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A note on heavy infection of diplostomid metacercarial cysts in Gar fish, *Xenentodon cancila* (Beloniformes: Belonidae) from River Penna, Andhra Pradesh, India

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Abstract

Digeneans of the Diplostomidae are the fish-borne pathogen and a digenetic trematode of birds. The metacercarial forms of these digeneans infect the vital organs such as the liver of the fish. The metacercariae of *Neascus hepatica* (Digenea: Diplostomidae) were observed in the liver of the infected adult Gar fish (*Xenentodon cancila*). The gross pathological examination revealed that the liver extremities were severely infected with the parasites, almost encompassing the entire organ. With metacercarial cysts embedded in it, only the outline of the liver structure is visible. At the site of parasite attachment, the liver tissue appeared transparent with no cellular organization i.e., parenchymal atrophy was observed. The description of the parasite and its clinicopathology is provided. The finding of the study suggests that infection with this metacercariae severely damages the vital organs, stunts the growth of the fish and inclines the fish towards the opportunistic pathogens in the environment.

Key words : Metacercariae, *Neascus hepatica*, Diplostomidae, liver, Infection, *Xenentodon cancila.*

Background :

Parasitic diseases cause a brutal menace to the culture and wild fishes and have become a major constraint in aquaculture[3]. These parasites directly show its impact on the fish's health thereby making fish more susceptible to predation to secondary infections[38]. Parasitic infections are more common, especially in wild populations where parasite transmission occurs effectively through intermediate hosts[16,40]. The round or bead-like immature or

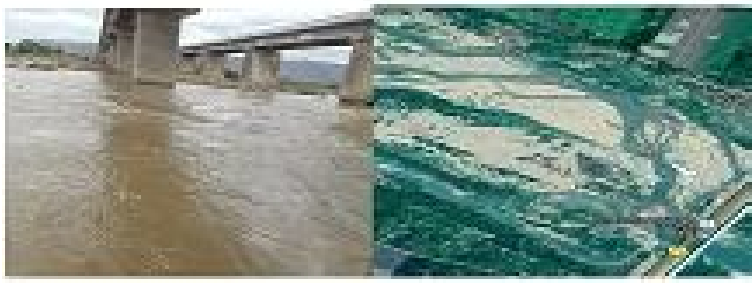
larval form of adult digenean flukes which are known as 'Grubs' cause a variety of disease in fishes such as yellow grub disease, white grub disease and black grub disease[23]. Usually the term 'grub' is a true larval form of insect (Beetle), but the 'grubs' found in fish are not insects but the larval forms of digeneans[17]. Digenean trematodes are the main fish parasites with fish serving both as intermediate (grubs) and final hosts (flukes). These digeneans have complicated life-cycles with numerous levels regarding a couple of hosts, ideally mollusc being the primary intermediate host, fish being the secondary transitional host and striding bird being the ultimate host[15]. Gross or microscopic examination of tissues or body cavities reveals cercarial, metacercarial, or adult worms. The fish secretes black, yellow and white pigmentation as an immune response against the parasite. Pigmentation depends on the color of the cysts and the condition is known as black, white or yellow grub disease[24, 36]. The white grubs among all the grubs have potentially harmful effects on its host-fish as they can travel through the bloodstream of fish to survive in the fish's visceral organs like liver, kidneys and ovaries. Severely parasitized fish are feeble, lean, immobile, and feed inadequately. In a heavily infected fish, massive numbers of white grubs can dislocate or rupture organs, ensuing in death. The presence of white grub is capable of deteriorating the fish immune system and making it more susceptible to secondary diseases. Usually these forms are harmless to the human beings; however, their appearance on the muscles of fish gives a nasty visual appeal to consumers and makes them less marketable. Heavily parasitized fish become weakened, skinny, lethargic, and have poor feeding habits. Most of the studies were focused on yellow grubs-*Clinostomum* sp., black grubs- *Uvulifer* sp. and white grubs-*Posthodiplostomum* sp., Neascus sp. as they can be a significant problem in pond fisheries[7,12,13,17,26,31-33,37]. An overview of the morphology, taxonomy, and clinicopathology of infection is presented in this study.

