

Aeromonas Hydrophila Induced Haematological Changes In Cyprinus Carpio

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Abstract- The alterations in the quality of water can adversely affect its inhabitants too. These changes may act as stressors. As fishes form one of the major parts of aquatic biota, the analysis of blood tissue gives a clear picture about physiology and environmental conditions of fish. *Aeromonas hydrophila* an opportunistic fish pathogen was used for the study. A known initial inoculum density of 10^5 cfu/ml bacteria was prepared and inoculated to the experimental groups. Blood was collected on every 5 days based on standard methods and haematological parameters were analysed. The results were submitted to the variance analysis (ANOVA) and F test ($P < 0.05$). The findings indicated significant changes in the values of experimental group like lower red blood cell count, and lower haematocrit and haemoglobin levels. Blood cells also showed slight morphological variations during the period of study.

Keywords- Cyprinus carpio, *Aeromonas hydrophila*, haematological parameters, infection, fish, haematological indices, blood, anaemia

I. INTRODUCTION

As a consequence of rapid industrialization our water bodies are getting polluted at a faster rate altering the physico-chemical and biological properties, affecting the aquatic organisms adversely. Fishes form major part of the aquatic biota. Fish are susceptible to a wide variety of bacterial pathogens especially when the fishes are physiologically unbalanced or nutritionally deficient, or subjected to stressors, i.e., poor water quality, and overstocking. Infectious diseases are the main cause of economic losses in aquaculture industry which is negatively impacted by various pathogenic organisms¹. Tolerance range to various stressors is variable among fish species. *Aeromonas hydrophila*, an ubiquitous free living, facultative, anaerobic, rod, gram negative bacterium of Vibrionaceae family, in aquatic habitats with cosmopolitan distribution. Ulcerous dermatitis on fish caused by *A. hydrophila* is one of the useful biomarkers of polluted or other stressful environment². The haematological parameters are an important tool of diagnosis that reveals the state of health of fish^{3, 4}. Blood tissue of fish gives clue about physiology and environmental conditions of fish⁵. Hence

haematological assays can be used in the detection and diagnosis of metabolic disturbance and diseases in fishes. Generally saying the status of a disease can be pointed out from the haematological failure such as drastic drop of hemoglobin level, hematocrit value, red blood cell counts etc. The evaluation of blood chemistry parameters in animals is a routine and important tool in clinical veterinary medical practice⁶.

The objective of the present study is to assess the haematological variations under the stress induced by *Aeromonas hydrophila*.

II. MATERIALS AND METHOD

The fishes for the study were collected from Pannivelichira fish farm at Kozhencherry, Pathanamthitta district, Kerala. At the laboratory they were maintained in aquarium tanks and acclimatized for 14 days. For the study they were divided into a sample size of 20 each and grouped A as control, B as experimental. Aeration was provided to each tank and they were fed daily. An inoculum of *Aeromonas hydrophila* was prepared. The inoculation of bacterial load required an inoculum of pure culture with known initial inoculum density of 10^5 cfu/ml. The bacterial stock culture was inoculated into 10ml nutrient broth for the preparation of pure culture. It was allowed to incubate at 37°C for 16-18 hours. Followed by centrifugation, washed for 3 times and resuspended in 10ml isotonic saline. By plating appropriate dilutions initial inoculum density was determined. To find out the stress of *Aeromonas hydrophila* on the haematology of *Cyprinus carpio* the prepared bacterial culture with known initial inoculum density was inoculated into fiber tanks containing fishes. For every 5 days interval, the haematological parameters were estimated up to a period of 30 days. For the analysis, the fish were caught individually in a small hand net from the control and test tanks.

The blood was withdrawn from the caudal vessel into a syringe containing a drop of 10% EDTA solution). For differential counting of blood cells, the smears were stained by Wright's stain⁷. For determining haemoglobin, the blood samples were treated with N/10 HCL and the colour of the

acid haematin was matched with given standards using Sahil's hemoglobinometer. By Wintrobe's method using haematocrit tube which was centrifuged for 10 - 15 minutes at 3000 - 4000 rpm³ the packed cell volume (PCV) or haematocrit values were measured. The mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) etc were calculated using the standard formulas. The results were submitted to the variance analysis (ANOVA) and F test ($P < 0.05$).

III. RESULTS

The haematological parameters under the stress of *A. hydrophila* were noted and represented in Table 1. All the blood parameters of the experimental group such as RBC, haemoglobin and haematocrit under the stress of *A. hydrophila* are found to be decreasing up to 30th day and this decrease was significant. However the WBC count increases up to 10th day and then decreases significantly till the end of the study period. The haematological indices such as MCH, MCV and MCHC values and O₂ carrying capacity of the experimental groups showed high variations when compared to control.

