

Study Of Digenea Parasite Clinostomum Phalacrocoracis (Dubois, 1930) Infection In Liver Of Freshwater Fish Channa Punctata (Bloach, 1793) From Pune District, (Maharashtra) India

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Abstract

Freshwater fish are prone to parasitic infection. These parasites fall under two types of endoparasites and ectoparasites. The current study is of Channa punctata (Bloch 1793), consumed locally by a large portion of the population. We found metacercariae stage of Clinostomum phalacrocoracis (Dubois, 1930) that infects the liver, kidneys and oesophagus of fish. Histopathological study was performed to study the body structure and anchoring of sucker to fish tissue. This can lead to anaemic condition in fish and may affect the fish's survival and reproductive capacity. Thus infected fish become unfit for human consumption.

Key words: Channa punctata, endoparasites, Clinostomum phalacrocoracis

Introduction

Channa punctata (Bloch 1793) commonly known as the spotted snakehead and its vernacular name is murrel, belong to the Channidae family. *Channa punctata* (Bloch 1793) is a species found in Sri Lanka and is found in countries such as India, Pakistan, and Afghanistan and in parts of Myanmar (1). It is found in fresh water and brackish water. Channa punctata (Bloch 1793) is a benthopelagic fish (2). Channa punctata (Bloch 1793) is highly carnivoros and are voracious feeders (2). They eat small fish, fry, mollusks, crustaceans, and insects (3) (2). It reaches a length of 15 cm, but males can grow up to 31 cm. Channa punctata (Bloch 1793) is a low-fat high protein source (4) and is consumed by most of the local population in India (5) (6) (7). They are known for their high nutritional value and are therefore the most sought after fish in India and eaten by a large portion of the Asian subcontinent population (5) *Channa punctata* (Bloch 1793) are also used as a live bait to angle large snakeheads (1). Asia contributes 90 percent of the world's aquatic production; in addition, FBT (food-borne trematodiases) by fish consumption is more common in Asia (8).

Classification of Channa punctata (Bloch 1793)



Fig. 1.1 Channa punctata (Bloch 1793) (Murrel)

Kingdom: Animalia Phylum: Chordata Class: Actinopterygii Order: Anabantiformes Family: Channidae

Genus: Channa

Species: C. punctata (Bloch 1793)

Channa punctata (Bloch 1793) is prone to ectoparasitic and endoparasitic infections in their natural environment. Trematodes are commonly found to infect fish, which are usually endoparasitic in nature. Metacercariae of different types of trematodes are known to infect Channa punctata (Bloch 1793) which is *Euclinostomum heterostomum, Polylekithum sp., Posthodiplostomum sp. Allocredium sp. Genarchopsis sp. Euclinostomum multiceacum* (9) (10) (11) (12) (13). *Clinostomum phalacrocoracis* (Dubois, 1930) trematodes are found in fish eating birds (definite host), while *Channa punctata* (Bloch 1793) acts as a second intermediate host. The life cycle of trematodes is complex and involves multiple hosts (14) (15) (16) (17). Indoplanorbis exustus is a freshwater snail found on Indian continent as the primary host and Channa punctata servers as the second intermediate host (18). Birds that feed on fish are definite hosts *of C. phalacrocoracis*. Approximately 750 million people are at high risk of FTB (8) (9). Studies have revealed that due to parasitic infections of trematodes conditions like retarded growth, anemia, and death especially in juvenile fish occurs (18) (19) (20) (21). Due to infestation of such parasites in fish that live in their natural habitat and in the cultured unit cause loss of nutritional value of fish, leading to economic losses. They may also cause physiological disorder, there by affecting the normal functioning of the fish. Heavy parasite infection may affect the reproductive capacity of a fish population (22). This present study will help to understand the nature of the damage, and will help the fishery community to design plans to avoid infection and there by breed a healthy population of the fish under study.

