Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 6094 - 6101
Received 15 April 2021; Accepted 05 May 2021.

# Haemato logical Studies on Fresh Water Fish, Xenentod on cancila affected by Nematode Parasite (Philometrapellucida)

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## ABSTRACT

Reservoirs are considered as one of the valuable sources of fish production which have a major role in the inland fish production of our country. Parasitic nematodes represent an important group of fish parasites. Many species are highly pathogenic, often cause serious diseases or even death of hosts. This study has been done to understand the haematologicaleffects on the host fish*Xenentodoncancila* due to the nematode parasite *Philometrapellucida*.The mean values of red blood corpuscle [RBC] count, hematocrit, and hemoglobin were significantly higher ($P<0.01$) in non-infected fish, while the values of white blood corpuscle [WBC] count was significantly higher ($P<0.01$) in infected fish. It can be concluded that *Philometrapellucida*has a significant impact on its host.

**Keywords:** *Xenentodoncancila*, *Philometrapellucida*, Hematocrit, WBC**,** RBC **.**

## INTRODUCTION

*Xenentodoncancila*, the freshwater garfish, is a species of needlefish found in freshwater and brackish habitats in South and Southeast Asia (Froese, 2013). The air-breathing teleost, being carnivorous in nature, acts as an intermediate or final host of many helminth parasites. Potentially, all freshwater and brackish water fish may be affected by nematodes, with heavier infections, utilizing fish as an intermediate or transient host. Parasitic infection in fish results in heavy mucous secretion and discoloration and in severe cases causes high mortalities, which results in huge economic losses to fisheries. The nematodes cause damage to the hosts by depriving them of digested food and by feeding on host tissues, sera, or blood. In some cases, direct mechanical damage results from them fixing to host tissues and developing or migrating in them. Among fish nematodes, *Philometrapellucida* infection has attracted considerable attention

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as it has been reported in various regions of the world and these nematodes exhibit a great potential for transmission and pathogenicity.

They parasitize the body cavities, tissues and ovaries of both marine and freshwater fishes. When still in the larval stages, these worms move to the body cavities or subcutaneous tissues in the host. This migration can cause damage to skeletal joints, result in internal bleeding, and inflame visceral organs. Hence, to maximize productivity and to reduce fish mortality due to diseases and parasites, continuous evaluation of the physiological status of the fish is essential in the fishery sector. Blood parameter analysis have proven to be valuable tools for diagnosing the health status of fish as these indices provide reliable information on metabolic disorders, deficiencies, and the chronic stress status before clinical symptoms appear (Bahmani, 2001). Thus, hematological tests and the analysis of serum constituents have proven useful in the detection and diagnosis of metabolic disturbances and disease processes (Shahsavani, 2008).

## MATERIALS AND METHODS

*Xenentodoncancila*, the freshwater garfish, is a species of needlefish found in freshwater and brackish habitats in South and Southeast Asia. Freshwater garfish inhabits large and medium-sized rivers with adults occurring in areas that lack floating vegetation (Pethiyagoda, 1991*).*

A total of 221 samples of *Xenentodoncancila* (the freshwater garfish) were examined. Out of observed fishes ,a total of 3428 parasites were found .The total number of parasites in male fish is 1381 and 2047 in female fish.

*Xenentodon cancila* (the freshwater garfish)

Nematode parasite-*Philometrapellucida*

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Blood samples were collected by the caudal puncture, and immediately transferred into EDTA. Enumeration of formed elements (blood cells) is a quantitative measure of the population of blood cells in circulation. The counting of cells was done manually with the help of a microscope after diluting blood and making a special type of wet mount as per method given by Rusia and Sood (1992). The total number of RBC's per cubic millimeter were calculated by using the following formula:

$$\text{Total No. of RBC} = \frac{\text{No of RBC x Dilution counted (million cu.mm)}}{\text{Area counted x Depth of fluid}}$$

Blood collection and processing procedure was same as described in the above except for the dilution factor which is 1: 20. As far as the counting (Neubaur counting chamber) procedure of WBC is concerned, each of these 4 square millimeter area is subdivided into 16 squares. The following formula was taken for the estimation of the total number of WBCs per cubic millimeter.

$$\text{Total No. of WBC} = \frac{\text{No of WBC x Dilution counted (million cu.mm)}}{\text{Area counted x Depth of fluid}}$$

Hemoglobin was determined by the more commonly used Sahli's method.

