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Research paper

A Study on the Hematology of Golden Mahseer *Tor putitora* (Hamilton) in Relation to *Aeromonas hydrophila*

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KEYWORDS

Golden masheer
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ABSTRACT

Present investigation deals with the haematological studies on freshwater fish Toriput tora, an economical fresh water fish, with reference to Aeromonas hydrophila infection. The haematological parameters included Total Erythrocyte Count (TEC), Total Leucocytes Count (TLC), Packed Cell Volume (PCV), Haemoglobin concentration (Hb.C), Differential Leucocyte Count (DLC), Mean corpuscular Volume (MCV), Mean Corpuscular haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC). Thrombocytes, Lymphocytes percentage, eosinophil of infected fish decreased when compared with the healthy fish but MCV, TEC, neutrophil percentage. Monocytes of the infected fish increased when compared with the normal fish.

1. Introduction

Aeromonas hydrophila is a Gram-negative, rod-shaped bacterium that is commonly found in warm climate areas and freshwater environments. It exhibits heterotrophic behavior and can survive in both aerobic and anaerobic conditions. This bacterium is capable of digesting materials such as hemoglobin and gelatin. Infections caused by A. hydrophila, particularly aeromonas wound infections, are most commonly associated with gastrointestinal or non-gastrointestinal complications. These infections often occur following accidental skin punctures and subsequent exposure to contaminated water or soil. The enterotoxin produced by A. hydrophila, known as anarolysin-related cytotoxin, possesses multiple biological activities, including lysing capabilities, evoking a fluid secretary response in ligated intestinal loop models, and reducing lethality in mice.

In recent years, there has been an increased interest in studying fish parasites and eco-pathological aspects, as highlighted by the works of Malhotra (1989), Watson et al. (1956), Smirnova (1971), Tandon and Joshi (1973), Joshi (1979, 1981, 1989), Joshi and Dabral (1981), Kumar et al. (1984), Rehulka (2002), Harikrishnan et al. (2003), Ranzani et al. (2005), Garcia et al. (2007), and Kharat and Sothavade (2012). However, detailed studies on the diagnostic aspects of fish diseases and the physiological and biochemical alterations in fish tissues, including blood, are still needed.

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Hematological parameters play a crucial role in the diagnosis and assessment of fish diseases. The analysis of blood tissue provides insights into fish physiology and biochemistry. Several researchers, such as Blaxhall (1972) and Joshi (1989), have recognized the significance of hematological indices in determining the health status of fish. By examining characteristics of blood cells, the presence of diseases can be identified (Anderson, 2003). Bruno and Munro (1986) have observed that hematological indices aid in the diagnosis and assessment of diseases in fish. Early detection of illnesses in fisheries is essential as some causes may not be easily detectable during the initial stages of infection. Certain blood parameters serve as reliable indicators of fish health, especially in cases where parasites reside in a host and cause harm. Hematological analysis provides valuable information for monitoring the health and condition of fish, enabling the diagnosis of both structural and functional abnormalities within their bodies.

2. Materials and Methods

Live specimens of Tor putitora (Golden Mahseer) were brought to the laboratory and allowed to rest to recover from transportation stress. They were housed in glass aquariums under suitable conditions of food and aeration. Fish of the same size and age were selected for the experiment. Blood samples were collected from the caudal vein of live fish using 5 ml heparinized syringes. The sampled fish were then returned to the aquarium. Infection was confirmed by observing live wriggling parameters in fixed (methanol for 5 minutes) and stained (Giema + buffer pH 7.2 for 30 minutes) preparations under 40X and oil immersion microscopy to confirm the species. Negative fish were considered as control, while positive fish were classified as infected.

The total erythrocyte count (TEC) was determined by the standard clinical method described by Dacie and Lewis (1984) and Blaxhall and Daisely (1973). Hemoglobin estimation was performed using the acid hematin method with Sahli's hemoglobinometer, and the values were expressed in g%. The packed cell volume (PCV) was measured using the microhematocrit tube method, and the PCV percentage was calculated using the formula:

PCV = (Height of the TEC column after centrifugation) / (Total height of the blood column)

TEC, total leukocyte count (TLC), and total thrombocyte count were determined using a Neubauer hemocytometer under 40X and 10X magnification with a research microscope. Hendricks solution was used for thrombocyte counting. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated based on the methods suggested by Dacie and Lewis (1984). For differential leukocyte counts, six blood smears per fish were prepared from fresh blood, air-dried, stained with Leishman-
Giemsa stain, and fixed in methanol. The percentage of neutrophils, eosinophils, lymphocytes, and monocytes in the blood smears was determined.

Statistical Analysis: A comparison between hematological parameters of normal and infected fish was conducted using Student's t-test.