MATERIALS AND METHODLOGY

Study Area

The fish were collected from two localities from Pune city. These two places have huge fish market, whereby the fishes from nearby freshwater are harvested and brought to sell to the local population. Swargate is located at latitude: 18.5018° N, longitude: 73.8636° E and Nana Peth fish market it is located at 18.5144° N, 73.8654° E. Methodology

A total of 15 individuals of *Channa punctata* (Bloch 1793) with length ranging between 15 - 26 cm were collected (regardless of sex) from the Swargate fish market and Nana Peth Pune. The fish were packed in ice-containing polythene to maintain a low temperature and were immediately brought to the laboratory for further examination. The fish were dissected under laboratory condition and thoroughly examined for parasitic infection. Severe infection of the metacercariae stage of *Clinostomum phalacrocoracis* (Dubois, 1930) was found in various parts of the fish including the kidneys, esophagus, liver, and fat and muscle tissues. The encysted metacercariae of *Clinostomum phalacrocoracis* (Dubois, 1930) was extracted from the host tissue and stored immediately in 70% alcohol for preparation of histological slides.

The encysted metacercariae were then dehydrated in ascending grades of ethanol, then cleared with xylene and embedded in paraffin wax. Upon fixation sections were cut of thickness 5-6 µm using rotatory microtome. Hematoxylin and eosin were used to stain the sections (P. A. Ahammed Shareef, Syed M. A. Abidi, 2015). Permanent slides were prepared using DPX as mounting medium.

Measurements of different parts of the parasite were also taken, including:

- 1. Length of parasite (cyst)
- 2. Width of parasite (cyst)
- 3. Length of ventral sucker
- 4. Width of ventral sucker
- 5. Length of testes
- 6. Width of testes

RESULT:

In this study in all 15 fish were collected from the study site and brought to a laboratory where they were dissected so as to check for the parasitic infection. On average 9 metacercariae are found attached to the peritoneum membranes of the liver and muscles (Fig. 4.1) The general observation after dissecting the fish under laboratory condition revealed heavy infection of the metacercariae stage of *Clinostomum phalacrocoracis*. White or brown cysts (2-3mm) were present in the peritoneal membrane of liver, kidney and muscle. It was observed that the liver lobes were the most infected site in the fish. There is no literature available on the parasitic infection of *Clinostomum phalacrocoracis* (Dubois, 1930) in *Channa punctata* (Bloch 1793).



Fig. 4.1 A: Dissected *C. punctata* (Bloch 1793) infested with the encysted metacercariae of *Clinostomum phalacrocoracis* (Dubois, 1930) (white arrows). B: metacercariae of *Clinostomum phalacrocoracis*

The cysts were removed from the host tissue and were washed using 0.74% NaCl and were fixed in 10% neutral buffer formalin, later histological slides were prepared. Histological section of parasite reveals different parts of the parasite. (Fig 4.2)



Fig. 4.2 section of parasite with fish tissue, (V= ventral sucker, T= testis, F= fat tissue, I= intestinal caecum, P= peritoneum) image B show presence of two testis (T₁ and T₂)

The histological section clearly reveals the presence of ventral sucker or the acetabulum is clearly visible; with the help of ventral sucker the parasite is able to anchor itself to the host tissue. In trematode digestion takes place in the lumen of caeca (J. D. Smyth, 1962). The cyst damages the tissue around it and derives the nutrition from the host tissue. Due to heavy infection of *Clinostomum phalacrocoracis* (Dubois, 1930) efficiency and functioning of liver is greatly reduced.

Morphological analysis:



FIG. 4.3 Measuring the size of parasite and its different organs.

The metacercariae were measured using calibrated ocular micrometer. The obtained result is tabulated in (Table no.4.2.1).

	Calibration Table					
SR. No.	No. of ocular division line	No. of stage division line of	Calibration factor			
	of coincidence (X)	coincidence (Y)	F = 10(X/Y)			
1.	11	35	10×3.18= 31.8			

Sr. No.		Cyst1	Cyst 2	Cyst 3	Average
1.	Length of cyst	2.48 mm	2.70 mm	2.73 mm	2.63mm
2.	Width of cyst	1.90 mm	1.68 mm	2.28 mm	1.95mm
3.	Length of ventral sucker	0.63 mm	0.63 mm	0.76 mm	0.67mm
4.	Width of ventral sucker	0.66 mm	0.66 mm	0.85 mm	0.72mm
5.	Length of testis	0.28 mm	0.28 mm	0.31 mm	0.29mm
6.	Width of testis	0.25 mm	0.25 mm	0.28 mm	0.25mm

Table no. 4.2.1	Calibration	of ocular	micrometer.
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Table no. 4.2.2 table of measurements

The morphometric measurements of the six parameters under studies are been tabulated in the (Table no- 4.2.2). Three parasites were studies and mean values of each parameter has been tabulated. The length of the cyst was 2.63mm; width of the cyst was 1.95mm, length of ventral sucker 0.67mm. The length of testis was found to be around 0.29mm while the width of testis was found to be 0.25mm.

