wild fishes.

Fishes			Load of MC	
Family	species	Sample infected (%)	Range (Mean ± SD)	Parasites
Cyprinidae	Ticto barb	14/16 (87.5[a])	13–19,200	*Clonorchis/Opisthorchis*
	n = 16		(1978.8[a] ± 5053.7)	*Metagonimus, Metorchis*
	Flying barb	5/12	14–200	*Clonorchis/Opisthorchis*
	n = 12	(41.7[b])	(117.6[b] ± 102.3)	
	Banded gourami	8/12	27–363	*Clonorchis/Opisthorchis*
	n = 12	(66.7[a])	(134.3[b] ± 109.2)	*Metagonimus, Metorchis*
	Reba carp	7/11	15–1,250	*Metorchis, Metagonimus Clonorchis/Opisthorchis*
	n = 11	(63.7[a])	(268.3[b] ± 440.7)	
Belonidae	Freshwater garfish	9/14	23–223	*Clonorchis/Opisthorchis*
	n = 14	(64.3[a])	(140[b] ± 105.4)	*Metorchis, Metagonimus*
				Echinostoma
	Total	43/65 (66.2[a])	13–19,200	
	n = 65		(748.3[b] ± 2,947.5)	

n, Sample number; MC, metacercariae
Values with different superscripts in the same column are significant ($P < 0.05$).

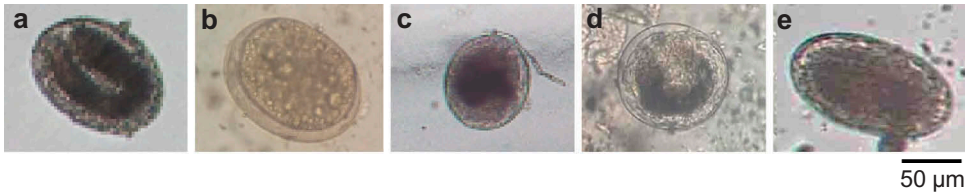

Figure 3. MC isolated and identified from wild fishes. Five species of fishes were collected, processed, and digested using artificial gastric juice as described in the Materials and Methods. Digested fishes was sieved and washed extensively with PBS. The sediment was examined under a microscope and microphotographs were taken. MC of (a) *Clonorchis* spp., (b) *Echinostoma* spp., (c) Metagonimus spp., (d) *Metorchis* spp., and (e) *Opisthorchis* spp.

Table 3. Relative distribution of metacercariae in different body parts of fishes.

	No. of infected body parts (%)		Load of MC Range (Mean ± SD)	
Fish species	Head	Body	Head	Body
Ticto barb	13/16 (81.3[a])	12/16 (75[a])	13–19200 (2082.7[a] ± 5245.7)	6–3376 (625.5[b] ± 1016.2)
Flying barb	2/12 (16.7[a])	3/12 (25[a])	14–44 (29[a] ± 21.2)	80–250 (176.7[b] ± 87.4)
Banded gourami	3/12 (25[a])	8/12 (66.7[b])	47–83 (62.3[a] ± 18.6)	27–363 (131.8[a] ± 111.7)
Reba carp	5/14 (35.7[a])	9/14 (64.3[a])	127–303 (215[a] ± 78.8)	23–106 (49.6[b] ± 25.1)
Freshwater garfish	4/11 (36.4[a])	4/11 (36.4[a])	150–1250 (450.5[a] ± 533.8)	15–50 (31.5[b] ± 17.1)

MC, metacercariae
Values with different superscripts in the same row and parameter are significant ($P < 0.05$).

banded gourami, freshwater garfish, and reba carp. The MC of *Metagonimus* spp. were also detected in ticto barb, banded gourami, freshwater garfish, and reba carp fishes, whereas *Echinostoma*-MC were only detected in freshwater garfish (Table 2).

