



# Infection of Cestode Parasites in *Channa punctatus* Fish in Sagar, Madhya Pradesh, India

Raghvendra Niranjana\*, Raghveer Kumar Gupta<sup>a</sup>  
and Malabika Sikdar<sup>a</sup>

<sup>a</sup> Department of Zoology, Dr. Hari Singh Gour University Sagar, (M.P.), India.

## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

Snakehead fishes are widely consumed throughout India because of their good taste of meat and high nutrient values. Parasitic infection constitutes significant economic loss in fish production. The present study deals with histopathological Study of Cestode parasites in *Channa punctatus* from Sagar region of Madhya Pradesh. Such type of study has been not conducted in this region. Cestode lives in a very hazardous environment as there is continuous movement of the gut lining, and causes severe damage to the overall health of fishes. Hematology plays a crucial role in diagnosis of fish diseases. In the presented study, a total of 150 individuals of *Channa punctatus* were examined. A total 45 were found to be infected with cestodes parasites, during the period from February 2022 to January 2024. The histopathology of the infected tissues indicated the following interface: tissue damage and infected liver showed degeneration of hepatocytes as well as

\*Corresponding author: Email: [raghvendraniranjana9556@gmail.com](mailto:raghvendraniranjana9556@gmail.com);

cytoplasmic vacuolation, nuclear alterations and necrosis. Parasite derives nutritive material required for growth, from host tissue by causing damage to it. Blood parameters are useful for the measurement of hematological disturbances in parasitized fishes and provide information about the disorders in the host. So, the present investigation was carried out to study the effects of parasites on the blood cells of a freshwater fish, *Channa punctatus*. The hematological manifestation of the infected *Channa punctatus* (host) showed remarkable decrease in red blood cells (RBC), increased white blood cells (WBC) and it is found that neutrophils, lymphocytes, monocytes, eosinophils and basophils of infected fishes were remarkably reduced, and hemoglobin content were also significantly reduced compared to uninfected fishes. Infected fishes showed symptoms like anemia.

**Keywords:** *Channa punctatus*; cestode parasites manifestation; histopathology; hematological alterations.

## 1. INTRODUCTION

The majority of freshwater fish are extremely nutritious diet for humans. A few of them are regarded as delicacies items. The market value and nutritional content of these edible fish are impacted because it is known that they are infected with several cestode parasites that affect their health.

With an annual production of 312.8 million tones, India is the second-largest producer of aquaculture globally, an industry that is expanding quickly [1].

Fish production accounts for 44% of total fish production in 2014, which was 74 million tons of fish valued at 160 billion dollars, with all of the production going toward human consumption. Fisheries are important for food security, as well as a source of income and social development in developing nations [2]. In many countries, the production of fish is crucial not only for financial gain but also for food security and social advancement. Therefore, it is vital to address the widespread disease that arises from the interaction of pathogens, hosts, and environments in order to address this issue [3]. However, health-related issues represent a significant limiting factor, and parasitic diseases rank among the top barriers to aquaculture output in terms of both qualitative and quantitative limitations [4].

Trematodes, cestodes, nematodes, and acanthocephalans are the main parasitic taxa that infect freshwater fish. In addition to this, there are numerous parasites that can only infect humans through fish [5]. A broad category of helminths known as cestode parasites is known to infect a range of vertebrate hosts, including

fish species like *Channa punctatus*, or snakehead fish. The increased attention that cestode infections in *C. punctatus* have collected is a result of their impact on conservation efforts, fishery management, and aquaculture techniques. For efficient disease management and the establishment of sustainable fisheries, it is essential to comprehend the dynamics of these diseases, including species diversity, host-parasite relationships, and ecological consequence.

Adult cestode parasites are frequently found in the digestive tract of fresh water fishes. Hemostasis is a very sensitive aspect that is vital in identifying cellular changes that may occur in target organs, such as the gills, gut, liver, and gonad [6], but adult helminth in general and cestode in particular are looked upon as of the pathogenicity of cestodes on fishes, compared to larval stages [7].

