



Full Length Article

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

Bannak G.D. Sumuduni^{a,*}, Dona H.N. Munasinghe^a, Appudurai Arulkanthan^b

^a Department of Zoology, Faculty of Science, University of Ruhuna, Matara, Sri Lanka

^b Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, Sri Lanka

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ABSTRACT

Centrocestus formosanus is a digenean that requires three host species to complete its life cycle. This study was conducted to observe the damage caused by two life stages of the *C. formosanus* on its host species. The snail *Melanoides tuberculata* was induced to shed cercariae by exposing to sunlight and specimens of koi carps were experimentally infected with cercariae. Gills of two infected fish were killed and fixed in Bouin's solution daily for 21 days. Infected fish were continuously fed to a pond heron (*Ardeola ralloides*) for three weeks and thereafter the bird was killed. Small intestine was resected as duodenum, jejunum, and ileum and fixed in formol saline. Gills and small intestine were prepared to study the histopathological damages. Flared opercula with protruding gills and increased respiratory rate were identified as the primary clinical signs of the fish. Encysted metacercariae were observed in the basal, middle and in the apical portion of the gills' filaments and gradual distortions and extensive proliferation of the cartilage of the gills resulted in loss of the respiratory epithelium. A progression of fibroblast to chondroblast encapsulation of the parasite was observed in the gill of fish as a host response. The duodenum of the heron was severely infected with adult parasites than jejunum and ileum. Flukes were observed in the villi, mucosae, submucosae, and also in the tunica muscularis of the duodenum. In conclusion, this study revealed that the heavy infection of *C. formosanus* could cause severe pathological lesions in both koi carps and pond heron.

1. Introduction

Sri Lanka has gained a good reputation in the international market for exporting high-quality ornamental fish species [1]. However, during culture practices, parasitic diseases harm the quality of fish on different levels. Certain parasites have the ability to reproduce rapidly to pathogenic levels and could cause high mortalities especially in young fish while some parasites have complex life cycles and they cannot reproduce in fish, but cause severe damages to fish even in lower numbers.

The heterophyid trematode *Centrocestus formosanus* (gill fluke) completes its life cycle using a snail and a fish as first and second intermediate hosts, respectively, and fish-eating birds or mammals as the definitive hosts [2]. This fluke is native to Asia, but it has now been recorded from the United States and other parts of the world [3–6]. The metacercariae of *C. formosanus* encyst in the gills of many species of

cultured fish and cause severe alterations in the architecture of gills, which could lead to respiratory difficulties, loss of production and death especially in young fish due to heavy infections [7,8]. Further, zoonotic cases due to species of the genus *Centrocestus* have been recorded from few Asian countries (*C. armatus* and *C. formosanus* in Korea, *C. caninus* in Thailand, *C. kurokawai* in Japan) as a result of eating raw or partially cooked freshwater fish, frogs and toads [9–11].

Among many parasite species, *Centrocestus* sp. have been recorded from many species of food fish, such as common carp (*Cyprinus carpio* L.), grass carp (*Ctenopharyngodon Idella* Valenciennes), silver carp (*Hypophthalmichthys molitrix*, Valenciennes), bighead carp (*Aristichthys nobilis* Richardson), catla (*Catla catla* Hamilton – Buchanan), Rohu (*Labeo rohita* Hamilton), Mrigal (*Cirrhinus mrigala* Hamilton) and common labeo (*Labeo dussumieri* Valenciennes), which were harvested from the mud ponds of fish culturing stations in Sri Lanka [7,12]. In addition, this parasite occurs in many ornamental fish species in this

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* Corresponding author.

E-mail address: deepikasumuduni@gmail.com (B.G.D. Sumuduni).

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country, including koi carps and goldfish from mud ponds [13,14].

The previous studies conducted on *Centrocestus* sp. in Sri Lanka were primarily focused on documenting this parasite in many species of fish, and there is a dearth of information on the histopathological lesions caused by this parasite in both second-intermediate (fish) and definitive hosts (piscivorous birds). Therefore, the first objective of the current study was to determine the sequence of histopathological lesions caused by this parasite in the gills of the koi carp (*C. carpio*) and an additional objective was to determine the lesions produced by this parasitic infection in the small intestine of the pond heron (*Ardeola ralloides*).

2. Materials and methods

2.1. Ethical approval

Ethical clearance for the study was obtained from the Institute of Biology (IOB), Sri Lanka (Permit No: ERC IOBSL136 11 15).

2.2. Collection and maintaining the snails

The first intermediate host *Melanooides tuberculata* snails (> 2cm in length) naturally infected with *Centrocestus* sp. were collected from two ornamental fish breeding centers in Sri Lanka: Rambadagalle in Northwestern province (GPS points: 7° 51'N; longitude-80° 50'E) and Ginigathhena in Central province (GPS points: 7° 00'N; longitude-80° 49'E). They were maintained in well-aerated tanks and fed with microalgae (*Chlorella* sp.).

