The Effect of Aeromons Hydrophila Stress on The Haematology of Anabas Testudineus

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Abstract- *An attempt has been made in the present investigation to determine the haematological parameters of Anabas testudineus inoculated with known inoculum density of Aeromonas hydrophila after thirty days of exposure.An experimental group and control group were setup for the study. There was noted alterations in most of the blood parameters measured such as packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC) and RBC indices and WBC. Haematological indices like mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated. White Blood Cell (WBC) counts increased on 10th day of exposure at the same time RBC counts decreased. The results are statistically significant at $p < 0.05$ level. These reports indicate that hematological parameters may be useful as a diagnostic test for Aeromonas exposure in A. testudineus.*

Keywords- Haematocrit, Anabas testudineus, WBC, RBC, Aeromonas hydrophila, inoculums ,stress

I. INTRODUCTION

The use of haematological technique in fish culture for toxicological research, environmental monitoring and fish health conditions have grown rapidly in recent times (Gabriel *et al*., 2007).Hematological parameters changes would be sign of fish physiological responses against environmental stresses e.g. such as heavy metals in water pollution (Vosyliene, 1996) or bacterial infections (Austin, 1987). Therefore hematological changes would occur subsequently in response to the invading pathogens. In many cases of fish infectious diseases diagnosis could be assisted by hematological study (Holway *et al.*, 1975). Bello-Olusoji *et al*., (2006) further explained that changes in haematology of fish in response to stressing agents are indicators of the stressful stage of fish producing useful information to curb any unfavourable condition that may affect the fish health. The blood parameters in fishes are influenced by many factors (Mishra *et al*., 1977). Quality of water, temperature, food availability and physiological status of fish either or indirectly influence on blood constituents of fish (Iqbal *et al*., 1997). Changes in physicochemical parameters may be reflected haematological parameters of the fishes (Abdul Naveen *et al*., 2011).

The main objective of this study is to analyse the haematological variations in *Anabas testudineus* after the inoculation of *Aeromonas hydrophila.*

II. MATERIALS AND METHODS

The selected fish *A. testudineus*, is a very hardy fish, which can tolerate extreme conditions. It is widely seen in canals, lakes, ponds, swamps and inland water bodies of India, hence selected as biological indicators of ecotoxicological studies(Babu Velmurugan *et a l*;2016). Fourty fishes were kept in three aerated aquarium tanks of 25L capacity with A as control and B, C as experimental groups respectively. Before assay the fishes were acclimated for 10 days and fed with commercial diet. *Aeromonas hydrophila* is a ubiquitous gram negative rod shaped opportunistic pathogen causing infections in fish especially during stressful conditions.A known inoculum density of 10^5 CFU/ml was prepared under standard microbiological methods.This was inoculated into the experimental tanks and the haematological parameters were analysed on ever 10^{th} day for a period of 30 days.

The blood samples were drawn by caudal vein puncture using 21 gauge hypodermic needle in two different vials, one containing the anticoagulant EDTA (Heparin sodium 1%) for blood cell studies. The collected blood samples were immediately subjected to haematological analysis. Hematological parameters was done by diluting the blood with appropriate diluting fluids for RBCs and WBCs and thereafter counts were determined using improved Neubauer haemocytometer and calculations by Blaxhall and Daisley, (1973). Sahli's haemoglobinometer was used to estimate haemoglobin percentage (Hb %). Haematocrit (Hct) was determined using micro haematocrit capillaries filled with blood and centrifuged at 8,700×g for 5 min and expressed as percentage of the total blood volume (Wintrobe, 1974). Mean corpuscular volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were

calculated from the average values of Hb% (Dacie and Lewis, 1984).

Differences in haematological parameters between the three freshwater fishes were statistically analysed by one-way analysis of variance (One way ANOVA).

III. RESULT

Table: 1 Changes in the haematological parameters of *Anabas testudineus* inoculated with *Aeromonas hydrophila* for a period of 30 days. (n = 10).