3. Results and Discussion

In the present study, the average mean value of TEC in normal fish was 3.60 ± 0.10 × 10^6/mm^3, while in infected fish, it was 2.82 ± 0.10 × 10^6/mm^3. There was a significant decrease in TEC count ($P < .0001$) in the infected fish compared to the normal fish. The average mean value of TLC in normal fish was 3.10 ± 0.10 × 10^4/mm^3, whereas in infected fish, the mean value was 4.02 ± 0.02 × 10^4/mm^3. There was a significant increase in TLC ($P < .0001$) in the infected fish compared to the normal fish. The average mean value of hemoglobin percentage in normal fish was 12.0 ± 0.42 gm%, whereas in infected fish, the mean value was 9.2 ± 0.30 gm%. There was a significant decrease in hemoglobin percentage ($P < .0001$) in the infected fish compared to the normal fish. The average mean value of PCV percentage was 42.60 ± 1.84% in normal fish and 30.15 ± 2.0% in infected fish. There was a significant decrease in PCV value ($P < .0001$) in infected fish compared to the normal fish. The average mean MCV in normal fish was 82.50 ± 2.6 µm, while in infected fish, it was 89.2 ± 2.11 µm. There was a significant increase in MCV level in the infected fish ($P < 0.001$) compared to the normal fish.

The decreased hemoglobin trend may indicate swelling of the RBCs and poor mobilization of hemoglobin from the spleen to other hematopoietic organs (Kumar & Ramulu, 2013). The data support the present investigation, suggesting that the significant decrease in RBC and hemoglobin content is possibly due to hypochromic microcytic anemia caused by A. hydrophila. Decreased TEC, PCV, and hemoglobin concentration indicate that TECs a

re being destroyed by leukocytic activity, resulting in erythrocytic anemia with subsequent erythroblastosis (Joshi, 1989). Rehulka (2002) observed decreased RBC and PCV in Asian Cichlid fish Etroplus suratensis with epizootic ulcerative syndrome. Similar decreases in blood values were recorded in rainbow trout Oncorhynchus mykiss infected with A. hydrophila (Rehulka, 2002). Harikrishnan et al. (2003) reported decreased RBC and PCV in Cyprinus carpio experimentally infected with A. hydrophila.

An increase in mean cell volume (MCV) is also associated with RBC swelling resulting from hypoxic conditions, impaired water balance, or macrocytic anemia in fishes exposed to stress (Tort et al., 1988). Decreased hemoglobin (Hb) and TEC values with an increased number of phagocytic leukocytes were observed in Perch, Lotalota infected with Trypanosomes (Smirnova, 1971). Prevailing anemia and leukocytosis were also reported in Clarias batrachus and Heteropneustes fossilis (Kumar et al., 1984). Kharat and Kothavade (2012) described severe infection of Trypanosomiasis in Clarias batrachus resulting in decreased RBC count, small lymphocytes, and various polymorphic stages of trypanosomes. Parasitic infestations have been found to destroy erythrocytes and cause anemia (Soivio & Nikinmaa, 1981). The decreased TEC values observed in the infected fish in this study are clear indications of anemia. The infected fishes exhibited abnormal behavior and became lethargic, consistent with previous studies (Engel & Dvevis, 1964; Rao & Shyamsundari, 1974; Joshi, 1989; Kumar & Ramulu, 2013).

The decreased number of thrombocytes, monocytes, and neutrophils could be due to the inability of hemoblasts to mature into different cell types, and the decreased number of eosinophils observed in this study is in agreement with the findings in Piaractus mesopotamicus following infection with A. hydrophila (Garcia et al., 2007) and common carp infected with A. hydrophila (Selvaraj et al., 2004).

Table 1 Range, Mean and Standard Deviation difference between normal and infected fish *Tor putitora*

Parameters	Normal	Infected
TEC × 10^6 / mm^3	3.60 ± 0.10	2.82 ± 0.10
TLC × 10^4 / mm^3	3.10 ± 0.12	4.02 ± 0.2
Hb. g%	12.0 ± 0.42	9.2 ± 0.30
PCV %	42.60 ± 1.84	30.15 ± 2.0
ESR mm/h	0.8 ± 0.4	2.6 ± 0.6
MCV	82.50 ± 2.6	89.2 ± 2.11
MCHC %	34.2 ± 0.60	30.1 ± 0.50
MCH pg	26.50 ± 1.58	24.10 ± 2.0
Thrombocytes (%)	15.4 ± 0.36	10.20 ± 0.30
Neutrophil (%)	24.02 ± 1.40	30.0 ± 1.2
Lymphocyte (%)	72.02 ± 1.10	59.5 ± 1.4
Monocytes (%)	2.0	3.0
Eosinophil (%)	1.1	1.0

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Declaration of Conflict

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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