PCV is the volume of erythrocytes expressed as a percentage of the volume of whole blood in a sample. Packed cell volume was calculated according to microhematocrit method, Mean Corpuscular Volume refers to the average volume of red cells. Because the size of the cell is very small, volume is expressed in cubic microns ($\mu m^3$). It is calculated by using the following formula:

$$\text{MCV} = \frac{\text{Hctx10}}{\text{RBC}}$$

Mean Corpuscular Haemoglobin (MCH) is the average haemoglobin content of the red blood cell. MCH is influenced by the size of the cell and concentration of haemoglobin. It is derived by the following formula:

$$\text{MCH} = \frac{\text{Total mass of Hb}}{\text{No. of blood cells}}$$

The MCHC is an expression of the average haemoglobin concentration per unit volume (100) of packed cells (W/V). Hence it is expressed in g/dl which is the same as percent (%).

$$\text{MCHC} = \frac{\text{Hbx10}}{\text{Hct}}$$

**RESULTS AND DISCUSSION**

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*X.cancila*,the freshwater garfish, it is a solitary fish that swims in midwaterusually against the current and is capable of bursts of speed , especially when in pursuit of itsprey.The freshwater garfish feeds exclusively on crustaceans ,small fishes and insects in the wild ; but takes live fish only when in an aquarium ( Pethiyagoda 1991; Rainboth1996).Moreover ,*X.cancila* is oviparous and eggs may be found attached to objects in the water by tendrils on the egg's surface Breder and Rosen (1966).

The parasitic infections of this fish results in economic losses due to not only mortality,but also treatment costs , decreasing growth that reduces the expansion of aquaculture .Some studies of *X.cancila* have been done in biology ,mainly in the breeding program of fishSrivastava and Singh ,(1994).The distribution ,Prevalence, parasitic intensitypathogenic effects and control of most of the parasitic diseases in natural population of freshwater fish *X. cancila* has been obtained . As *X.cancila* is most popular fish throughout the country ,their abundance is reducing due to the massive parasitic invasion and the occurrence of diseases.

In this study the RBC count, hemoglobin value, and packed cell volume were found to be significantly reduced in infected fish, which occurs as a result of the parasitic infestation that often leads to anemia. Martins (2004) evaluated, the infected *L. macrocephalus* showed reduced RBC count, hemoglobin value, and packed cell volume. This suggests the occurrence of a microcytic-hypocromic anemia. Similar results were described in carp infected with *Bothriocephalusacheilognathi* , while no difference in hematocrit values were seen in rainbow trout infested with *Lepeophtheirussalmonis*. Anemia was also associated to *Heteropneustesfossilis* infected with metacercariae and carp infected with *Myxobolusartus* . No changes in erythrocytic results were observed in parasitized *C. carpio M. platanus* ,

*P. mesopotamicus* and *L. macrocephalus*. The present observations on the hematocrit values were in accordance to Kelly *et al*. (2000) who have reported significant reduction in eels infected with *Anguillicolacrassus*. Schuwerack*et al*. (2001) related decreased neutrophil, thrombocyte and lymphocyte percentages in lymphoid organs of carp infected with *Sanguinicolainermis*.

**Table 1: Overall prevalence of parasites in sample site (n=221)**

| | **No.of Fishes** | **No.of Parasites** | **% of Prevalence** | **Intensity** | **Relative Intensity or Abundance** |
|---|---|---|---|---|---|
| Male Uninfected | 26 | _ | 66.51 | - | - |
| Female Uninfected | 48 | _ | 34.69 | - | - |
| Male Infected | 51 | 1381 | 34.69 | 27.07 | 6.24 |
| Female Infected | 96 | 2047 | 65.30 | 21.32 | 9.26 |

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| | | | | | |
|---|---|---|---|---|---|
| Total | 221 | 3428 | - | - | 15.51 |

A total of 221 *XenentodonCancila* caught randomly out of observed fishes a total of 96 (Female) and 51 (male) fishes were found to be infected and 26 (male), 48 (female) fishes were found to be uninfected. The infected 96 (female) fishes have a total of 2047 parasites prevalence is 65% and out of observed infected 51 male fishes have total of 2047 parasites , prevalence is 34%.