Approximately 60 adult gar fish (*Xenentodon cancila*) (19.6±2.21cm, mean total length ± standard deviation; total length ranged from 14.6-24.7 cm and 27.4.9±7.86g, mean total weight ± standard deviation; total weight ranged from 12.9-41.9 g) were procured from three diverse sampling locations of YSR Kadapa district (Lat. 14°28′N 78°49′E, 137 m altitude), located in Andhra Pradesh state during August, 2017 to February, 2018 (Fig. 1) *i.e*., Location-1: Mylavaram reservoir across Penna River in Mylavaram village, YSR Kadapa District; Location -2: Aadinimma-yapalle Dam across the Penna River in Chennur village, YSR Kadapa district and Location-3: Backwaters of Somasila reservoir across the River Penna in Somasila village, Nellore district, Andhra Pradesh reach near Vontimitta Village, Kadapa. Microscopic examination of wet mounts scraping of skin, gills and fins and internal visceral organs was carried out to observe ectoparasites and endoparasites under stereozoom microscope (LM-52-3621 Elegant). The live encysted metacercarial forms were recovered from the infected liver of the fish and morphological information was recorded for their taxonomic identification. Metacercarial forms were excysted and fixed in alcohol/formalin/acetic acid (AFA) fixative, preserved in 70% ethanol, and processed for identification. Parasites were further stained with alum carmine followed by dehydration with a graded series of alcohols

(70%, 80%, 95% and 100%), cleared in xylol and lastly mounted on a glass slide with DPX mount. Observations, identifications and microphotographs of these parasites were taken using Lynx trinocular microscope (N-800M) at three different magnifications (10X, 20X and 40X) and scale was consequently indicated. An attached drawing tube to the microscope was used to illustrate line diagrams at different magnifications (4X, 10X, and 40X). All measurements were expressed in millimeters, unless otherwise mentioned. For

Fig. 1: Geographical location of the three fish sampling sites of YSR Kadapa District, Andhra Pradesh

Site-1: Mylavaram reservoir across Penna River in Mylavaram village, YSR Kadapa District



Site-2: Aadinimmayapalle Dam across the Penna River in Chennur village, YSR Kadapa district

Site-3: Backwaters of Somasila reservoir across the River Penna in Somasila village, Nellore district, Andhra Pradesh reach near Vontimitta Village, Kadapa.

histopathological studies, infected and uninfected (control) liver were fixed in neutral buffered formalin, processed for paraffin wax sections of 4-5 µm, and further stained with haematoxylin-eosin counterstains.

This study documents the heavy infection of *Neascus* type metacercarial cysts of Diplostomid digeneans (40-50 numbers/fish) in Gar fish, *Xenentodon cancila* (Fig. 2). The *Neascus* type larval metacercarial form of the Diplostomidae is a relatively large genus infecting the liver (Fig. 3) and other visceral organs of freshwater fishes which are in turn eaten by the fish eating birds and become adult diplostomid digeneans. The metacercarial cysts appear transparent through which the live metacercaria can be seen moving within the cyst (Fig. 4 & 5). The metacercaria was excysted from the cyst and its body is elongate, smooth, with rudiments of lateral sucking cups (1.18-1.34 x 0.34-0.39). Body well-demarcated into a long, broad fore-body (0.74-0.79 x 0.37-0.47) and slightly truncated posterior hind-body

Fig.2

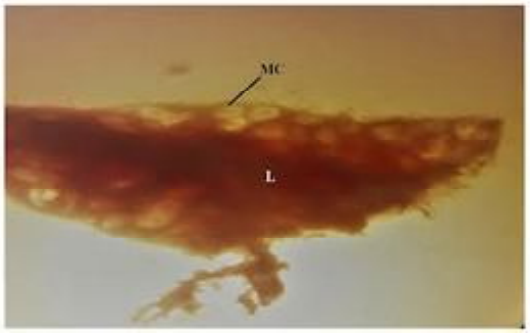


Fig.3

Fig.4

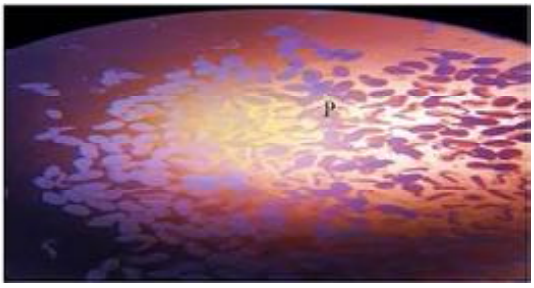


Fig.5

Fig. 2: *Xenentodon cancila* infected with metacercarial cysts

Fig. 3: Heavily infected liver of *X. cancila* showing Neascus type metacercarial cysts of diplostomid digeneans

Fig. 4: Neascus type metacercarial cysts of diplostomid digeneans detached from the liver and live excysted larvae

Fig. 5: Excysted and encysted Neascus type metacercarial cysts of diplostomid digeneans fixed in FAA fixative

