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Detection and Identification of Helminth Parasites in Fish by Pepsin Digestion Method

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ABSTRACT

Fish-borne zoonotic parasites (FZPs), particularly helminths such as trematodes, pose significant threats to global aquaculture, public health, and food safety. These infections commonly occur through the consumption of raw or undercooked fish harboring infectious larval stages. In the present study, parasitic larvae were detected in the muscle tissues of edible fish species obtained from local markets in Lucknow, India, using the pepsin digestion method. Among the various species examined, *Channa punctatus* showed the highest prevalence of *Lophosicya diplostomum* metacercariae (7.14%), while *Echinostoma* spp. was observed at a lower prevalence (1.42%). Additionally, metacercariae of *Metagonimus yokogawai* and *Echinostoma* spp. were detected in *Heteropneustes fossilis*, and *Haplorchis yokogawai* was found in *Mystus tengra* at low abundance. The findings highlight the zoonotic potential of commonly consumed freshwater fish and underline the need for comprehensive epidemiological surveys, especially in regions with traditional raw fish consumption practices. Routine parasitological screening and public awareness are essential to mitigate the risks associated with FZPs.

KEYWORDS: Fish-borne Zoonotic (FZP), *Channa punctatus*, Pepsin, Larvae, Muscle tissue, Metacercariae, Trematode

Parasitic infections in fish are a major concern due to their impact on aquaculture productivity, food safety, and the risk of zoonotic disease transmission. Larval stages of nematodes, particularly *Anisakis, Contracaecum*, and *Gnathostoma*, often inhabit fish musculature, where they remain encysted and undetectable without specific diagnostic methods (Bao et al., 2017; Ziarati et al., 2022). These larvae pose a significant public health threat, especially in regions where raw or undercooked fish is commonly consumed. Traditional microscopic examination frequently fails to detect early or low-intensity infections, particularly when larvae are embedded deep within muscle tissues (Aibinu et al., 2019; Yadav et al., 2022).

To address this, artificial digestion methods have been developed to mimic gastric conditions, allowing for the release and recovery of larval parasites. Among these, the pepsin digestion technique, using pepsin and hydrochloric acid, followed by sedimentation and microscopic examination, is widely regarded as a sensitive, cost-effective tool for isolating viable larvae for diagnostic and epidemiological studies. In addition to nematodes, fish-borne trematodes (FBTs) are a growing global health concern, particularly in Asia. Human infections with liver and intestinal flukes, such as those caused by species in the families Opisthorchiidae, Heterophyidae, Nanophyetidae, and Echinostomatidae, have been linked to the consumption of raw or undercooked fish. According to WHO estimates, nearly 680 million people are at risk globally, and these infections contribute significantly to liver diseases and gastrointestinal disorders ((Infections and World Health, 1995; World Health Organization, 2004; Chai et al., 2005; Chai and Jung, 2022).

Fish act as key intermediate or transport hosts in the life cycles of many parasitic worms. Helminth larvae, including trematodes, cestodes, nematodes, and acanthocephalans, are commonly found in both wild and cultured fish, with infection severity influenced by species, environment, and aquaculture practices. The transmission of zoonotic parasites through fish consumption is an emerging public health concern, particularly in regions where raw or undercooked fish is traditionally consumed (Le, 2000; Taylor et al., 2007; Scholz and Kuchta, 2016; Shamsi, 2019; Jaiswal et al., 2022). The snake-headed freshwater murrel, *C. punctatus*, is a carnivorous species rich in omega-3 and omega-6 PUFAs. Native to freshwater habitats across Asia, it is valued not only for its nutritional content but also for its potential pharmaceutical properties in disease prevention and health promotion. Fish parasites and other genotoxic agents can also cause damage to DNA and lead to mutations, which can contribute to various human diseases through food chain (Srivastava et al., 2016; Jaiswal et al., 2022).

The present study uses the pepsin digestion method to detect larval parasites in commonly consumed fish from Lucknow, India, including *C. punctatus* from the Gomti River. It aims to assess helminth diversity and zoonotic risk, providing baseline data for monitoring and public health awareness.