3.4. Distribution of FBTs in the head and body of fishes

During the pr o head and body segments and digested separately using artificial gastric juice, as described in the Materials and Methods section. The infection rate was significantly higher (P < 0.05) in the body (55.4%) than in the head (40%) of fishes (data not shown). The infection rate and load both differed in the head and body of the same species of fishes. The infection rate with MC was the highest in the head (81.3%) of ticto barb, but was higher in the body than in the head of the other fishes examined. The lowest infection rates with MC were detected in the head (16.7%) and body (25%) of flying barb. There was no tissue preference in freshwater garfish; MC were equally detected in both the head and body (36.4%) (Table 3). On the other hand, the mean load of MC was significantly higher in the heads of ticto barb (2082.7 ± 5,245.7), reba carp (215 ± 78.8), and freshwater garfish (450.4 ± 533.8). In contrast, the load of MC was significantly higher in the bodies of flying barb (176.7 ± 87.4) and banded gourami (131.8 ± 111.7) (Table 3).

4. Discussion

FBT infections, particularly diseases caused by human liver flukes (*C. sinensis* and *O. viverrini*), are neglected tropical diseases (NTDs). FBTs have a negative impact on public health and are of great concern due to the associated complications [25]. We herein investigated the current status of FBT infections in wild, small, freshwater fishes in Bangladesh. The results obtained revealed that FBT infections in wild freshwater fishes were very high in Bangladesh, with 66% of the pool samples examined being infected. Although the MC of FBTs had not previously been identified in freshwater fishes in Bangladesh, adult flukes were detected in reservoir animals and birds [15,16], indicating that these parasites had existed for a long time. Since wild fishes are available throughout the country and are in high demand both in rural and urban areas in Bangladesh, the entire population is at high risk of FBT infections.

The present study revealed that four species of wild freshwater fishes belonging to the family Cyprinidae, namely, ticto barb, flying barb, banded gourami, and reba carp, and one species of the family Belonidae (e.g. freshwater garfish) play roles as the second intermediate hosts to complete the lifecycle of different FBTs prevalent in the study areas. In Bangladesh, the types of freshwater fishes that act as the second intermediate hosts of FBTs have not yet been identified. However, previous studies conducted in other countries suggested that

cyprinid fishes typically play central roles in the transmission cycle of FBTs [20,26–28]. In addition to cyprinid fishes, MC have been identified in freshwater garfish (Belonidae) in Thailand [29] and *Tilapia* spp. (family: Cichlidae) in northern Vietnam [30], suggesting that fishes other than cyprinids can act as the second intermediate hosts for FBTs.

In the present study, the MC of *Clonorchis/Opisthorchis* were detected in all types of fish species examined, suggesting that these fishes play roles as the second intermediate hosts of opisthorchid flukes. *C. sinensis* is the most significant species of FBTs in Southeast Asia [31–33]. Until now, endemic areas of clonorchiasis included China, South Korea, North Vietnam, Taiwan, and Far East Russia [34]. A nationwide survey in China revealed that 102 species of fishes act as the second intermediate hosts of *Clonorchis* [35]. In Korea, *Pungtungia herzi, Ladislabia taczanowskii, and Acheilognathus rhombeus* fishes were identified as the second intermediate hosts of *C. sinensis* [36]. On the other hand, only two fish species have been shown to act as the second intermediate hosts for *O. viverrini* in Phu Yen province, Vietnam, which is considered to be the 'hot spot' of the parasite. The prevalence of *O. viverrini* was 10–29% in the crucian carp, *Carassius carassius*, in Phu Yen province [37] and 1.9% in the snakehead fish, *Channa striata*, in An Giang province in southern Vietnam [38]. The MC of *O. viverrini* have been detected in fishes of the genera *Carassius, Channa, Cyclocheilicthys, Hampala, Esomus, Osteochilus, Puntioplites*, and *Puntius* [39].