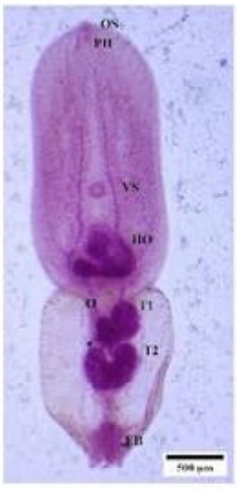


Fig.6

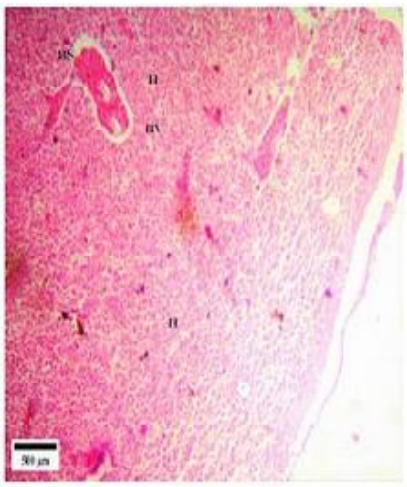
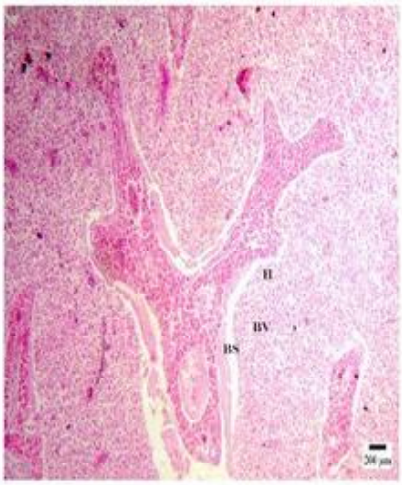


Fig.7



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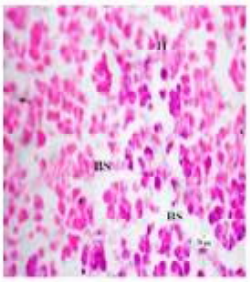

Fig.9

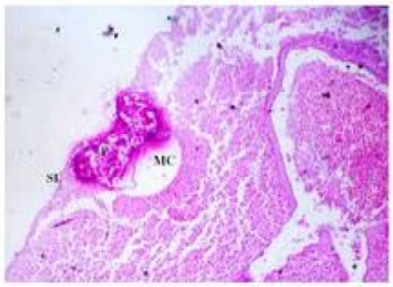

Fig.10

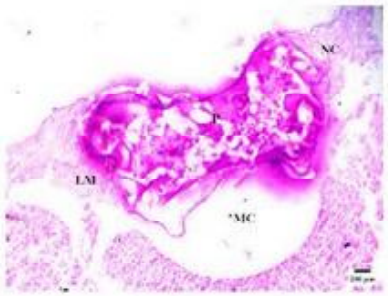

Fig.11

Fig. 6: Whole mount of excysted Neascus type metacercaria of Diplostomid digenean (H&E-500µm)-OS-Oral sucker; PH-Pharynx; VS- Ventral sucker; HO- Holdfast organ; T1-Testis-1; T2- Testis- 2; O-Ovary; EB- Excretory bladder

Fig. 7: Transverse section of normal liver (H&E-500µm) showing polyhedral hepatocytes (H) with blood vessels (BV) and Blood sinusoids (BS) with intact serosal layer (SL)

Fig. 8: T.S of normal liver at higher magnification (H&E-200µm) showing polyhedral hepatocytes (H) with blood vessels (BV) and Blood sinusoids (BS)

Fig. 9: T.S of normal liver at higher magnification (H&E-50µm) showing polyhedral hepatocytes (H) with blood vessels (BV) and Blood sinusoids (BS)

Fig. 10: T.S of infected liver section (H&E-200µm) showing parasites (P) within the metacercarial cyst (MC) disrupting the outer serosal layer (SL) of liver

Fig. 11: T.S of infected liver section (H&E-200µm) showing parasites (P) within the metacercarial cyst (MC) disrupting the outer serosal layer (SL) of liver, necrotic cells (NC) and cellular infiltration with lymphocytes (LM) around the cyst