Studies on blood parameters are important because it provides relevant data regarding fish physiological capabilities and are a helpful instrument for assessing the immune system. Hematological parameters may offer extra information about the host's health and immune status, which are crucial indicators of changes to the host, as they can be viewed as indicators of the ability to transport oxygen (hemoglobin and hematocrit) [8]. It is commonly acknowledged that hematology plays a crucial role in both the diagnosis of fish diseases and the evaluation of pollution's consequences. Worldwide commercial freshwater fish utilization, including that of decorative and food species, is restricted by some diseases spread by water [9]. Fish habitat, seasonal variation, and epizootic ulcerative syndrome all contribute to changes in haematological parameters [10].



Map 1. Study site

By assessing a few hematological and biochemical parameters for both infected and non-infected fish, the current study aimed to assess cestode species infections based on morphological traits in *C. punctatus* as well as their effect on their health state. Additionally, histopathology images were included to identify the harm caused by the parasite. The current study intends to examine the changes in *C. punctatus* inhibiting haematology and biochemistry in the nearby contaminated waterbody, Sagar Lake. Such type of study has been not conducted in this region.

### 1.1 Study Area

The Sagar district is situated between latitudes 23° 10' and 24° 27' north and longitudes 78° 04' and 79° 21' east, in the north central region of the state of Madhya Pradesh. The district is located. Sagar Lake (23° 50' N, & 78° 45' E) spans 82 hectares, of which 68 hectares are occupied by the main lake and the remaining 14 hectares by a tiny marsh. The lake is centrally located in Sagar city. This small natural lake has become contaminated as a result of careless pollution releases from nearby communities.

## 2. MATERIALS AND METHODS

### 2.1 Examination of Fishes for Parasitic Infection

Between February 2023 and January 2024, live *C. punctatus* in the size group of 7–16 cm was brought to the lab in water containers from the Sagar Lake (Also called Lakha Banjara Lake) and nearby fish market in Sagar. They underwent dissection via the peritoneal cavity,

gills, and body surface, and every internal organ was checked for parasitic infection. Using forceps and a needle, the cestodes were extracted from the host tissue and transferred in 0.9% NaCl. The worms were then processed to make permanent slides and stained in carmine for microscopic examinations.

### 2.2 Histopathology

The liver from *C. punctatus* infected with the cestode and uninfected tissue samples were washed with 0.9% saline and fixed in 10% neutral buffer formalin for 48 hours. The liver was then dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax to cut sections that were 5-7  $\mu\text{m}$  thick using a rotatory microtome. Hematoxylin and eosin were used to stain the sections, and slides that would be permanently mounted were made. Under a light microscope, the gross pathology was examined and captured on microscope (EVOS microscope).

### 2.3 Fish Blood Biochemical Parameters

Blood samples from both infected and uninfected fishes were taken from the caudal vein using a heparinized syringe and placed in glass jars with EDTA as an anticoagulant in order to examine hematological parameters. Collected blood was kept cold in polyethene cool bags until analysis [11]. The first vial was placed into blood tubes labeled with EDTA to be used for hematological evaluations. The measurements included total red blood cells (RBCs), white blood cells (WBCs), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean cell hemoglobin (MCH). The serum from

the second vial was taken for additional biochemical testing to measure the liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in dry, clean tubes (without anticoagulants). Additionally, measurements of globulin (GL), albumen (ALB), and total protein (TP) were made in accordance with [12,13].

## 2.4 Statistical Analysis

The experiments were conducted in five replicates. Each value is expressed as the standard error of the mean (S.E.M.)  $\pm$  the mean.

Graph Pad Prism 7 software was utilized to perform a statistical comparison of the hematological and biochemical data values between the infected and non-infected groups of fish blood using the student's t test (2-tailed). In order to compare the mean values, significance was set at 5% ( $P < 0.05$ ).

Control liver of *C. punctatus* showed normal hepatocytes and sinusoids. The infected liver displayed Hepatocyte degeneration, vacuolation, and focal necrosis, activation of Melano macrophage cells and moderate hemorrhage.