2.3. Screening of infected snails and collection of cercariae

Fifty snails were placed individually in small beakers containing 25 mL of de-chlorinated water and exposed to sunlight to induce the shedding of cercariae larvae and few drops of water from each beaker were observed under the microscope to confirm the shedding of larvae. Cercaria of *Centrocestus* sp. was identified using morphometric and morphological characters such as 263.07 µm in length with a heart-shaped body, two eyespots as well as single tailed and compared with published descriptions [15,16]. Among the 17 snails that released cercariae, 12 of them shed larvae of *C. formosanus*. The cercariae suspensions of *C. formosanus* were collected into a beaker and the density was estimated.

2.4. Artificial infection of koi carp (*Cyprinus carpio*) from the cercariae

Matured Koi carps (*Cyprinus carpio*) up to 45 days were selected. Before the experiment, the fish were checked for external parasites and were treated with freshly prepared 2.5 ppm KMnO₄ [17] in order to clear the ectoparasites. All the experimental and control fish were fed twice a day with a commercial feed containing 35% protein and the temperature of the water was maintained at 29 °C. Three hundred fish (mean weight-3.0 ± 0.4 g, mean length-3.8 ± 0.5 cm) were kept individually in separate beakers with 300 mL of water. Approximately 200 cercariae were introduced to each beaker and after 2 h; the infected fish were stocked in two well-aerated treatment tanks (50 individuals per tank with 150L water). Furthermore, an extra tank was maintained as a control by stocking non-infected fish. The remaining infected fish (n = 200) were kept in a well-aerated tank as an extra stock.

2.5. Observation of infected fish

The experimental tanks were inspected daily and the abnormal behavior and clinical signs of the fish were recorded.

2.6. Chronological analysis of the damages caused by the metacercariae on the gills of fish

Wet mounts of the gills of some infected fish were prepared and observed by the microscope. Two infected fish from each treatment tank were killed daily for 21 days and the gills were fixed in Bouin's solution to examine histopathology in order to determine the chronological lesions caused by the metacercariae.

2.7. Infection of metacercariae to the pond heron (*Ardeola ralloides*)

To determine the damage caused in the small intestine of the definitive host by the adult fluke, the pond heron (*A. ralloides*) was selected as a possible natural definitive host of *C. formosanus* under Sri Lankan conditions. The wild caught heron was treated with Drontal plus® (a broad spectrum anthelmintic containing praziquantel, pyrantel, and febantel) to clear any intestinal parasitism, and the bird was housed in a cage and provided with fish and water *ad libitum*. Three days after the treatment, the bird was continuously fed with 28 days old metacercaria infected fish up to 3 weeks. The feces of the heron were observed daily under the microscope for the presence of the eggs of the parasite.

2.8. Investigation of histopathological damage caused by adult fluke in the small intestine of the heron

After 3 weeks of experimental infection, the heron was dissected and the small intestine was resected into three parts such as the duodenum, jejunum, and ileum and fixed in formal saline for histopathology.

The fixed tissues (gills and small intestine) were dehydrated using a series of ethanol and embedded in wax. Thin sections of the above tissues were stained with Haematoxylin and Eosin (H&E) and observed under the microscope and photographs of the lesions were taken.

3. Results

3.1. Clinical signs of the infected fish

Highly infected fish showed restless behavior and often came to the water surface for air breathing. Increased respiratory rate as evidenced by the rapid rate of opercula movements, outwardly flared opercula flaps (Fig. 1) and red colored inflamed gills were observed in heavily infected fish which died more readily than those fish in the control tank.



Fig. 1. Infected Koi carp (*C. carpio*) with *C. formosanus*: Infected Koi carp (*Cyprinus carpio*) showing outwardly flared opercula flaps (arrow).

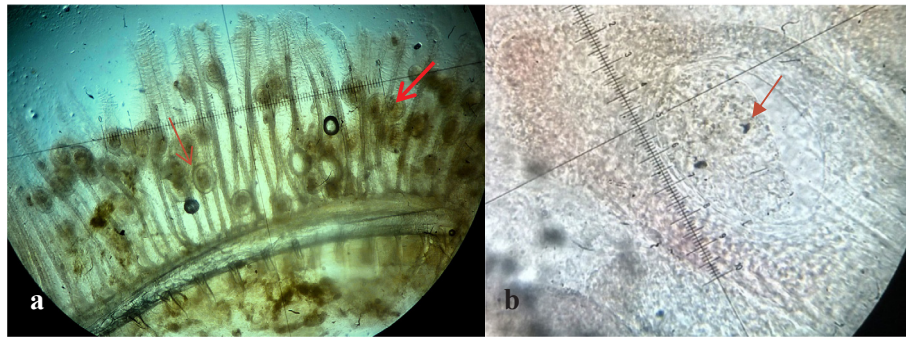


Fig. 2. Encysted metacercariae. (a) Wet mounts of the gills of an infected Koi carp with encysted metacercariae (arrow) of *C. formosanus* ($\times 40$). (b) Encysted metacercaria with eye spots (arrow) after 6 days of infection ($\times 100$).