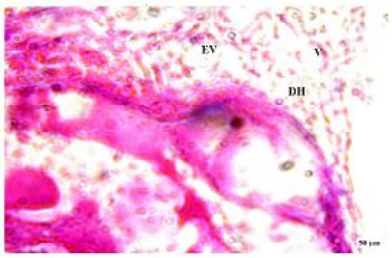

Fig.12

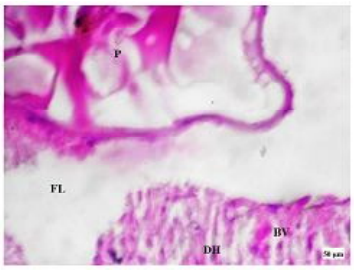

Fig.13

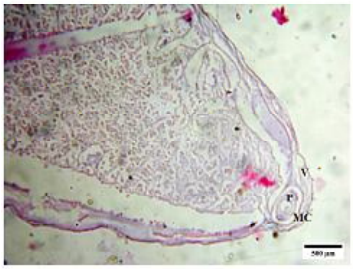

Fig.14

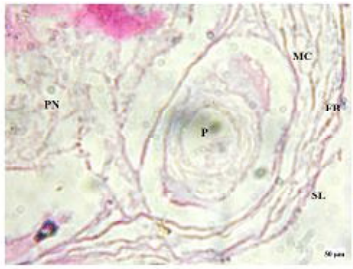

Fig.15

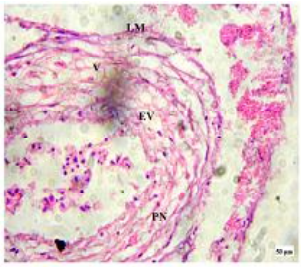

Fig.16

Fig. 12: Infected liver showing extensive vacuolization (EV) and dilatation of most hepatocytes (DH) (H&E, 50µm)

Fig. 13: Extensive vacuolization (EV) around the metacercarial cyst (MC) (H&E, 50µm)

Fig. 14: Tip of the section of infected liver showing an extensive damage to the hepatic cells (H) (H&E, 500µm)

Fig. 15: Tip of the infected liver showing the attachment of metacercarial cyst at higher magnification (H&E, 200µm) with vacuoles (V) around the cyst, dispersed hepatocytes (DH) and fibrosis (FB) around the cyst.

Fig. 16: Infected liver showing the dispersed hepatocytes (DH) with pyknotic nuclei (PN) near attachment of metacercarial cyst at higher magnification (H&E, 50µm) with vacuoles (V) around the cyst (MC).

(0.53-0.58 x 0.29-0.45) (Fig. 6). Forebody has fairly developed suckers with a small terminal oral sucker (0.03 x 0.03-0.04) and a large, round acetabulum (0.04-0.05). The digestive tract shows a short pre-pharynx and a small, muscular pharynx (0.01-0.02 x 0.01-0.02); short oesophagus bifurcating to form intestinal caeca terminating in the posterior extremity of the body. Hold fast organ (0.11-0.13 x 0.14-0.21) posterior to acetabulum, at the posterior margin of the fore body followed by a granular, transversely elongated hold fast gland (0.04-0.06 x 0.12-0.17). The short hind body has 3 well- defined, densely stained masses of genital rudiments with two tandemly arranged large testes (small anterior pear-shaped testis- 0.09-0.10 x 0.05-0.09; crescent-shaped posterior testis- 0.13-0.16 x 0.15-0.19) and one

small ovary (0.06-0.08 x 0.03-0.04). Genital pore terminal, surrounded by copulatory bursa of the adult. Copulatory bursa when protruded through the genital pore shows one median and two lateral pieces (Fig. 6). The gross examination of the normal liver of gar fish, *X. cancila* revealed that it is yellowish brown in colour with a single lobe lying on the right side of the body besides the alimentary canal and enclosed in a fibrous tissue capsule (Fig. 7). The cells of the liver are polyhedral (Fig. 8). Among the liver cells are found the blood sinusoids and blood vessels (Fig. 8). Between any two blood capillaries, at least 2 to 3 hepatic cells (H) are seen (Fig. 9). Bile capillaries appear as minute canals between the hepatic cells (Fig. 9). However, the gross examination of the infected liver of the fish showed a complete degeneration with parenchymal atrophy, pale liver due to the encystment of the nearly 40-50 cysts deeply into the liver. The metacercarial cysts totally occupied the spaces in the liver cells and only the outline of the liver structure was visible with metacercarial cysts embedded in it (Fig. 10). At the site of parasite attachment, the liver tissue appeared transparent with no cellular organization (Fig. 11). Large number of inflammatory cells accumulated around the parasite cyst which is surrounded by a fibrous layer. The liver tissues adherent to the parasitic cyst showed a layer of degenerated hepatocytes, necrotic cells and cellular infiltration (Fig. 11). Cysts were elongate, oval and transparent, found deeply embedded in the liver of the fish. Cysts single layered devoid of pigments (Fig. 12). Cysts contain transparent, colourless, gelatinous and adhesive fluid (Fig. 13). The metacercarial body within the cysts was discovered moving freely in the spacious accommodation available (Fig. 14-16).