In this study, the parasites were isolated from the targeted organs of the fish like ovary, liver, kidney, air bladder, muscle, intestine gills and testis. Liver showed the higher number of parasites (n =815) and the percentage of distribution is 23.77. The minimum number of parasites found in Gill(n =146) and the percentage of distribution is 4.25.

**Table 2: Percentage distribution of parasites in various organs of *Xenentodoncancila***

| **Organs** | **No. of parasite** | **% of distribution** |
|---|---|---|
| Liver | 815 | 23.77 |
| kidney | 702 | 20.47 |
| Muscle | 729 | 21.26 |
| Gill | 146 | 4.25 |
| Intestine | 343 | 10.00 |
| Testis | 278 | 8.10 |
| Ovary | 251 | 7.32 |
| Air bladder | 167 | 4.87 |
| Total | 3428 | |

**Table 3: Hematological Parameters of blood**

| **Parameters of blood** | **Nematode -Non Infected** | **Nematode Infected** |
|---|---|---|
| RBC× $10^6$/$mm^3$ | 4.5 ± 0.21* | 1.8 ± 0.21* |
| WBC × $10^3$/$mm^3$ | 14.4 ± 0.42* | 18.2 ± 0.45* |
| Hb (g/100ml) | 13 ± 0.35* | 9.5 ± 0.35* |
| PCV (100%) | 38.3 ± 1.08* | 27.3 ± 1* |
| MCV (F1) | 107.6 ± 1.08* | 169 ± 1.4* |
| MCH (Pg) | 33.3 ± 1.08* | 58 ± 0.72* |
| MCHC (%) | 33 ± 0.70* | 35.5 ± 0.35 |

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Each value is mean ±SD of observations (+ indicates increase over control, - indicates decrease over control, *indicates significant ($P < 0.05$).

Furthermore, the parasites act as a stressor and during primary stages of stress, PCV changes due to the release of catecholamine, which can mobilize RBC's from the spleen or induce RBC swelling as a result of fluid shift into the intracellular compartment. The WBC count was found to be enhanced due to parasitic infestation, as WBCs are key components of innate immune defense and leukocytes are involved in the regulation of immunological function in the organism.

The erythrocyte counts of normal fish showed a mean value of 4.5 ±0.21$mm^3$where as the nematode infected fish showed a mean values of RBC's 1.8±0.21$mm^3$. The nematode infected fishwas found to inflict a drastic reduction in the total count of RBC's (Table 1). The values mentioned above showed a significant decrease when compared to the control ($P< 0.05$).

The results of the total count of white blood cells revealed that the blood of the control fish showed a mean value of 14.4± 0.42$mm^3$. The nematode infected fish showed the mean values of WBC as 18.2± 0.45$mm^3$(Table 1). The values mentioned above showed a significant increase when compared to the control ($P< 0.05$).

The nematode non infected fish showed mean value of 13± 0.35$mm^3$ for hemoglobin. The nematode infected fish the hemoglobin mean values of 9.5± 0.35$mm^3$(Table 1). The values for treatments showed a significant decrease when compared to the control ($P< 0.05$).

The erythrocyte counts of nematode normal fish showed a PCV value of 38.3± 1.08$mm^3$. The nematode infected fish showed mean values of RBC'sPCVvalue is27.3± 1$mm^3$. The nematode infected fish was found to inflict a drastic reduction in the PCV value of RBC's(Table 1). The values mentioned above showed a significant decrease when compared to the control ($P< 0.05$).

In this study, the value of MCH is 33.3 ± 1.08 in normal fish and 58 ± 0.72 in nematode infected fish. The value of MCHC is 33 ± 0.70 in nematode normal fish and 35.5 ± 0.35 in nematode infected fish. The MCV and MCH values recorded in infected fish were enhanced, which confirmed the pathological occurrence of pernicious anemia. The MCHC value is found to be insignificant, when compared to normal and infected fish. Ivy *et al.,* (2016) study thepatho physiological effects of the nematode parasite*Eustrongylides sp*. on freshwater fish *Channapunctatus* by hematology, serum biochemical, and histological studies. He found out the mean values of red blood corpuscle [RBC] count, hematocrit, and hemoglobin were significantly higher in normal fish, while the values of white blood corpuscle [WBC] count, mean corpuscular volume [MCV], and mean corpuscular hemoglobin [MCH] were significantly higher in infected fish, which resembles the results of the present investigation.