MATERIALS AND METHODS

Study Area, Host Collection and Identification

Lucknow district was selected for the experiment, which is in northern India. Some of the fish varieties found in the Gomti River, such as murrel snakehead fish include *C. punctatus* were transported to

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nearby local fish markets. Fish were collected from local markets in Lucknow, including Keserbagh, Rajajipuram, Rajnikhand, Telibagh, Dubagga, Pakka Pull, Kali Paschim, and Ghaila Bridge. Species in the Channidae family have broad heads and large, snake-like scales, earning them the name "snakeheads." They host several parasites, notably the nematode *Gnathostoma spinigerum*, which uses these fish as intermediate hosts, encysting in their muscle tissues.

Fish Processing and Separation

Fish samples (100–150 g per species) were either processed immediately or stored at −20 °C for up to one week. After removing the viscera, the head and body were skinned, chopped, and homogenized with a few drops of PBS. The homogenate was then mixed with artificial gastric juice containing pepsin, concentrated HCl, and double-distilled water for metacercariae detection.

Artificial Digestion and Recovery of MC

The digestion technique was followed with minor adjustments. Processed samples were stirred overnight in artificial gastric juice at 37 °C. The next day, undigested materials such as scales, fins, and bones were removed using a 1×1 mm mesh sieve. The filtrate was washed with 2 L of normal saline, stirred, and allowed to settle for 30 minutes. The supernatant was discarded, and washing was repeated until the solution became clear. The final sediment was collected and examined.

Detection of MC

The sediment was gradually transferred into a small Petri dish containing 6–7 ml of 0.85% saline and gently swirled by hand to concentrate the metacercariae (MC) in the center. This step was repeated until all MC were concentrated. An aliquot of the suspension was then placed on a clean glass slide and examined under a compound microscope at 10× magnification for MC identification. Each sample was examined in triplicate, and the average number of MC was recorded. The average was multiplied by 100 to estimate the total number of MC in 15 ml of suspension, and the number of MC per 100 g of fish was calculated. Microphotographs were taken using an inverted microscope fitted with a digital camera (Labomed, USA).

Tissue Digestion Method

Samples of fish flesh were dissected from various parts of the body, including the head, gills, muscles, fins, scales, intestines, and other viscera. Each tissue sample (10–20 g) was ground separately using a mortar and pestle or a tissue homogenizer. The homogenized tissue was transferred into a 100 ml beaker containing 50 ml of artificial gastric juice, prepared by dissolving 8 ml of concentrated HCl and 2 g of pepsin (1:10,000) in 1,000 ml of distilled water. The mixture was thoroughly mixed and incubated at 37.3 °C for 2–3 hours with occasional stirring. After digestion, the mixture was passed through a 1 × 1 mm mesh brass sieve and washed with 0.85% saline. In some cases, the digested sample was left overnight in 250 ml of artificial gastric juice before sieving and washing. The sediment was allowed to settle, and the supernatant was carefully discarded. This washing step was repeated several times until the supernatant became clear. The final sediment was transferred in small amounts to a Petri dish containing 6–7 ml of 0.85% saline. The dish was gently swirled to concentrate the sediment at the center, and excess fluid was removed using a pipette. This process continued until all metacercariae were concentrated in the central portion of the dish. The metacercariae were then observed and identified under a compound binocular microscope, isolated, and stored in a small dish in the refrigerator for further analysis.

RESULTS

The current state of metacercarial infections in commonly eaten fish hosts, like *C. punctatus*, from various research areas, is represented in Table 1.

Table 1: The status of metacercarial infection in *C. punctatus* surveyed from different local fish markets in Lucknow

Name of Host	Local fish market-number of hosts examined	Location of the parasite in the host	Metacercaria recovered
Channa punctatus	Rajaji Puram- 20	Body muscle	*Neascus*
	Kali Paschim-20	Body muscle	*Heterphyopsis continua*
	Pakka Pull -20	Body muscle	*Procerovum varium*
	Ghiala Bridge -20	Body muscle	-
	Rajnikhand -20	Body muscle	*Opisthorchis viverrini*
	Dubagga -20	Body muscle	-
	Keserbagh -20	Body muscle	*Echinostoma recurvatum*

A total of 140 fish were sampled and found to be infected with metacercariae, with a low infection rate. Based on morphological and morphometric criteria, the recovered metacercariae were identified as belonging to genera -*Procerovum varium, Heterophyopsis continua, Opisthorchis viverrini, Echinostoma recurvatum, Posthodiplostomum sp., Haplorchis Yokogawai, C. sinensis, M. orientalis, Ehinochasmus sp. and Metagonimus Yokogawi* (Figure 1).