DISCUSSION

Fish consumed by human should be free of any sort of parasitic infections. It is estimated that approximately 1.3% of the world's population is infected with other trematode infections (8) and approximately 750 million people are at high risk of foodborne trematodiases (8) (9). It is therefore very important to study the fish to check for parasitic infections. The current study is an attempt to study the type of damage caused by trematode to infected fish. Such research can help fishing institutions develop programs that will reduce the risk of parasitic infections that lead to poor health and quality of the fish meat.

C. phalacrocoracis (Dubois, 1930) are trematode of family Clinostomidae. They have two suckers one oral and other ventral sucker with the help of which they anchor themselves to the host tissue. These trematodes are hermaphrodites as they have both a pair of testis and ovary as well. The present results are in accordance with the studies carried about various workers. The anterior testis has a blunt end and posterior testis is fan shaped (23) (24). The uterus extends from ventral sucker to the anterior testis shaped (23) (24). *C. phalacrocoracis* (Dubois, 1930) is also reported in cichlids (24). P. A. Ahammed Shareef, Syed M. A. Abid (2015) reported E. heterostomum infects the liver, kidney, peritoneal membrane, ovary, and muscle of *C. punctata* (Bloch 1793). In present study it was observed that *C. phalacrocoracis* (Dubois, 1930) infects the liver, kidney, peritoneal membrane and muscle. In comparison to other organs and tissue liver was most heavily infected by the parasite. Liver being the centre of biochemical, metabolic activities, furthermore it is the site where processes like detoxification and biosynthesis occur, so any factor that affects the functioning of liver will have direct impact on the physiology of the causing stress to the fish (25) (26) (27) (28) (18). For juvenile fishes the infection can be fatal and leading to economic loss to the framer (19) (18). Such parasitic infection can reduce the reproductive potential of the fish, decreased number and size of eggs, and lowered fecundity, (22) (29). Damage to the muscles was comparatively low but since *C. phalacrocoracis* (Dubois, 1930) is closely related to *C. complanatum* which is known for high zoonotic potential (18) it is risky to consume the fish which is infected by such parasites.

It is ironic that Asia contributes to the 90% of world's aquaculture production but still there is very less study regarding prevention of diseases and parasitic infection in fishes. There is an urgent and high demand for relevant research and documentation for fish health testing and prevention strategies.

CONCLUSION

Fresh water fishes are prone to parasitic infections. Parasites like *C.phalacrocoracis* (Dubois, 1930) affect the health of fish and its reproductive potential as this leads to huge economic loss to the framers. In addition to economic loss fishes which are infected by flukes have low nutritive value and are not fit for consumption as well. If humans consume uncooked parasitized fishes, they can get infected by the trematodes too, leading to discomfort and condition like Petechial hemorrhage.

There is need of more research on fish health assessment as well as on the fecundity and the reproduction capacity of the fish from its natural habitat. It will help to design conservational program for the fish population in the natural habitat. It will also help to prevent economic loss for the fishing community.