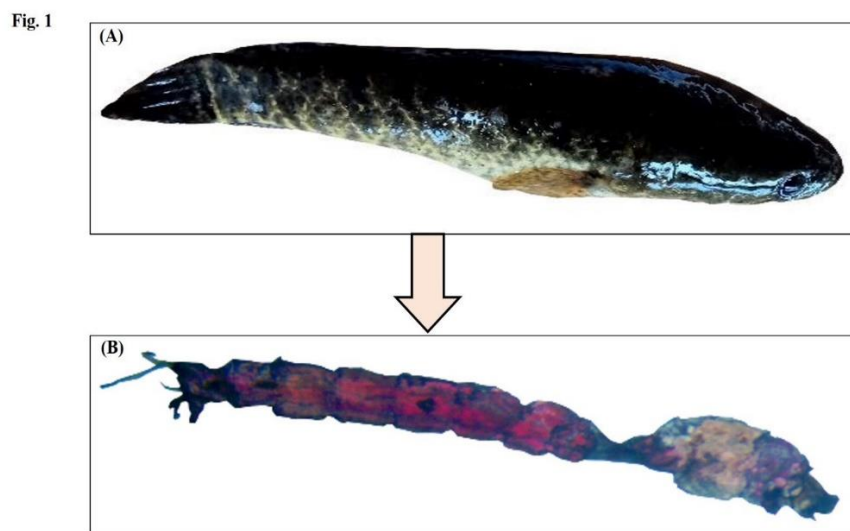


Fig. 1. (A) *Channa punctatus* (B) cestode parasite

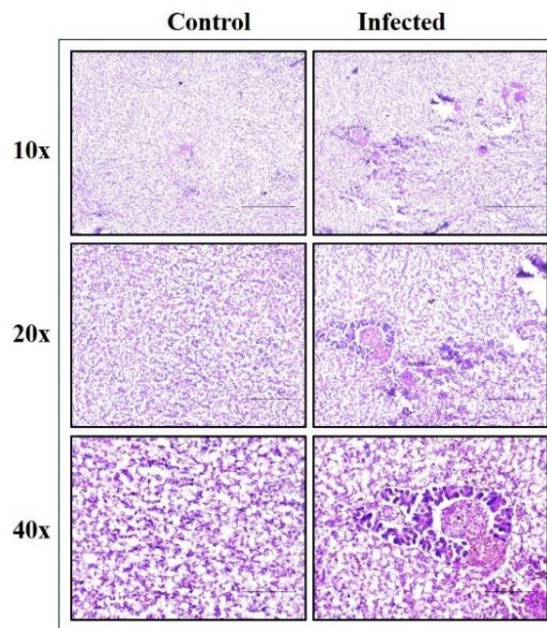


Fig. 2. Histopathology of liver of *Channa punctatus*

### 3. RESULTS

*C. punctatus* was severely infected with cestodes spp. during the current study, causing liver damage. There were no histopathological changes found in the non-infected (control) liver of the fish samples. The liver of the infected fish had a number of pathological symptoms; in addition to activation of Melano macrophage cells and moderate hemorrhage, most of the liver examined displayed vacuolar degeneration and sinusoidal congestion. *C. punctatus* had a cestode infection in its liver, which showed a variety of cellular and histological abnormalities. A sizable portion of the liver tissue affected with the disease had cestode growth on it. The liver segment displayed vacuolar degeneration in the hepatocytes which are spherical aggregates of cells with granules of varying sizes that are typically found adjacent to the artery.

Freshwater fish infections with helminths (cestodes) can cause a variety of harmful histopathological changes, especially in cases of heavy infection (Taraschewski, 2000).

Both morphological and histopathological alterations were included in the pathological changes. The morphological alterations in the infected liver of fish suggested an alteration in color and inflammation. Significant histological alterations included hepatocyte expansion, loss of morphology, and cytoplasm vacuolation. There was blood vessel-filled, burst sinusoids. Bile channels were blocked as a result of the parasites entry into the liver. The infections

altered the histological structure of liver. There are extremely few documented cases of mature cestodes intestinal parasites occurring in the liver. These results are complicated by the recent revelation by Thanopon Yooyen (2006) of an adult cestode parasite called *Senga chiangmaiensis* in the liver of *Mastacembelus armatus*. The histological harm caused by *Penetrocephalus ganapatii* in the liver of *Saudina tumbil* (Bloch) is comparable to the harm caused by *Senga kaigaonesis* [14]. Therefore, it can be said that the worm makes contact with the host tissue, uses the nutrients to its advantage for growth and feeding, weakens the host and inhibits the ability to grow, resulting in damage to the liver tissue of host.