3.2. Wet mounts of the gills of infected koi carps

Microscopic examination of the wet mounts of infected gills revealed many metacercariae encysted along the entire length of the gill filaments and few of them were found to be encysted on the gill arch (Fig. 2a). Infected metacercarial cysts found in gills were ranged from 10 to 20 in fish showed clinical signs and they were 20–25 cysts in dead fish. The cyst wall surrounding the metacercariae consisted of concentric layers of cells and two eyespots were visible in the metacercariae (Fig. 2b).

3.3. Histopathological changes caused by the metacercariae on gills of koi carp

The encysted metacercariae were observed at different sites of the gill filaments such as base, middle and apical portions (Fig. 3a) and the majority of them were found adjacent to the cartilage of gill filaments and on the gill arch (Fig. 3b). Overall, the gill architecture was found to be altered by the cartilage proliferation, epithelial hyperplasia followed by the fusion of primary lamellae, increased number of mucous cells, fusion of secondary lamellae and clubbing of gill filaments (Fig. 3c).

The metacercaria was encapsulated by a thin, non-cellular homogeneous and retractile cyst wall, which was surrounded by layers of chondroblasts or chondrocytes of host origin (Fig. 3d). The eosinophilic chondroblast or chondrocyte layers around the parasitic cyst consisted of granulated nuclei and varied in size. The cells adjacent to the parasite were flattened while the cells at the periphery were hypertrophied (Fig. 3d).

3.4. Sequence of chronological damage caused by the metacercaria stage on gills of koi carp

Cercariae were found to be attached to the cartilaginous rod after 2 h of infection. Mild hemorrhage and oedema with few necrotic cells were seen around the parasite (Fig. 4a). At 15 and 24 h post-infection, hemorrhage, oedema, inflammatory cells and fibroblast-like cells were observed around the parasite (Fig. 4b & c). An organized encapsulation of the parasite by 2–3 layers fibroblast-like cells was apparent by 48 h (Fig. 4d).

By 72 h of infection, there were up to 3 layers of eosinophilic and rectangular cells surrounding the cyst (Fig. 4e). The thickness of the encapsulation around the parasite had increased up to 3–4 layers of the