REFRENCES

- 1. Abdus Salam Bhuiyan, Shamima Afroz and Tanjeena Zaman. J. FOOD AND FEEDING HABIT OF THE JUVENILE AND ADULT SNAKEHEAD, CHANNA PUNCTATUS (BLOCH) Life Earth Sci., Vol. 1(2): 53-54, 2006.
- 2. Chaudhry, S., de Alwis Goonatilake, S., Fernado, M. & Kotagama, O. 2019. Channa punctata. The IUCN Red List of Threatened Species 2019: e.T166437A60584432.
- 3. Fatma A.S. Mohamed, Histopathological Studies on Tilapia zillii and Solea vulgaris from Lake Qarun, Egypt, World Journal of Fish and Marine Sciences 1 (1): 29-39, 2009
- 4. Fernando Jonas Sutili 1, Luciane Tourem Gressler 1, Luis Fernando Vilani de Pelegrini 2*, Clinostomum complanatum (Trematoda, Digenea): a parasite of birds and fishes with zoonotic potential in southern Brazil. A Review, Revista Brasileira de Higiene e Sanidade Animal Print version ISSN 1981 2965 Revista Brasileira de Higiene e Sanidade Animal, v. 08, n. 1, p. 99 114, jan-mar
- 5. GERALD W. ESCH, 2 MICHAEL A. BARGER, AND K. JOEL FELLIS, The Transmission of Digenetic Trematodes: Style, Elegance, Complexity, INTEG. AND COMP. BIOL., 42:304–312 (2002)
- 6. Heins D.C., Baker J.A. 2003 Reduction of egg size in natural populations of threespine stickleback infected with a cestode macroparasite J. Parasitol. 89: 1-6
- Ileana C. Troncoso, Jimena Cazenave, Carla Bacchetta, Mari'a de los A' ngeles Bistoni. Histopathological changes in the gills and liver of Prochilodus lineatus from the Salado River basin (Santa Fe, Argentina), Fish Physiol Biochem (2012) 38:693–702