According to statistical analysis the hematological and biochemical profiles of the non-infected fish were significantly different from those of the infected fish. Fish that are not sick and those who are affected have their hematological parameters compared in (Table 1). The findings demonstrated that the red blood cell counts and hemoglobin concentrations, which are the key erythrocyte indices, were considerably ( $P < 0.001$ ) lower in infected fish as compare to non-infected fish. On the other hand, the MCV, MCH, and MCHC of infected fish that were also showed a substantial drop ( $P < 0.001$ ), ( $P < 0.05$ ), and ( $P < 0.001$ ) in comparison to fish that were not infected. These derived erythrocyte indicators. The infected fish had a considerably greater total leucocyte count (WBC) ( $P < 0.001$ ) than the non-infected fish. When compared to the non-infected fish, there was a significant

**Table 1. Hematological and Biochemical parameters between non- infected and infected fishes**

Parameter	Non infected fishes		Infected fishes		T test/ significance
	Range	Mean± SEM	Range	Mean ± SEM	
RBC ( $10^6/\text{mm}^3$ )	3.4-4.7	4.12 ± 0.229	1.3-2.5	1.94 ± 0.214	6.96 ***
WBC ( $10^3/\text{mm}^3$ )	7.9-9.8	8.72 ± 0.343	11.1-13.8	12.8 ± 0.49	6.82 ***
Hb (g/dl)	12.1-13.3	12.8 ± 0.213	9.8-11.2	10.4 ± 0.265	7.05 ***
MCV (ft)	170.2-205.8	188 ± 6.14	108.3-118.5	113 ± 1.76	11.8 ***
MCH (pg)	33.4-62.9	51.8 ± 5.11	29.7-41.7	35 ± 2.42	2.98 *
MCHC (%)	30.3-31.1	30.6 ± 0.16	28.1-29.7	28.9 ± 0.273	5.44 ***
Lymphocytes (%)	86.7-91.8	89.5 ± 1.08	70.5-73.1	71.8 ± 0.521	14.8 ***
Monocytes (%)	2.63-3.15	2.93 ± 0.088	5.83-6.74	6.24 ± 0.152	18.7 ***
Eosinophils (%)	2.87-3.26	3.08 ± 0.074	1.82-2.13	1.99 ± 0.0558	11.7 ***
Neutrophils (%)	5.63-6.27	5.91 ± 0.111	18.5-21.1	19.5 ± 0.459	28.7 ***
SGOT (Unit/gm)	63.8-65.7	65 ± 0.442	67.5-70.1	68.7 ± 0.44	5.84 ***
SGPT (Unit/gm)	31.7-33.4	32.6 ± 0.323	34.6-38.3	36.4 ± 0.627	5.5 ***
Total Protein(g/dL)	2.36-2.74	2.54 ± 0.066	2.19-2.32	2.24 ± 0.0334	3.94 **
Bilirubin (mg/mL)	2.52-3.15	2.87 ± 0.127	2.07-2.45	2.2 ± 0.0694	4.62 **
Albumin (g/dL)	1.75-1.89	1.83 ± 0.025	0.21-0.91	0.61 ± 0.118	10.1 ***
Globulin (g/dL)	0.59-0.85	0.71 ± 0.047	1.67-1.86	1.77 ± 0.0393	17.1 ***
ALP(IU/L)	109.43-128.52	121 ± 3.23	140.63-190.56	170 ± 8.63	5.31 ***
LDH (IU/L)	560.63-625.51	594 ± 12.9	621.91-683.53	658 ± 10.7	3.83 **

increase in the percentage of neutrophils ( $P < 0.001$ ) and monocytes ( $P < 0.001$ ). It was found that the blood of infected fish had a lower percentage of lymphocytes ( $P < 0.001$ ) and eosinophils ( $P < 0.001$ ) than that of non-infected fish. Table 1 presents a comparison of the biochemical parameters of non-infected and infected *Channa punctatus*. When compared to non-infected fish, the concentrations of SGOT, SGPT, globulin, ALP, and LDH are markedly higher in infected fish. When compared to fish that are not sick, the other biochemical markers, such as total protein, bilirubin, and albumin, are considerably lower in infected fish.