In the present study, the MC of *Metagonimus* spp. were found in ticto barb, banded gourami, freshwater garfish, and reba carp. The MC of *Metagonimus* have been reported in several species of fishes, such as *Abramis brama, A. ballerus, Aspius aspius, Blicca bjoerkna, C. carassius, Chondrostoma nasus, Hemibarbus labeo, Leuciscus idus, Pseudobagrus fulvidraco, Plecoglossus altivelis, Tribolodon taczanowskii* (*T. hakonensis*), and *Lateolabrax japonicus* in China, Taiwan, and Korea [19,35]. In the present study, ticto barb, banded gourami, freshwater garfish, and reba carp were positive for the MC of *Metorchis* spp. *Metorchis* spp. have been detected in humans in Eurasia, North America, and East Asia [40]. Anisuzzaman et al. [16] reported that 48% of ducks in Bangladesh were infected with *M. orientalis*. Reservoir animals (dogs and cats) act as the source of *M. orientalis* in Japan and China. The MC of *M. orientalis* were detected in *Pseudorasbora parva, Hemiculter leucisculus, Saurogobio dabryi, Rhynchocypris lagowskii, C. auratus, Rhodeus ocellatus, Perccottus glehnii, P. herzi, Misgurnus anguillicaudatus, Microphysogobio koreensis*, and *Gnathopogon strigatus* in China and Korea [41,42], indicating its very wide host preference.

Overall, we found very high loads of MC in all five fish species examined. Previous studies reported that the average load of MC ranged between 1 and 485 [43,44]. Variations among previous findings on the load of MC obtained from different studies may be attributed to the fish species examined, the methods employed, the geoclimatic conditions of the countries, the availability of reservoir hosts, and even cropping patterns. In Bangladesh, measures to control snails have not yet been implemented, and all types of natural water bodies harbor varieties of snail species. The entire country is crisscrossed with rivers and other natural water sources, and most parts of the country are flooded during the long rainy season. The temperature is generally >30 °C throughout the year and rarely decreases to ~15 °C in the very short winter (from the second week of December to the first week of the February) [45]. Moreover, countrywide faulty drainage systems used for irrigation, particularly in the dry seasons, also favor the survival and propagation of snails, intermediate hosts, throughout the year. Thus, wild fishes are continuously exposed to the cercariae released from snails. Furthermore, in the present study, we digested fish samples for a long time, which possibly caused the release of all MC from the muscles and head tissues of the fishes, leading to as the detection of high infection rate and recovery of MC.

5. Conclusions

FBT infection rates are high in small wild fishes in Bangladesh, which is a public health concern. The present study confirmed that Bangladesh is an endemic area of fish-borne liver and intestinal parasites of humans, which is an emerging threat particularly to those who consume improperly cooked fish and fish products. The present results will be the basis for advanced studies on FBTs in Bangladesh. A countrywide extensive survey and further studies on more fish species are needed to reveal the extent of this infection.

Acknowledgments

We thank the fishermen of the study area for providing information on fishes.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The present study was supported by grants (to Anisuzzaman) by the Ministry of Education, Government of Bangladesh, Bangladesh.

References

[1] Andrews RH, Sithithawarn P, Petney TN. *Opisthorchis viverrini*: an underestimated parasite in world health. Trends Parasitol. 2008;24:497–501.