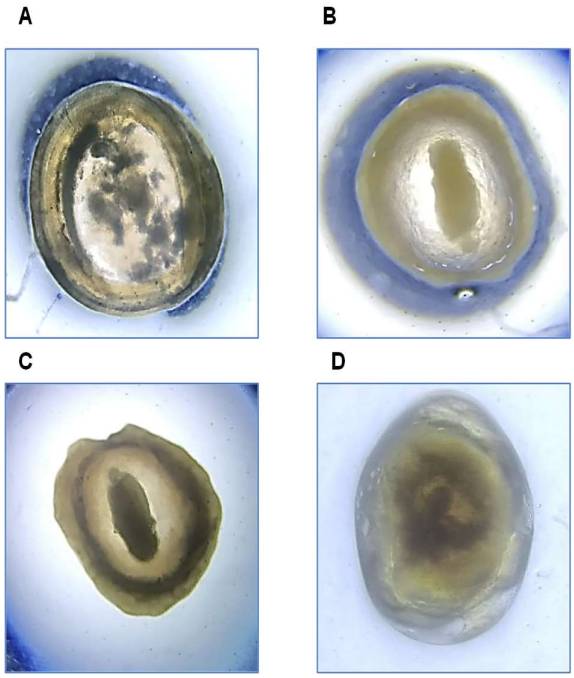

Figure 2: Light Microscopy images of the encysted (a), (b), (c), and (d) metacercariae of *Posthodiplostomum* spp recovered from fish host *C. punctatus*

Posthodiplostomum spp. metacercarial cyst oval in shape, 0.64-0.67* 0.53 -0.56 mm, and the excysted metacercaria with the distinctly bipartite body, forebody lanceolate, hind body oval, oral and ventral suckers feebly developed; holdfast almond-shaped; testes tandem; ovary ellipsoidal, testicular. The host fish *C. punctatus* was found to harbor metacercarial cysts in the fish muscle tissue with a prevalence of 2.85%, a mean intensity of 1, and an abundance of 0.02.

DISCUSSION

The detection of parasitic larvae in fish tissues is critical for understanding parasite prevalence, assessing public health risks, and improving food safety standards. In this study, the pepsin digestion method proved to be a reliable and effective technique for isolating helminth larvae from various fish species commonly sold in local markets in Lucknow, India. This enzymatic technique offers a significant advantage over traditional dissection and direct microscopic examination, as it facilitates the release of deeply embedded larvae from muscle tissues, thereby increasing the detection sensitivity. The presence of larval forms of trematodes, cestodes, and nematodes in *C. punctatus* suggests a high level of parasite transmission in the aquatic ecosystem. These findings align with earlier studies that identified fish as both intermediate and paratenic hosts in the life cycles of zoonotic helminths (Chai et al., 2005; WHO, 2004). The detection of such larvae is of public health concern, particularly in regions where cultural practices include the consumption of raw or undercooked fish.

Fish-borne zoonotic trematodes (FZT), along with nematode species like *Anisakis*, have been recognized as emerging global threats due to increasing international trade and changing food habits (Nguyen et al., 2013; Aibinu et al., 2019). The prevalence of larval stages in edible fish species highlights the need for effective monitoring tools like the pepsin digestion method in both surveillance and food quality assurance. The study also demonstrates the utility of this method in differentiating larval types based on their morphology under microscopy, which is crucial for proper species identification and risk assessment. The data generated from such assessments can be instrumental in designing control strategies, including public awareness campaigns, better fish handling practices, and deworming policies in aquaculture.

However, it is important to acknowledge the limitations of this method. While highly effective in detecting motile and intact larvae, the pepsin digestion method may not differentiate between viable and non-viable larvae unless coupled with further viability assays or molecular diagnostics. Additionally, some larval stages may be morphologically indistinguishable without further genetic identification techniques.

In conclusion, this study confirms the effectiveness of the pepsin digestion technique as a sensitive tool for detecting tissue-dwelling larval helminths in fish, including zoonotic parasites found in edible species like *C. punctatus* from local Indian markets. The detection of such parasites underscores the public health risks associated with consuming raw or undercooked fish. These findings highlight the importance of routine parasitological screening, public awareness, and preventive strategies to enhance food safety. Future research should integrate pepsin digestion with molecular tools for comprehensive parasite

profiling and improved risk management, particularly in tropical and developing regions.

Conflicts of Interest: The authors declare no conflicts of interest.

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