The erythrocyte counts of nematode normal fish showed a MCV value of 107.6 ± 1.08$mm^3$. The nematode infected fish showed mean values of RBC'sMCV value,169 ± 1.4$mm^3$. The nematode infected fish was found to inflict an increasein the MCV value of RBC's. The

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value of MCH is 33.3 ± 1.08mm$^3$ in normal fish and 58 ± 0.72mm$^3$ in nematode infected fish. The value of MCHC is 33 ± 0.70mm$^3$in normal fish and 35.5 ± 0.35mm$^3$in nematode infected fish(Table 1).

This study reveals that *Philometrapellucida*causes a series of hematological changes in the host fish. Glucopyruvic intoxication leads to a marked decrease in the glucose level of serum due to the consumption of an abundant amount of glucose content from the host by the nematode. Blood acts as a patho physiological reflector of the whole body (Sharma, 2006). Hence, hematological parameters are important in diagnosing the functional status of the fish infected with nematode parasites (Joshi, 2002).

In conclusion results of the present investigation show that nematode caused immunological impairments in *Xenentodoncancila*, which suggests that the nematode parasites may weaken the immune system and may result in severe physiological problems, ultimately leading to the death of fish.The results of this study provide information regarding the characteristic features of hematological changes in *Xenentodoncancila*due to *Philometrapellucida*infection, suggesting that blood parameters studies may be effective in monitoring the effects of nematode infestation in fish; this knowledge would be effective in fishery management programs

## REFERENCES

1. Bahmani M, Kazemi R, and Donskaya P.,(2001) A comparative study of some hematological features in young reared sturgeons (*Acipenserpersicus*and Husohuso). Fish PhysiolBiochem,; 24: 135-40.
2. Breder,C.M.&.,Rosen,D.E.1996.Modes of Reproduction in Fishes.NeptuneCity,New Jersey T.F.H.Publications.
3. Froese, Rainer Pauly, and Daniel, eds. (2013). "*Xenentodoncancila*" in Fish Base.
4. Ivy Chiocchia G, Motais R.,(2016) Effect of *catecholamines*on deformability of red cells from trout: relative roles of cyclic AMP and cell volume. J Physiol,; 412: 321-32.
5. Joshi P. K., Bose M, Harish D.,(2002) Change in certain Haematological parameters in suliroid catfish *Clariasbatrachus* (Linnaeus) exposed to cadmium chloride. PollutResour,; 21: 119-22.
6. Kelly, C. E, Kennedy, C. R, Brown, J.A.,(2000) Physiological status of wild European eel (*Anguilla anguilla*) infected with the parasitic nematode, *Anguillicolacrassus*. Parasitology,;120: 195-202.
7. Martins M. L, Tavares-Dias M, Fujimoto R. Y, Onaka E. M, and Nomura D T, (2004). Haematological alterations of *Leporinusmicrocephalus*(Osteichtyes: Anostomidae) naturally infected by Goezialeporine(Nematoda: Anisakidae) in fish pond. Arq Bras Med Vet Zootec,; 56: 640-6.
8. Pethiyagoda, R. 1991. Freshwater Fishes of Sri Lanka. The Wildlife Heritage Trust of Sri Lanka, Colombo.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 6094 - 6101
Received 15 April 2021; Accepted 05 May 2021.

9. Rainboth,W.J(1996) fishes of the Cambodian Mekong ,FAO species Identification field Guide for fishery purposes,FAO,Rome.
10. Russia, U. and Sood, S.K. 1992. Routine Haematological tests. In: Kanai, Mukherjee, L. (Ed) Medical Laboratory Technology, Tata McGrow Hill Publishing Company Limited, New Delhi : 252-258.
11. Schuwereck, P. M, Lewis, J. W,Hoole, D,(2001) Ammonia-induced cellular and immunological changes in juvenile *Cyprinuscarpio* infected with the blood fluke *Sanguinicolainermis*. Parasitology;122: 339-345.
12. Shahsavani D, Mohri M, and GholipourKanani H.,(2008) Determination of normal values of some blood serum enzyme in *Acipenserstellatus*. FishPhysiolBiochem; 36: 39-43.
13. Sharma G, Singh S.,(2006) Assay of some blood parameters of the fish,*Channapunctatus*(Bloch.) after intoxication of indofil. Bionotes,; 8: 21
14. Srivastava,S.J.and Singh,R.1994 .Seasonal changes in the testes of a freshwater murrel,*Channapunctatus* .Naturalia,19 119-130.