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Research paper

Haematological Study of Golden Mahseer *Tor putitora* (Hamilton) with reference to *Aeromonas hydrophila*

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ABSTRACT

Present investigation deals with the haematological studies on freshwater fish *Tor putitora*, 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

Junk *Aeromonas hydrophila* is a heterophic, Gram-negative, rod-shaped bacterium, mainly found in area with a warm climate. This bacterium can be found in fresh water. It can survive in aerobic and anaerobic environments and can digest materials such as haemoglobin and and gelatin. Infection with gastrointestinal or non-gastrointestinal complication aeromonas wound infections are most commonly caused by *A. hydrophila* and have been reported after accidental puncture of the skin followed by exposure to contaminated water or soil. Anarolysin-related cytotoxin enterotoxin of *A. hydrophila* possesses multiple biological activities, which include its ability to lyse, evoke a fluid secretary response in ligated intestinal loop models and reduce lethality in mice.

During past few years interest in the study of fish parasites and piscine eco-pathological aspects has increased as reviewed by Malhotra (1989). However,

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studies related to the diagnostic aspects of fish diseases and diseases related physico-biochemical alternations in fish tissues, including blood are yet to be made in needed details. Important contribution of these lines have come from the work of 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), Kharat and Sothavade (2012).

The haematological parameters are an important tool of diagmnosis that reveals the state of health of fish (Blaxhall, 1972; Joshi, 1989). Blood tissue of fish gives clue about physiology and the biochemistry of blood. By analysing blood cell characteristics, disease status can be identified (Joshi, 1989; Anderson, 2003). Bruno and Munro (1986) have observed that haematological indices aid in the diagnosis and assessment of disease in fish. In fisheries it is important to find out our illness as the source of these causes and may not be generally detectable in early period of the infection. Certain blood parameters serve as reliable indicators of fish health as many parasites can live in a host, causing damage to it. Haematological analysing can provide valuable knowledge for monitoring the health and condition of fish and are important in diagnosing the structural and functional status of the body.

2. Materials and Methods

Live fishes, *Tor putitora* were brought to the laboratory allowed to rest in order to recover the stress caused by transportation and maintained the glass aquarium, under proper conditions of food and aeration. Care was taken to select the experimental fish of the same size and age. Blood was collected from live fish from the caudal vein using 5 ml heparinized syringes. The sampled fish were reverted back into the aquaria. Infection was confirmed by detecting live wriggling parameters in fixed (methanol 5 min) and stained (Giema + buffer pH 7.2 for 30 mins) preparation observed under 40X and oil immersion for the confirmation of species. The negative fish were treated as control and the positive as infected. Total erythrocyte count us the number of red cells per volume of blood. The preparation for TEC in infected and non-infected blood were made by standard clinical method (Dacie & Lewis, 1984; Blaxhall & Daisely, 1973). Haemoglobin estimation was done by acid Haematin method using sahli's haemoglobinometer and the values were expressed in g%. The PCV was determined by micro haematocrit tube method and PCV has been calculated using the following formula:

$$\text{PCV} = \frac{\text{Height of the TEC column after configuration}}{\text{Total height of the blood column}}$$

TEC, TLC and Total thrombocytes were determined using Neubauer Haemocytometer after examining at 40 and 10X magnification using a research microscope. Hendricks solution used as the thromobocytes. The data was used to calculate The mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC) suggested by Dacie and Lewis (1984). For differential leucocyte, six blood smears per fish were prepared from fresh blood, air dried stained with Leishman-Giemsa stain and fixed in Methanol. The percentage of neutrophil, eosinophil, lymphocyte and monocyte tissues was determined.

***Statistical Analysis*:** A comparison between haematological parameters between normal and infected fish was made by student's t-test.

3. Results and Discussion

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

The decreased haemoglobin trend may be swelling if the RBC as well as poor mobilization of haemoglobin from the spleen to other hamatopoietic organs (Kumar & Ramulu, 2013). The data support the present investigation that the significant decrease in RBC and haemoglobin content is possibly is due to hypochromic microcytic anemia caused by *A. hydrophila*. Decreased TEC, PCV and haemoglobin concentration indicate that TEC are being destroyed by leucocytosis activity in an erythrocytic anemia with subsequent erythroblastosis (Joshi, 1989). Rehulka (2002) observed decreased RBC and PCV iin asian Cichlid fish *Etroplus suratensis* with epizootic ulcerative syndrome. Rehulka (2002) recorded some decreased blood values due to infection of *A. hydrophila* in rainbow trout *Oncorhynchus mykiss.* In *Cyprinus carpio* experimentally infected with *A. hydrophila* (Harikrishnan et al., 2003).

According to Tort et al. (1988) on increase in mean cell volume is also linked to the swelling of the RBC as a result of a hypoxic condition or impaired water balance or macrocytic anaemia in fishes exposed to stress. Smirnova (1971) in Perch, *Lota lota* infected with Trypanosomes, noted decreased Hb and TEC but increased number of phagocytic leucocytes. Researchers also reported of prevailing anaemia and leucocytosis in the *Clarias batrachus* and *Heteropneustes fossilis*. Kumar et al. (1984) noted in hillstream fish *Schizothorax plagiostomus* infected with black spot disease noted decreased values of TEC, Hb and PCV but increased number of phagocyctic leucocytes. Kharat and Kothavade (2012) described severe infection of Trypanosomiasis in *Clarias batrachus* resulted decreased in RBC, small lymphocytes and various polymorphic stages of trypanosomes. Swelling of body at pectoral fin region, lesions on symptoms of infection. Parasitic infestations have been found to destroy erythrocytes and cause anaemia (Soivio & Nikinmaa, 1981). Decreased TEC value of the infected fish in the present study are clear indications of anaemia. The infected fishes exhibited abnormal behaviour and become lethargic. The present study lends support of Engel and Dvevis (1964), Rao and Shyamsundari (1974), Joshi (1989) and Kumar and Ramulu (2013).

The number of thrombocytes, monocytes and neutraphils might have decreased because of the inhabitability of haemoblasts to attain maturity to form different cell types and decreased number of eosinophilis observed in this study were 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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