

Hematological and Biochemical Profile of *Oreochromis mossambicus* Naturally Infected with Isopod *Cymothoaeremita*

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Abstract

Hematological and biochemical profile of *Oreochromis mossambicus* naturally infected with isopod *Cymothoaeremita* was studied. The isopods were identified morphologically and by molecular method following PCR amplification of 28S rDNA and sequence analysis. Infected fishes had significantly ($P<0.05$) lower RBC, MCH and PCV, MCHC and MCV values compared to the healthy uninfected fishes. Biochemical analysis revealed that infected fishes had significantly ($P<0.05$) decreased triglycerides, cholesterol, total protein, albumin, and globulin levels compared to the uninfected healthy *O.mossambicus*. This is the first report on the hemato-biochemical responses in *O.mossambicus* infected with isopod, *C.eremita*.

Key words: Isopod infection, Host-parasite interaction, 28S rDNA sequencing

Cymothoids are obligate ectoparasitic isopods, commonly infest various fish species, especially in tropical and subtropical waters (Trilles *et al.*, 2011). These isopods engage in blood-feeding and tend to inhabit the buccal and gill cavities, as well as the fins of fish hosts (Ravichandran *et al.*, 2010). Isopod-induced fatalities are frequently observed in mariculture facilities, leading to significant mortalities among cultured fish populations (Rajkumar *et al.*, 2005, Abdelkhalek *et al.*, 2017).

Blood parameters can be used to measure the health status of parasitized fish based on

the physiological abnormalities caused due to the disease and the extent of damage to the host (Tavares-Dias *et al.*, 2007). *C. eremita* has been identified in the disease surveillance as a significant isopod infecting *O.mossambicus* in the Pulicat Lake. As there have been no studies on blood parameter profiles of this kind, this study was carried out with an objective to assess the hematological and biochemical profile of *O.mossambicus* when infected with isopod *C.eremita*.

Materials and Methods

Healthy *Oreochromis mossambicus* (110 fishes) were collected from the Pulicat Lake (13.4177° N, 80.3185° E) and isopod infected - uninfected fishes were segregated and maintained in the rearing tank during October to December 2021. The isopods were identified initially based on the literature identification key provided by Hadfield *et al* (2013). For molecular characterization, total DNA was extracted from the parasites using Qiagen DNA easy Blood and Tissue Kit following the manufacturer's instructions. Partial 28S rDNA was amplified using the universal primers and protocol of Roy *et al* (2015) and Nucleotide sequencing of the amplified products was carried out by outsourcing (Eurofins, Bangalore, India). About 40 fishes from parasites infected and 40 from healthy uninfected stocks were selected for the study to assess the hematological and biochemical characteristics.

A Neubauer hemocytometer was used to count the red blood cells (RBCs), while the microhematocrit method was used to determine

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haematocrit (Ht) (Nelson and Morris, 1979). The erythrocyte indices such as MCV, MCH and MCHC were calculated according to Wintrobe (1934). Serum cholesterol (CHO) and triglyceride (TG) levels were estimated following the method of Parekh and Jung (1970) and Rice (1970) respectively. Globulin were analyzed using a commercial kit (DIATEK, Kolkata) following the manufacturer’s instructions. The data were statistically examined by one-way ANOVA using SPSS 20.

Prevalence (%) = Number of infected host/ Number of hosts examined X 100

Results and Discussion

During the present investigation, out of 110 *O.mossambicus* examined, 42 fishes were found infected by the isopod *C.eremita* with a mean prevalence of 38.18%. The isopod was observed on the buccal cavity of *O.mossambicus* establishing the site specificity nature of the parasite and classical identification based on the dorsal and ventral structural revealed that the isopod infecting the *O.mossambicus* was *C.eremita* (Fig 1). Nucleotide sequencing and the BLAST analysis of the sequence (NCBI) confirmed the identity of the isopod as *C.eremita* (Accession No. ON561786).

The hematological and biochemical parameters of healthy and *C. eremita* infected *O.mossambicus* are presented in (Table I). The RBC, Hb, MCH, MCHC, MCV and PCV levels were significantly (P<0.05) decreased in the *C.eremita* infected fishes compared to the healthy ones. The triglycerides, cholesterol, total protein, albumin and globulin were significantly decreased (P<0.05) in the infected fishes.

Table I: The hematological and biochemical parameters of healthy and *C.eremita* infected *O.mossambicus*

Parameters	Healthy fishes (n = 40)	Parasitized fishes (n = 40)
Hemoglobin (g/dl)	7.97 ^a ±.57	4.50 ^b ±1.40
RBC	2.48 ^a ±.45	1.49 ^b ±0.42
Hct (%)	39.86 ^a ±1.15	24.12 ^b ±1.03
PCV (%)	24.53 ^a ±4.40	15.73 ^b ±4.85
MCV (fl)	160.4 ^a ±12.95	129.3 ^b ±4.67
MCH (pg)	60.8 ^a ±8.19	42.87 ^b ±3.03
MCHC (%)	37.16 ^a ±2.29	28.66 ^b ±1.35
Triglyceride (mg/dl)	177.8 ^a ±7.20	125.3 ^b ±9.31
Cholesterol (mg/dl)	182.67 ^a ±9.16	85.93 ^b ±9.02
Protein (g/dl)	10.06 ^a ±0.15	3.94 ^b ±0.06
Albumin (g/dl)	6.58 ^a ±0.17	2.84 ^b ±0.50
Globulin (g/dl)	3.81 ^a ±0.38	1.36 ^b ±0.12

Mean values marked with distinct superscripts between columns exhibit statistically significant differences (P<0.05).

Parasite infestation causes a reduction in RBC count, haemoglobin (Hb) value, and packed cell volume (PCV) which typically leads to anemia in affected fishes (Nnabuchi *et al.*, 2015) and reduced blood Ht in affected fish might be attributable to parasite stress-induced rapid erythrocyte lysis (Celik *et al.*, 2006). Similar results as in this study, were observed in values of RBC, Hb and Ht in bogue fish infested with isopod *Ceratothoaoestroides* (Özdemir *et al.*, 2016) and *Dicentrarchuslabrax* parasitized by *C.oestroides* (Horton and Okamura, 2003). The MCHC is an index of RBC swelling (Wepener *et al.*, 1992). In this study, the levels of MCH,



Fig 1. *Cymothoaeremita* infecting the buccal cavity of *Oreochromis mossambicus*

MCHC significantly ($P>0.05$) decreased in the *C. eremita* infected fishes compared to the uninfected healthy fishes. Anaemia induced reduction in Hb production in RBC may be responsible for the reduction in the above values of MCH and MCHC during parasite infection (Soivio and Nikinmaa, 1981).

Plasma proteins viz., albumin and globulin are referred to as circulating mobile proteins since they are the most significant component in the serum. Similar results were observed by the effects of *Trachelobdellalubrica* and *Ceratothoaoestroides* infection in black scorpion and in boguefish respectively (Celik *et al.*, 2006; Özdemir *et al.*, 2016). Infectious disorders and kidney and liver function and lipid metabolism have been linked to lower TG and CHO. Furthermore, in this study it is suspected that decline in nutrition during isopod infection in the buccal cavity causes reduced TG values, and the necessary energy is provided by liver storage.

Summary

This study enhances our comprehension of how *O. mossambicus* responds hematologically and biochemically to isopod infection. The insights gained from this study will contribute to the formulation of effective control strategies aimed at mitigating losses in aquaculture resulting from comparable isopod infections.

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