[2] Chai JY, Murrell KD, Lymbery AJ. Fish-borne parasitic zoonoses: status and issues. Int J Parasitol. 2005;35:1233–1254.
[3] WHO. The World Health Report, Changing History. 2004; 170.
[4] Nguyen MH, Madsen H, Fried B. Global status of fish-borne zoonotic trematodiasis in humans. Acta Parasitol. 2013;58:231–258.
[5] Skov J, Kania PW, Dalsgaard A, et al. Life cycle stages of heterophyid trematodes in Vietnamese freshwater fishes traced by molecular and morphometric methods. Vet Parasitol. 2009;160:66–75.
[6] De NV, Le TH, Murrell KD. Prevalence and intensity of fish-borne zoonotic trematodes in cultured freshwater fish from rural and urban areas of Northern Vietnam. J Parasitol. 2012;98:1023–1025.
[7] Keiser J, Utzinger J. Food-borne trematodiases. Clin Microbiol Rev. 2009;22:466–483.
[8] Qian M, Utzinger J, Keiser J, et al. Clonorchiasis. Lancet. 2016;387:800–810.
[9] Qian M, Zhou X. Human liver flukes in China and ASEAN: time to flight together. PLoS Negl Dis. 2019;13:4.
[10] Yossepowitch O, Gotesman T, Assous M, et al. Opisthorchiasis from imported raw fish. Emerg Infect Dis. 2004;10:2122–2126.
[11] Hung NM, Dung DT, Anh NTL, et al. Current status of fish-borne zoonotic trematode infections in Gia Vien district, Ninh Binh province, Vietnam. Parasit Vectors. 2015;8:21.
[12] GBD 2017 DALYs and HALE Collaborators. Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2017: a systematic analysis for the GLOBAL Burden of Disease Study 2017. Lancet. 2018;392:1859–1922.
[13] Scholte LLS, Pascoal-Xavier MA, Nahum LA. Helminths and cancers from the evolutionary perspective. Front Med. 2018;5:90.
[14] Mac Lean JD, Cross JH, Mahanty S. Liver, lung and intestinal fluke infections. In: Guerran RL, Walker DH, Weller PF, editors. Tropical Infectious Diseases. Vol. II. Philadelphia: Churchill, Livingston; 2006. p. 1039–1057.
[15] Shaikh H, Huq MM A survey on the parasites of zoonotic importance in Bangladesh. Annual report of Bangladesh Agricultural Research Council. 1984: 9–10.
[16] Anisuzzaman, Alim MA, Islam MK, et al. Avian liver fluke infection in indigenous ducks in Bangladesh: prevalence and pathology. J Bangladesh Agrl Univ. 2005;3:87–94.
[17] Rahman AKA. Freshwater fishes of Bangladesh. 2nd ed. Dhaka, Bangladesh: Zoological Society of Bangladesh; 2005. p. 394.
[18] Talwar PK, Jhingran AG. Inland fishes of India and adjacent countries. Vol. 1&2. New Delhi, India: Oxford & IBH Publishing Co. Pvt. Ltd; 1991. p. 541–1158.
[19] Sohn W. Fish-borne Zoonotic trematode metacercariae in the Republic of Korea. Korean J Parasitol. 2009;47:103–113.
[20] Scholtz T, Ditrich O, Giboda M. Differential diagnosis of opisthorchid and heterophyid metacercariae (Trematoda) infecting flesh of cyprinid fish from Nam Ngum Dam Lake in Lao PDR. Southeast Asian J Trop Med Public Health. 1991;22:171–173.
[21] Kaewkes S. Taxonomy and biology of liver flukes. Acta Trop. 2003;88:177–186.
[22] Clausen JH, Madsen H, Murrell D, et al. Relationship between snail population density and infection status of snails and fish with zoonotic trematodes in Vietnamese carp nurseries. PLoS Negl Dis. 2012;6(12): e1945.
[23] Yamaguti S. Systema Helminthum. Vol. I. The Digenetic Trematodes of Vertebrates-Part I&II. New York, London: Interscience Publishers; 1958.