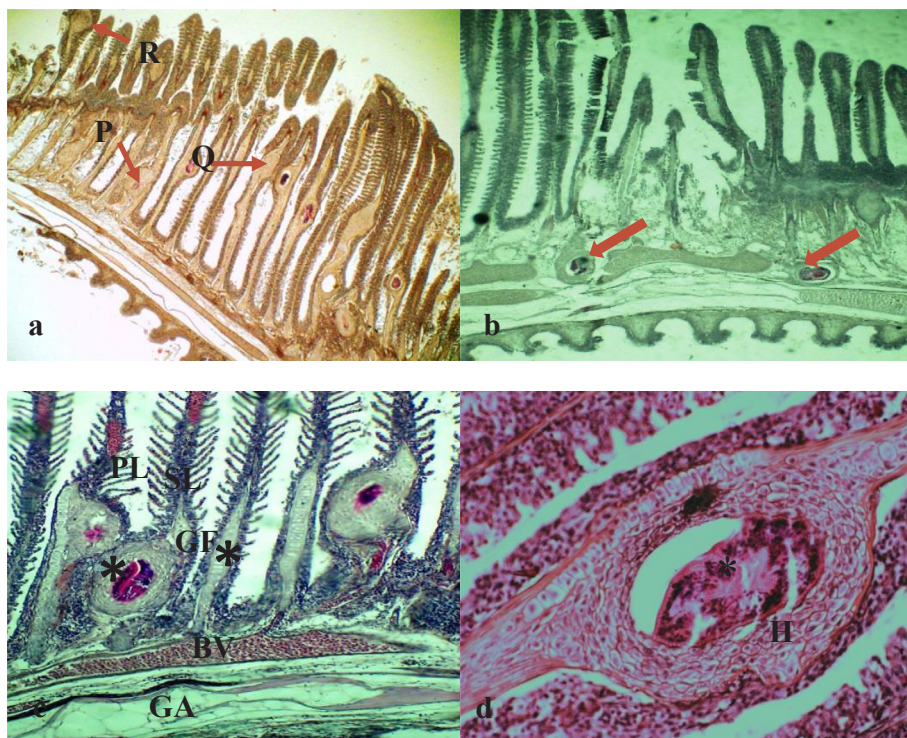
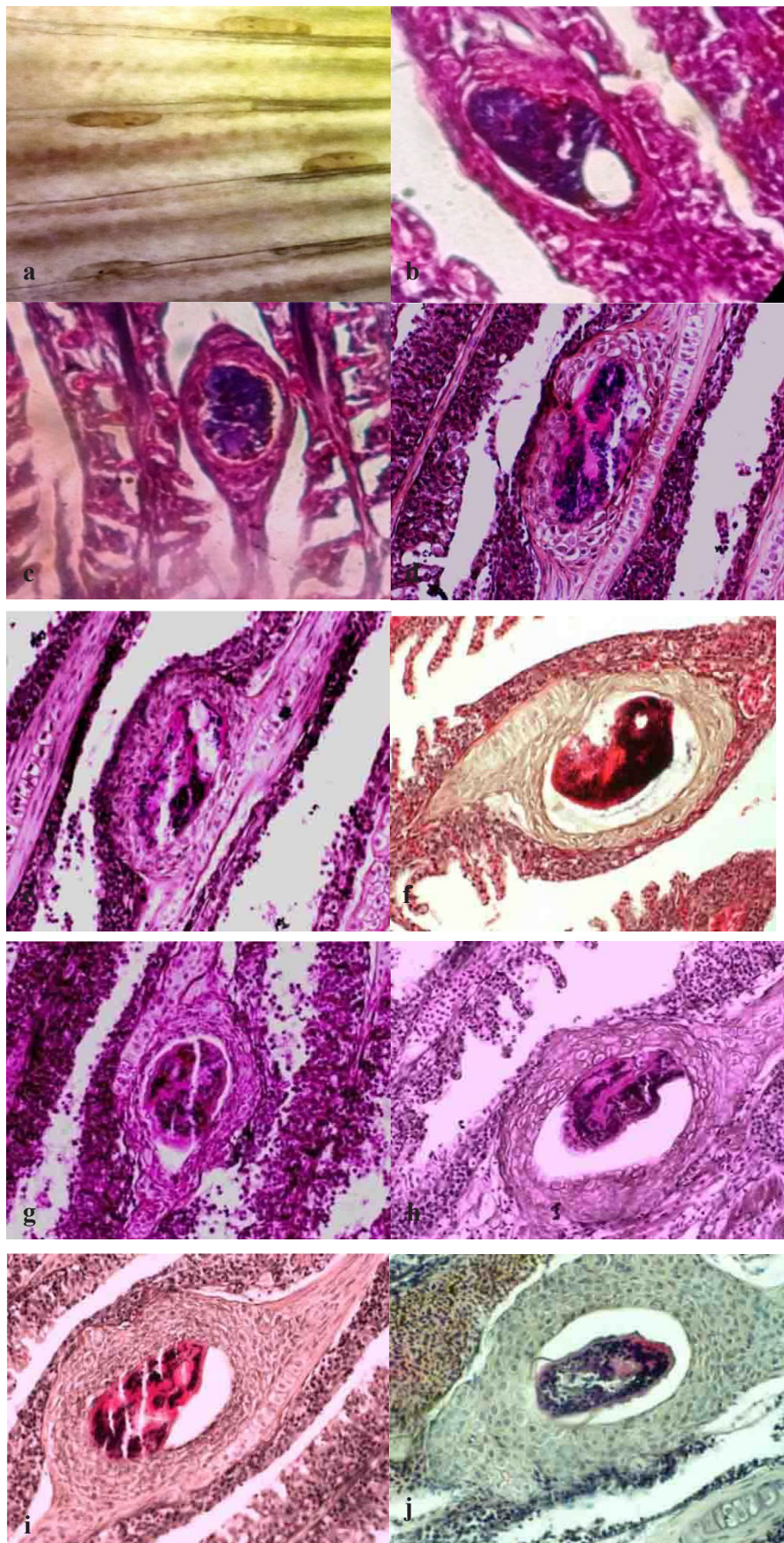


Fig. 3. Histopathological changes caused by *C. formosanus* on the gills of koi carp. (a) Gill of koi carp: Note the encysted metacercariae in the base (P), middle (Q) and the apical portion (R) of the gill filament ($\times 10$). (b) Metacercariae encysted on the gill arch (arrow) with hyperplastic tissue ($\times 40$). (c) Distortion of gill lamellae due to fusion of the hyperplastic tissues (*) surrounding the parasites which located in adjacent the lamellae ($\times 40$): Primary Lamellae (PL), Secondary Lamellae (SL), Blood Vessel (BV), Gill Filament (GF) and Gill Arch (GA). (d) Parasite (*) is obvious in the center of chondrodysplasia (H) ($\times 100$, H&E).