#### 4. DISCUSSION AND CONCLUSION

Histological sections of the infected fish's liver revealed moderate hemorrhage, necrosis, and hyperplasia, which suggested that the parasite Cestode sp. had minimal effect on the liver and more of an impact on the intestine. This might be brought on by the parasite's unrestricted mobility within the intestine and the poisonous compounds it generated, which spread to other tissues. According to [15], goblet cells scattered beneath the epidermis exhibit modest hyperplasia and hypertrophy, which are suggestive of an immune response brought on by the parasite. There exists a correlation between the depth of penetration of cestodes parasites into host tissues and the severity of histopathological alterations generated by them.

Hematological parameters are crucial for determining the nutritional and physiological status of fish as well as for identifying the health status of fish affected with helminthes parasites [16]. Numerous parasites, particularly internal ones, can coexist peacefully inside the host body without causing harm, but changes in the host immune system, parasite behavior, and environmental factors may upset this equilibrium [17]. When the host's parasitic load increases, hematological changes may take place. This can lead to a severe illness in the fish body due to parasite infestations, which function as a stressor and trigger a cellular immune response as one of the fish's defense mechanisms against parasites.

In this study, there is a significant difference in the counts of MCV, MCH, MCHC, and TLC, with the infected groups having lower values than the non-infested ones. This supported the findings of [18], who reported a decrease in the similar parameters following the infection of the

freshwater fish *Channa punctatus* by the nematode parasite *Eustrongylides* sp.

As reported by Tavares-Dias et al. [17], who observed notable alterations in the quantity of circulating neutrophils in the bloodstream of *P. mesopotamicus* when parasitized with *Argulus* sp. that may be related to the *Capillaria* sp., neutrophils demonstrated a significant difference between the two groups. The host cells were affected by the parasite extracellularly. Monocyte counts in the infected group were significantly higher ( $p \leq 0.001$ ) than in the non-infested group, which was consistent with findings from [15] who observed elevated monocyte counts in *Oreochromis niloticus* parasitized by *Argulus* sp., *Lamproglena* sp., and *Epistylis* sp.

Transaminase activity levels may rise in response to serum protein depletion, suggesting that under stress, reserve nutrients like protein and carbs are rapidly consumed (Goel and Gupta, 1985). In this study, there were notable variations in the levels of total protein, albumin, and bilirubin; the infected group's values were lower than those of the non-infected group. This is because of the parasitic infestation, which releases the amino acids and allows them to profit from various metabolic processes by proteolyzing the levels of TP. Additionally, compared to non-infected fish, infected fish had significantly higher SGOT (AST) and SGPT (ALT) activity, which is consistent with findings by [18] and [19]. Due to an infection with *Eustrongylides* sp. that caused disruptions in liver function and damage to the hepatocytes, they investigated an increase in the blood ALT and AST hepatic activities of *Channa punctatus*. The prior study found that blood parameters fluctuate when fish from sewage-fed, contaminated ponds have lower hemoglobin and red blood cells (RBCs) than fish from unpolluted fish ponds. Similar findings have also been reported by Shahi et al. [20] and Ghani and Bhuyan [21].

Saxena and Chauhan (1993) also observed that a parasite infection in the blood of *H. fossilis* affected blood parameters. According to the findings of Brown and McLeay [22], Hb molecules may have oxidized and become incapable of binding or carrying oxygen molecules, which is why the Hb concentration was noticeably reduced in the current study. Similar to the current experiment, Khan [23] found low levels of Hb content in infected *Gadus murhua* with *Trypanosoma muramenensis*. The

parasite infection, which frequently causes anemia, resulted in a decrease in Hb, RBC, and other blood components in the affected fish [24].

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### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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