[24] Kiyan VS, Bulashev AK, Katokhin AV. *Opisthorchis felineus* and Metorchis bilis metacercariae in cyprinid fish Leuciscus idus in Nura-Sarysu River, Kazakhstan. Korean J Parasitol. 2018;56(3):267–274.
[25] Keiser J, Utzinger J. Food-borne trematodiasis: current chemotherapy and advances with artemisinins and synthetic trioxolanes. Trends Parasitol. 2007; 23:555–562.
[26] Petney TN, Andrews RH, Saijuntha W, et al. The zoonotic, fish-borne liver flukes *Clonorchis sinensis, Opisthorchis felineus* and *Opisthorchis viverrini*. Int J Parasitol. 2013;43:1031–1046.
[27] Lun ZR, Gasser RB, Lai DH, et al. Clonorchiasis: a key foodborne zoonosis in China. Lancet Infect Dis. 2005;5:31–41.
[28] Conlana JV, Sripa B, Attwood S, et al. A review of parasitic zoonoses in a changing Southeast Asia. Vet Parasitol. 2011;182:22–40.
[29] Wongsawad C, Rojanapaibul A, Mhad-arehin N, et al. Metacercaria from freshwater fishes of Mae Sa stream, Chiang Mai, Thailand. Southeast Asian J Trop Med Public Health. 2000;31(Suppl 1):54–57.
[30] De NV. The fishborne trematodes in Vietnam. Southeast Asian J Trop Med Public Health. 2004;35: 299–301.
[31] Rim HJ. Clonorchiasis in Korea. Korean J Parasitol. 1990;28:63–78.
[32] Chen MG, Lu Y, Hua X, et al. Progress in assessment of morbidity due to *Clonorchis sinensis* infection: a review of recent literature. Trop Dis Bull. 1994;91:R7–R56.
[33] Hong ST. Clonorchis sinensis. In: Miliotis MD, Bier JW, editors. International handbook of foodborne pathogens. New York, Basel: Marcel Dekker, Inc.; 2003. p. 581–592.
[34] Mas-Coma S, Bargues MD. Human liver flukes: a review. Res Rev Parasitol. 1997;57:145–218.
[35] Yuan R, Huang J, Zhang X, et al. Modeling the transmission dynamics of clonorchiasis in Foshan, China. Sci Rep. 2018;8:15176.
[36] Shin HC, Lee W, Kim T, et al. Prevalence of zoonotic Trematode metacercariae in freshwater fish from Gangwon-do, Korea. Korean J Parasitol. 2014;52: 399–412.
[37] Chuong NV, Tuan BV, Chau LV, et al. Several epidemiological characteristics of Opisthorchis viverrini. Malar Parasit Dis Prevent Bull. 1997;2:85–90.
[38] Thu ND, Dalsgaard A, Loan LT, et al. Survey for zoonotic liver and intestinal trematode metacercariae in cultured and wild fish in An Giang Province, Vietnam. Korean J Parasitol. 2007;45:45–54.
[39] WHO. Control of food borne trematode infections. World Health Organ Tech Rep Ser. 1995;849:1–157.
[40] Lv S, Tian LG, Liu Q, et al. Water-related parasitic diseases in China. Int J Environ Res Public Health. 2013;10:1977–2016.
[41] Qiu J, Zhang Y, Zhang X, et al. Metacercaria infection status of fishborne zoonotic trematodes, except for *clonorchis sinensis* in fish from the Heilongjiang

Province, China. Foodborne Pathog Dis. 2017;14 (8):440–446.
[42] Sohn W, Na B, Cho S, et al. Prevalence of *Clonorchis sinensis* metacercariae in fish from water systems of Seomjin-gang (river). Korean J Parasitol. 2017;55 (3):305–312.
[43] Rim HJ, Sohn WM, Yong TS, et al. Fishborne trematode metacercariae in Luang Prabang, Khammouane, and Saravane Province, Lao PDR. Korean J Parasitol. 2013;51(1):107–114.
[44] Sarun T, Chalit K, Prayong R, et al. Discovery of *Opisthorchis viverrini* metacercariae in freshwater fish in southern Cambodia. Acta Trop. 2009;111: 108–113.
[45] Bammi YM. India Bangladesh relations: the way ahead. New Delhi, India: Vij Books India Pvt Ltd; 2010. p. 3.