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Fig. 4. The sequence of chronological lesions caused by metacercariae of *C. formosanus* in the gills of *C. carpio*. (a) Cercariae on the gill filaments after 2 h of infection ($\times 40$, H&E). (b) After 15 h of infection. (c) After 24 h of infection. (d) After 48 h of infection. (e) After 72 h of infection. (f) After 4 days of infection. (g) After 5 days of infection (h) After 7 days of infection. (i) After 10 days of infection. (j) After 21 days of infection (from (b) to (j) – magnification power: $\times 100$, H&E).

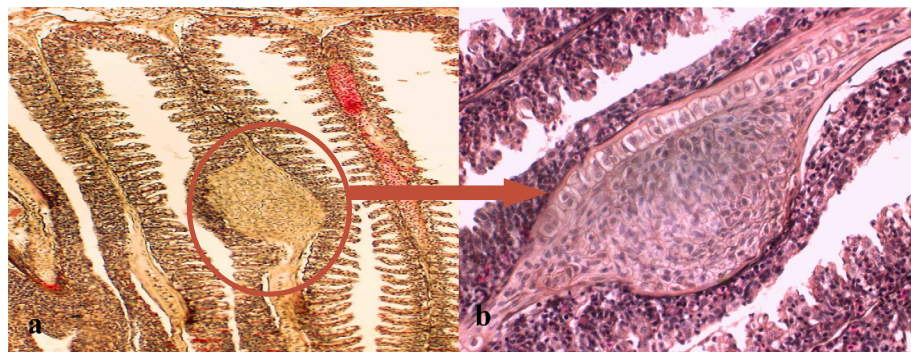


Fig. 5. (a) Hyperplastic cysts in the gill filament without the parasite ($\times 40$, H&E). (b) Higher magnification of Fig. a ($\times 100$, H&E).

cell by the 4th day post-infection (Fig. 4f). The outermost cells were flattened while the innermost cells were cuboidal to rectangular in shape. After 5 days post-infection, the encapsulation was increased up to 4 layers with basophilic cells with a large nucleus (Fig. 4g), and the number of cell layers was gradually increased up to 4–5 by 7 days (Fig. 4h).

Thickness of the cyst was increased up to 8–10 cell layers by 10 days post-infection (Fig. 4i). The cell layers were primarily cuboidal shaped chondroblasts or chondrocytes, which were surrounded by flattened fibroblast-like cells. More than 8 cell layers were observed in the cyst at 21 days post-infection (Fig. 4j). Some hyperplastic cysts were observed without the parasite (Fig. 5).

3.5. Histopathological changes caused by the adult stage of the parasite in the small intestine of pond heron (*Ardeola ralloides*).

While examining the histological sections of the duodenum, jejunum, and ileum, it was apparent that more flukes were found in the duodenum than in the ileum. The main lesions observed in the duodenum included fusion of the villi (Figs. 6 & 7a), hyperplasia of the crypt epithelium and epithelial damage around the site of worm attachment. Flukes with intrauterine eggs were observed in the sub-mucosal layer of the duodenum (Figs. 7b & c). Flukes were seen attached to the mucosae of the intestinal crypts by using their oral sucker. Moreover, they were found deeply invaded into the submucosa and tunica muscularis (Figs. 7d & e). The adult fluke collected from the small intestine was small, pyriform, consisted of 32 oral spines, with X-shaped excretory bladder, and the eggs with latticed design (Fig. 7f).

4. Discussion

Many studies have been carried out in different countries, including Sri Lanka to document lesions caused by the metacercariae of *C.*

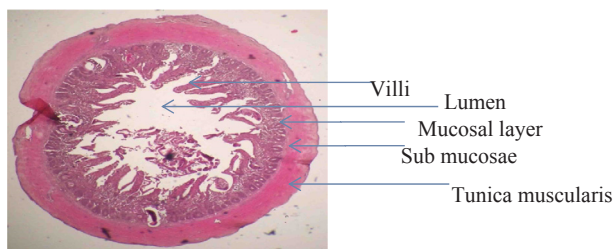


Fig. 6. Invasion of *C. formosanus* into the superficial and deep layers of the duodenum of *A. ralloides* ($\times 40$, H&E).

Formosanus in the gills of the second intermediate host [7,18–21]. However, the information on the chronological damage caused in the gills of fish by *C. formosanus* is scant, which precludes the meaningful comparison of this study with similar studies done on this parasite. This study indicated that the chronological lesions observed in the gills were related to the invasion and encystment of the parasite in the gill lamellae followed by the host responses to the parasite.