Table: 1. Changes in the haematological parameters of *Cyprinus carpio* inoculated with *A. hydrophila* for a period of 30 days. (n = 3).

Parameters	Days							F	Level of Significance
	Control	5	10	15	20	25	30		
WBC($\times 10^6/\text{mm}^3$)	6.92	7.08	7.84	5.44	1.61	1.10	1.54	21.73*	$p < 0.01$
RBC($\times 10^6/\text{mm}^3$)	16.46	13.5	4.63	2.32	1.53	3.20	2.10	263.35*	$p < 0.01$
Hb(g/ml)	7.3	9.35	6.5	5.5	4.2	6.0	3.5	30.96*	$p < 0.01$
PCV (%)	33.67	33.3	24.5	22.42	16.7	16.38	12.5	109.47*	$p < 0.01$
MCV (μ^2)	20.46	24.67	52.92	96.64	109.15	51.19	59.52		
MCH (pg)	4.43	6.93	14.04	33.71	27.45	18.75	16.67		
MCHC (%)	21.68	28.08	26.53	24.53	25.15	36.63	28.00		
O ₂ carrying capacity(mlO ₂ -1Hb)	9.13	11.69	8.13	6.88	5.25	7.5	4.38		

A similar analysis was carried out under the stress of *Aeromonas hydrophila* too (Fig. 1). The cell size of RBC showed slight variations during the experimental period than the control. The least RBC size was observed on the 20th day and maximum (11.25 μ) on the 15th and 25th day. There were no wide variations in the cell size of WBC during the period of study. But a minimum size (8.75 μ) and a maximum of 11.25 μ were observed on the 15th and 10th days.

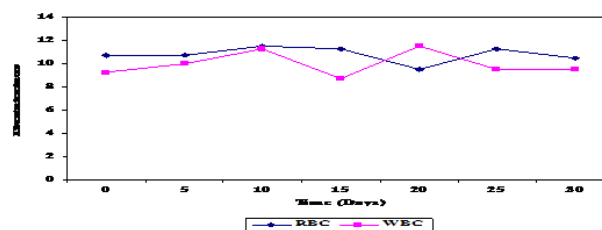


Fig. 1. Variations in the cell size of RBC and WBC of *Cyprinus carpio* subjected to the stress induced by *A. hydrophila*

IV. DISCUSSION & CONCLUSION

The degree of variation on the haematological response is an important tool to fish health diagnosis and may vary according to stressor stimulus, treatment, parasitic or infectious diseases^{8, 9, 3}. The significant reduction in total erythrocyte count in *Cyprinus carpio* inoculated with *A. hydrophila* indicates the anaemic state which may be resulted by the impaired osmoregulation across the gill epithelium¹⁰. The haemoglobin level as well as the O₂ binding capacity of the blood of *Cyprinus carpio* under the stress of *A. hydrophila* show a significant decrease. Here a steep decline was noted in the values of the Hb from the 5th day onwards and the decrease was significant. Similar results were reported by Harikrishnan *et al.*, (2005)¹¹ when *Cyprinus carpio* (L) infected with *Aphanomyces invadans* fungus. *Aeromonas hydrophila* infections also cause a significant drop in RBC and Hb levels in *Ictalurus punctatus*, the decreased Hb content was due to the swelling of RBC as well as the poor mobilization of Hb from the spleen and other haemopoietic organs¹². The data support the present findings that the significant decrease in RBC and haemoglobin content is possibly due to hypochromic microcytic anemia caused by *A. hydrophila*. Decreased RBC counts, hematocrit and hemoglobin concentration indicate that RBCs are being destroyed by the leucocytosis activity in an erythrocytic anemia with subsequent erythroblastosis¹³. In this present study, increase in mean cell volume was observed in fish infected with of *A. hydrophila*, it may be attributed to the swelling of the erythrocytes, resulting in a macrocytic anaemia. Barham *et al.*, (1980)¹⁴ recorded a reduction of Hb and Ht in rainbow trout infected with the *Aeromonas* and *Streptococcus* bacteria. Leucocyte belongs to an important cell involved in the immune response¹⁵. It can be added that lymphocytes have been reported as immunocompetent cells. The present study supported this data with an increase in leucocyte count during the initial days of infection. A significant reduction in the haematocrit values in the inoculated fish, *Cyprinus carpio* was observed may be due to severe bacterial infection affecting the haemopoiesis mechanisms¹⁶. The results depicts the distorted shape of erythrocytes, due to decrease of PCV, may cause an

imbalance in the respiratory physiology of the fish by reducing the surface area of haemoglobin and its access to oxygen¹⁷. The variations in the blood indices may be attributed to a defense reaction against toxicity formed by *A. hydrophila* through the stimulation of erythropoiesis.

From the present study it was observed that *A. hydrophila* can induce considerable changes in the haematological parameters of *Cyprinus carpio*.

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