- 8. Jhansilakshmibai K., Madhavi R. 1997 Euclinostomum heterostomum (Rudolphi, 1809) (Trematoda): life-cycle, growth and development of the metacercaria and adult Syst. Parasitol. 38: 51-64
- K. T. Waghmare* and V. V. Baile, Channa punctata (Bloch, 1793) As A Model For Toxicological Studies, IJRBAT, Special Issue (2), Vol-V, July 2017 ISSN No. 2347-517X (Online) SHRI SHIVAJI SCIENCE COLLEGE, NAGPUR ICRTS-2017 908 INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOG.
- M A Islama, Z Mawaa, M Y Hossain*, a, M A Rahmana, M R Hasana, D Khatuna, A A Chowdhurya, O Rahmana, M A Rahmana, S Tanjina, Habib-Ul-Hassanb & J Ohtomic Indian Journal of Geo Marine Sciences Vol. 49 (08), August 2020, pp. 1442-1446.
- Mai A. Salem1, Sahar Z. Abdel- Maogood1, Mohamed Abdelsalam2, Olfat A. Mahdy1 *, Comparative morphomolecular identification of Clinostomum phalacrocoracis (Dubois, 1930) and Clinostomum complanatum metacercaria coinfecting Nile tilapia in Egypt, ISSN 1110 – 6131 Vol. 25(1): 461 – 475 (2021)
- Mamun MAA1 *, Bari1 SM, Das H1, Islam S1, Iqbal MM2, Nasren S2, Parasitic Infestation in Channa punctatus (Bloch 1793) Collected From Lala Bazar Fish Market, Sylhet, Bangladesh, International Journal of Natural Sciences (2016), 6(1): 49- 53.
- 13. Manoj Kumar, Prevalence of trematode parasites in some species of genus Labeo and Channa of Bareilly district (Uttar Pradesh), ISSN (Print): 2328-3777, ISSN (Online): 2328-3785, ISSN (CD-ROM): 2328-3793.
- 14. Marina M. P. Camargo and Cláudia B. R. Martinez, Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream, Neotropical Ichthyology, 5(3):327-336, 2007.
- 15. Mark H. Armitage, COMPLEX LIFE CYCLES IN HETEROPHYID TREMATODES: STRUCTURAL AND DEVELOPMENTAL DESIGN IN THE ASCOCOTYLE COMPLEX OF SPECIES, Volume 4 Print Reference: Pages 21-34
- Monica Caffara1,*, Nadav Davidovich2, Rama Falk2, Margarita Smirnov2, Tamir Ofek2, David Cummings3, Andrea Gustinelli1, and Maria L. Fioravanti1, Redescription of Clinostomum phalacrocoracis (Dubois, 1930) metacercariae (Digenea: Clinostomidae) in cichlids from Lake Kinneret, Israel, Parasite 2014, 21, 32
- 17. Muhammad Moosa Abro, Ali Murtaza Dharejo, Muhammad Munif Khan and Nadir Ali Birmani, Description of a new species Clinostomum awadhi n.sp. (Trematoda: Clinostomidae) in Phalacorcorax niger (Aves: Phalacrocoracidae) of Sanghar, Sindh, Pakistan JNBR 5(3) 122 128 (2016).
- 18. N. KUMARI GAUTAM1 *, P. KUMAR MISRA2, A. MURARI SAXENA, Seasonal variation in helminth parasites of snakeheads Channa punctatus and Channa striatus (Perciformes: Channidae) in Uttar Pradesh, India, HELMINTHOLOGIA, 55, 230 239, 2018.
- 19. Poole, B. C., and Dick, T. A., LIVER PATHOLOGY OF YELLOW PERCH, PERCA FLAVESCENS (MITCHILL), INFECTED WITH LARVAE OF THE NEMATODE RAPHIDASCARIS ACUS (BLOCH, 1779), Journal of Wildlife Diseases, 20(4) : 303-307
- 20. Raghavan Kuppu, Shobana Manoharan and Ramesh Uthandakalaipandia, A study on the impact of water quality on the murrel fish: Channa striata and Channa punctata from three major Southern Tamilnadu rivers, India, : RSC Adv., 2018, 8,11375
- Sarmin Akther, Emrul Hakkani, Ashaduzzaman and Moni Krishno Mohanta* Int.J.Curr.Microbiol.App.Sci (2018) 7(4): 3431-3441.
- 22. SATISH CHANDRA1, NEELAM YADAV2 & A. M. SAXENA3, AN ECOLOGICAL ASPECT ON DIGENETIC TREMATODE PARASITE OF FRESH WATER FISHES FROM UTTAR PRADESH (INDIA), ISSN(P): 2319-4014; ISSN(E): 2319-4022 Vol. 5, Issue 4, Jun Jul 2016; 93-102.
- Schultz E.T., Topper M., Heins D.C. 2006 Decreased reproductive investment of female threespine stickleback Gasterosteus aculeatus infected with the cestode Schistocephalus solidus: parasite adaptation, host adaptation, or side effect? – Oikos. 114: 303-310.
- 24. Shareef P.A.A., Abidi S.M.A. 2012 Incidence and histopathology of encysted progenetic metacercaria of Clinostomum complanatum (Digenea: Clinostomidae) in Channa punctatus and its development in experimental host Asian. Pac. J. Trop. Biomed. 2: 421-426.
- Shareef P.A.A., Abidi S.M.A. 2015 Studies on the epidemiology and histopathology of Euclinostomum heterostomum (Trematoda; Digenea) infection in Channa punctata from North India Arch. Pol. Fish. 23: 133-140.
 Sharmiethe Chalmeberter 2* Biene des Kenner Benchmet NUTEDITIONAL BROEFLE OF SMALL NUDICENOUS.
- Sharmistha Chakraborty1,2*, Birendra Kumar Brahma1, NUTRITIONAL PROFILE OF SMALL INDIGENOUS FOOD FISH, CHANNA PUNCTATUS J. Assam Sc. Soc. Vol. 58. No. 1 ISSN 0587-1921 June 2017; Pp. 14 – 24.
 Nutricities and the second se
- 27. Sumeeta Khurana and Nancy Malla, Water- and Food-Borne Trematodiases in Humans, 10.1007/978-81-322-1029-0_15, Springer India 2014
- 28. Velisek J., Svobodova Z., Machova J. 2009 Effects of bifenthrinu on some haematological, biochemical and histopathological parameters of common carp (Cyprinus carpio L.) Fish Physiol. Biochem. 35: 583-590.
- 29. Voleentina Devi Athokpam Veena Tandon, A survey of metacercarial infections in commonly edible fish and crab hosts prevailing in Manipur, Northeast India, J Parasit Dis (July-Sept 2015) 39(3):429–440
- 30. Zemmer SA, Detwiler JT, Sokol ER, Da Silva Neto JG, Wyderko J, Potts K, et al. (2020) Spatial scale and structure of complex life cycle trematode parasite communities in streams. PLoS ONE 15(11): e0241973