Previous studies documented the occurrence of hemorrhages in the gills of fish infected with metacercariae [19,22]. Similarly, the hemorrhage observed in this study within hours after infection could be due to the mechanical damage of gill capillaries by the invading parasite. Oedema and infiltration of leucocytes observed in the gills were probably due to the onset of inflammatory reactions in the host in response to the injury. After the invasion of the bronchial tissues, the parasite was found to be adjacent to the cartilage of primary gill lamellae within two hours of infection and the parasites were surrounded by a few layers of host cells within 24 h post-infection (Figs. 4a & c). The host cell layer tencircled the parasite increased in thickness with time and gradually transformed into chondroblasts or chondrocytes (Fig. 4j). Blazer and Gratzek [18] have observed the chronological damage caused in the gills of guppies (*Poecilia reticulata*) and goldfish (*Carassius auratus*) by an unidentified metacercaria for 6 days, and their findings generally corroborate with this study. The hyperplasia of the cartilage cells around the parasite observed in this study is probably a defense mechanism of the host against the parasite as suggested in earlier studies [8,18,20,21]. The encystment of the metacercariae in the gill filaments and gill arches caused severe distortion of the gill architecture and reduction of the respiratory surface, which may explain the respiratory signs, such as air-breathing and increased respiratory rate of fish observed in this study.

Although the piscivorous birds are the main definitive host of *Centrocestus* sp., only a few studies had been carried out to document the lesions caused by this parasite in birds. In one study, Nath [23] used pigeons as definitive hosts to document the pathological responses against *C. formosanus*.

The main lesions observed in the duodenum of the experimentally infected *A. ralloides* were the fusion of the villi, hyperplasia of the crypt epithelium and epithelial damage around the site of worm attachment (Figs. 6 & 7). The adult *C. formosanus* has been found in all layers of the small intestine, such as mucosa, submucosa and tunica muscularis, of pond heron.

Studies conducted on the damage caused by *C. armatus*, *C. caninus*, *C. formosanus* in the intestine of *Rattus novogicicus* suggested that intestinal lesions in the host may both depend on the species of the parasite and the host [24–26]. Chai et al. [27] have reported the deep invasion of a heterophyid trematode *Metagonimus yokogawai* into the