Xenentodon cancila, commonly known as gar fish/needlefish is a freshwater fish mainly traded as aquarium fish feeding on small frogs, fishes and crustaceans[19]. A lot of work on metacercarial fauna of freshwater fishes from Indian sub-continent was contributed by scientists[2,4-11,14,18,21,22,25,27,29,30,34,35]. The larval genus *Neascus* was originally erected by Hughes (1927)[20] with eleven Indian species, *N. vetastai* Kaw, 1950, *N. elongatus* Singh, 1957, *N. chelai* Khera, 1958, *N. hepatica* Chakrabarti, 1971, *N. channi* Pandey, 1971, *N. xenentodoni* Pandey, 1971, *N. gussevi* Chakrabarti, 1974, *N. hoffmani* Pandey, 1976, *N. komiyai* Pandey, 1976, *N. indicus* Thapar, 1967 and *N. cirrhinus* (Hughes, 1927) Thapar, 1967. Pandey (1971)[28] provided a key for the separation of *Neascus* type of larva reported from India. Later, Agrawal and Khan (1982)[1] described *Neascus hanumanthai* from *Channa punctata*. The species diversity of diplostomid digeneans is not yet completely understood especially in Indian sub-continent as the species were described based on morphological characteristics only[7]. However, three neascus-type metacercaria have been described by Choudhary *et al*.[13], namely N. *hanumanthai, N. gussevi, and N. xenentodoni* from Indian freshwater fish, *Channa punctata* (Bloch, 1793), *Trichogaster fasciata* (Bloch and Schneider, 1801) and *Xenentodon cancila* (Hamilton, 1822) respectively. Though, there were many Indian metacercarial species described but according to WoRMS[39] (World register of marine species) there were only 12 valid species under the genus *Neascus* Hughes, 1927. Recently, Chaudhary *et al.*,[7] provided the morphological and molecular evidence for the larval trematodes infecting

the liver of gar fish, *Xenentodon cancila* and identified as *Posthodiplostomum pandei* (*Neascus pandei*) sp. However, the present species comes closer to *N. hepatica* from *Xenentodon cancila* described by Chakrabarti[4] in their body shape and size. Though these parasites are not harmful to human beings, but their appearance on the muscles and organs of fish make least saleable. However, severely parasitized fish become immunologically weak, immobile, and feed inadequately due to dislocation or damage to organs by the massive numbers of metacercariae and make them more susceptible to secondary diseases[23,24,31-33,36,37].

In the present study, we have observed that the presence of metacercarial cysts is harmless to humans if they are removed or properly cooked. But their occurrence on the main organs like liver cause histopathological alterations which in turn makes liver non-functional. The greatest histopathological damage in the liver of *X. cancila* was caused by parenchymal atrophy, cellular infiltration, necrosis, and fibrosis, all of which impairs the liver function. In heavy infections, the entire liver tissue is replaced by parasitic cysts which make the fish liver completely non-functional and leads to death. Thus, the current study concluded that metacercarial infestations are potential threats to fish and can cause significant damage to fish yield.

List of abbreviations

L-Liver; P- Parasites (*Neascus*.sp.); H- Hepatocytes (Hepatic cells); MC-Metacercarial cysts; BV- Blood vessels; BS-Blood sinusoids; SL- Serosal layer; NC-Necrotic cells; LM- Lymphocytes.

Declarations :

Ethics approval and consent to participate:

All procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals and have been approved and authorized by IAEC (Institution of Animal Ethics Committee-Regd. No.1460/PO/a/11/CPCSEA, dt. 20.05.2011), Zoology Department in Faculty of Life Sciences, Yogi Vemana University, Andhra Pradesh.

Consent for publication

Not applicable

Availability of data and material

The raw data used to support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that no competing interests exist.

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Authors' contributions

APV designed the work and MDP assisted late Dr. Asha Kiran Modi for the fish

sample collection to carry out the pathological work. The third author, RVS, helped in framing the manuscript and correction part. All the authors have read and approved the manuscript.

The corresponding author commemorates this work to late Dr. Asha Kiran Modi, who worked under the financial assistance of UGC Faculty improvement programme (FIP)-Award No.APSC021/001(TF)ZOOLOGY/PH.DXII PLAN/2016-17 dt. July 2016.

Significance statement

The significance of the present work is to show that even the metacercarial infestations can be potential threats for fish and aquaculture.

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