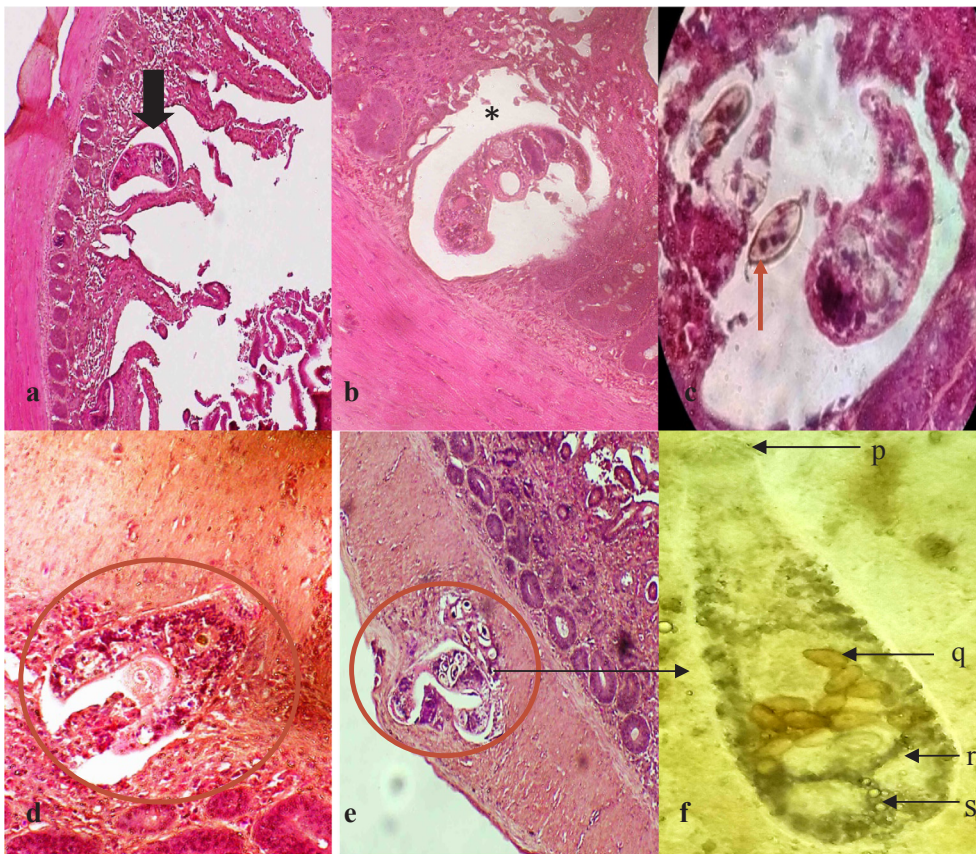


Fig. 7. Invasion of *C. formosanus* into the intestine of *A. ralloides*. (a) Worm found in between the fused villus ($\times 40$, H&E). (b) *C. formosanus* in the sub mucosae (*) destroying the intestinal glands ($\times 400$, H&E). (c) Fluke with released eggs (arrow) ($\times 400$, H&E). (d) Parasite Invading the tunica muscularis ($\times 400$, H&E). (e) Invasion of *C. formosanus* into the deeper layer of the tunica muscularis of the intestine ($\times 100$, H&E). (f) Adult fluke of *C. formosanus* found in the small intestine of *A. ralloides* (p: Oral sucker, q: Eggs, r: Testes, s: Excretory bladder, $\times 100$).

submucosal layer in mice. Africa et al. [28] recorded presence of the eggs of heterophyid flukes (*Haplorchis* sp., *Stellantchasmus* sp. and *Procerovum* sp.) in the heart and spinal cord of human due to deeper penetration and subsequent spread of the flukes to internal organs. The present study revealed the deep invasion of adult *C. formosanus* up to tunica muscularis layer of the small intestine of the pond heron.

The observed adult parasite morphology was consistent with the findings of earlier studies and confirmed as *C. formosanus* [16,29]. According to data and observations gathered from the current study, it was experimentally proved that *C. formosanus* could be survived successfully in the small intestine of the pond heron (*A. ralloides*), a common bird species live associated with water bodies in Sri Lanka. Therefore, it might be a potential definitive host for *C. formosanus* under local conditions.

5. Conclusions

A progression of fibroblast to chondroblast encapsulation of the parasite was observed in the gills of the fish as a host response. Adult flukes were observed in the villi, mucosae, submucosae and also in the tunica muscularis of the duodenum of pond heron. This study revealed that the heavy infection of *C. formosanus* could cause severe pathological lesions in both koi carps and pond heron. The findings of the present study are important for understanding the severity of the damage caused by the *C. formosanus* in cultural systems that assists in early detection and control through proper treatment methods.

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Competing interests

All authors declared that they have no conflicts of interest.

References

- [1] Gunasekara S. The export of indigenous freshwater fish species from Sri Lanka. *J WNPS* 2007;24:7–13.
- [2] Yamaguti S. *Systema Helminthum*. The digenetic trematodes of vertebrates, Interscience, New York, Druzno lake Part- 1, Acta Parasite Pole 1958;6: pp. 1–64.
- [3] Chai JY, Woon-Mok S, Tai-Soon Y, Keeseon SE, Duk-Young M, Mi YL, et al. *Centrocestus formosanus* (Heterophyidae): human infections, and the infection source in Lao PDR. *J Parasitol* 2013;99:531–6.
- [4] Foojan M, Hannah MJ, Per WK, Kurt B. Import of exotic and zoonotic trematodes (Heterophyidae: *Centrocestus* sp.) in *Xiphophorus maculatus*: implications for ornamental fish import control in Europe. *Acta Parasitol* 2014;59:276–83.
- [5] Krailas D, Namchote S, Koonchornboon T, Dechruksa W, Boonmekam D. Trematodes obtained from the thiarid freshwater snail *Melanooides tuberculata* (Müller, 1774) as vector of human infections in Thailand. *Zoosyst Evol* 2014;90:57–86.
- [6] Pinto HA, Mati VLT, Melo AL. Metacercarial Infection of wild Nile tilapia (*Oreochromis niloticus*) from Brazil. *Sci World J* 2014;2014:1–7.
- [7] Balasuriya LKSW. A study on the metacercarial cysts of a *Centrocestus* sp. (Digenea: Heterophyidae) occurring on the gills of cultured fishes in Sri Lanka. *J Inland Fish* 1988;4:3–10.
- [8] Mitchell J, Overstreet RM, Goodwin AE, Brandt TM. “Spread of an exotic fish-gill trematode: a far-reaching and complex problem. *Fisheries* 2005;30(8):11–6.
- [9] Kurokawa T. On a new species of *Stamnosoma* found from the man. *Tokyo Iji Shinshi* 1935;59:293–8. (in Japanese).
- [10] Hong S, Woo H, Chai J, Chung SW, Lee S, Seo BS. Study on *Centrocestus armatus* in Korea. *Korean J Parasitol* 1988;26:55–60.
- [11] Waikagul J, Wongsaraj T, Radomyos P, Meesomboon V, Praewanich R, Jongsuksantikul P. Human infection of *Centrocestus caninus* in Thailand. *Southeast Asian J Trop Med Public Health* 1997;28:831–5.
- [12] Wimalawickrama ND, Pathirathne A. Evaluation of the effects of infestation by trematodes and *Lernaea* on *Catla catla*, an Indian carp cultured in Sri Lanka. *Sri Lanka J Aq Sci* 2005;10:85–96.
- [13] Thilakarathne IDSIP, Rajapaksha G, Hewakopara A. Rajapakse RPVJ, Faizal ACM. Parasitic infections in freshwater ornamental fish in Sri Lanka. *Dis Aquat Organ* 2003;54:157–62.
- [14] Sumuduni BGD, Munasinghe DHN, Chandrarathna WPR, De S, Amarasinghe NJ. Relationship between condition factor and external parasite density of goldfish

- (*Carrasius auratus* (Linnaeus, 1758)) and Koi carp (*Cyprinus carpio* (Linnaeus, 1758)) during dry and wet seasons. *Int J Curr Res* 2014;6:8282–5.
- [15] Hernandez LE, Diaz MT, Bashirullah AK. Description of different developmental stages of *Centrocestus* sp. (Nishigori, Digenea: Heterophyidae). *Rev Cient* 1924;2003(6):285–92.
- [16] Yousif F, Ayoub M, Tadros M, Bardici SEL. The first record of *Centrocestus formosanus* (Nishigori 1924) Digenea Heterophidae in Egypt. *J Exp Parasitol* 2016;168:56–61.
- [17] Noga EJ. *Fish Disease Diagnosis and Treatment*. Iowa State University Press; 2000. p. 1st ed..
- [18] Blazer VS, Gratzek JB. Cartilage proliferation in response to metacercarial infections of fish gills. *J Comp Pathol* 1985;95:273–80.
- [19] Mitchell AJ, Goodwin AE, Salmon MJ, Brandt TM. Experimental infection of an exotic heterophyid trematode, *Centrocestus formosanus*, in four aquaculture fishes. *N Am J Aquac* 2002;64:55–9.
- [20] Gjurcevic E, Petrinec Z, Kozaric Z, Kuzir S, Gjurcevic Kantura V, Vucemilo M, et al. Metacercariae of *Centrocestus formosanus* in goldfish (*Carassius auratus* L.) imported into Croatia. *Helminthologia* 2007;44:214–6.
- [21] Rezaie A, Dezfuly ZT, Mesbah M, Ranjbar A. Histopathologic report of infestation by *Centrocestus formosanus* in Iranian grass carp and common carp. *Iran J Vet Sci Tech* 2017;9:49–53.
- [22] Velez-Hernandez EM, Constantino-Casas F, Garcia-Marquez LJ, Osorio-Sarabia D. Gill lesions in common carp, *Cyprinus carpio* L., in Mexico due to the metacercariae of *Centrocestus formosanus*. *J Fish Dis* 1988;21:229–32.
- [23] Nath D. Pathology of *Centrocestus formosanus* (Nishigori, 1924) infection in experimental pigeon. *Indian J Anim Sci* 1972;42:952–4.
- [24] Saenphet S, Wongsawad C, Saenphet K, Rojanapaibul A, Vanittanakom P, Chai J. Chronological observations of intestinal histopathology in rats (*Rattus norvegicus*) infected with *Centrocestus caninus*. *Southeast Asian J Trop Med Public Health* 2006;37:69–73.
- [25] Hong SJ, Han JH, Park CK, Kang SY. Intestinal pathologic findings at early stage infection by *Centrocestus armatus* in albino rats. *Korean J Parasitol* 1997;35:135–8.
- [26] Mati VLT, Pinto HA, Melo AL. Experimental infection of Swiss and AKR/J mice with *Centrocestus formosanus* (Trematoda: Heterophyidae). *Rev Inst Med Trop Sao Paulo* 2013;55:133–6.
- [27] Chai JY, Kim J, Lee SH. Invasion of *Metagonimus yokogawai* in to the sub mucosal layer of the small intestine of immune suppressed mice. *Korean J Parasitol* 1995;33:313–21.
- [28] Africa CM, De Leon W, Garcia EY. Visceral complications in intestinal heterophyidiasis of man. *Acta Med Philipp (Monographic series)* 1940;1:1–32.
- [29] Wongsawad C, Wongsawad P, Anuntalabhochai S, Chai JY, Sukontason K. Occurrence and molecular identification of liver and minute intestinal flukes metacercariae in freshwater fish from Fang-Mae Ai Agricultural Basin, Chiang Mai province, Thailand. *Asian Biomed* 2013;7:97–104.