Parameters	Days					Level of
	Control	10	20	30	F	Significance
WBC($x10^{-4}$/cmm)	0.99	1.30	0.64	0.40	845.10*	p<0.05
RBC($x10^{-6}$/cmm)	2.88	1.92	4.31	3.7	2069.86*	p<0.05
Hb (g/100ml)	14.38	11.36	17.61	16.07	405.15*	p<0.05
PCV (%)	41.66	33.73	51.61	46.94	1440.54*	p<0.05
MCV (μ^3)	144.61	175.36	119.33	126.91		
MCH (pg)	49.92	59.05	40.85	43.45		
MCHC (%)	34.51	33.69	34.12	34.24		

The haematological parameters of fish infected with *A.hydrophila* and control is presented in Table1 above. During the assay no mortality was observed after experimental infection. But the condition of the fishes depreciated slightly in all the experimental setup. The haematological parameters analysed reported a significant alterations in the values in comparison to control. The WBC increased on the 10^{th} day followed by a decrease in the coming days. On the contrary the RBC values decreased on the 10^{th} after that the values showed an increase than the control group. A similar pattern of changes in values were noted in the Hb, PCV and haematological indices.

IV. DISCUSSION

The haematological characteristics have been investigated with the aim of establishing normal blood values and ranges respect to *Aeromonas* stress. RBC is the dominant cell type in the blood of the majority of fish species. However, significant differences in the values of erythrocyte volumes between the three species were observed in this study, suggesting that, in the intensive river environments, the elevated RBC counts and Hct concentration are a response to the higher metabolic demand and have no impact on erythrocyte volume. The increased number of RBC indicates the oxygen requirement at higher metabolic rates (Engel and Davis, 1964). In this present study, Hb and ESR seemed to vary, significant at the $p < 0.05$ level between species. The low Hb value was associated with low active fishes; similar results were already reported by Engel and Davis, (1964) and Rambhaskar and Srinivasa Rao, (1986). Decreased WBC count was reported in the present study was in consistence with Shakoori *et al*., (1996) The environmental changes and the pollutants in the river water may increases the RBC/WBC ratio and also alter the physiological and chemical properties of fish blood parameters (Hughes and Nemcsok, 1988. Haematological analysis of common carp (Cyprinus carpio), old fish (Carassius auratus), Tilapia (Oreochromis mossambicus) and stinging cat fish (Heteropneustes fossilis) was carried out by Dhanaraj *et al*., (2008) following injection of *Aeromonas hydrophila*. They observed that WBC and RBC values exhibited a decrease from 0 to 2nd day in C. carpio, C. auratus and H. fossilis but in O. massambicus it decreased from 0-7 th day and increased from 7th day to touch high level on 14th day simply to decrease again up to 21st day. In the experimental group the homeostatic processes are extended beyond the normal level due to stress (Pickering, 1981). Due to A.*hydrophila* infection in test group fishes, the RBC count and haemoglobin level increased ($P<0.05$) from the day 20.

Panigrahi and Misra, (1978) observed reductions in haemoglobin percentage and red blood cell (RBC) count of the fish Anabas scandens treated with mercury. The increase in WBC observed in the present study could be attributed to a stimulation of the immune system in response to tissue damage caused by bacterial stress. Gill and Pant (1985) have reported that the stimulation of the immune system causes an increase in lymphocytes by an injury or tissue damage.In addition decreased RBC, hemoglobin and hematocrit in chum salmon infected with V. anguillarum, in rainbow trout infected with Aeromonas/Streptococcus and in cichlid fish with epizootic ulcerative syndrome were previously reported (Harbell *et al*., 1979; Barham *et al*., 1980; Pathiratne and Rajapakshe, 1998).

Decrease in the level of hemoglobin and MCV observed could induce anaemia condition clearly suggests that a hemodilution mechanism has occurred. The MCV gives an indication of the status or size of the erythrocytes and reflects an abnormal or normal cell division during erythropoiesis. Similar pattern has been detected in Moggel fish (Labeo umbratus) after exposure to various pollutants (Nussey *et al*., 2000). The MCHC is a superior indicator of erythrocytes swelling (Wepener *et al*., 1992).

The decrease in the PVC in C. carpio treated with sublethal concentration of chlorpyrifos due to either rapid oxidation of hemoglobin to methemoglobin or release of oxygen radical brought about by toxic stress of insecticide (Ramesh and Saravanan, 2008). In the present study, significant decrease in MCV, MCH and MCHC in rosy barb fishes exposed to calcium carbide could be probably due to stress induced by acetylene gas and confirm the occurrence of

haemolytic anaemia in experimental fish which exaggerates further disturbances in haemopoitic activity. Howeverer, the decrease in MCV and MCH might be due to high percentage of immature red blood cell in the circulation.

V. CONCLUSION

The approach may provide a rapid means for determining the physiological status of the fish, thereby enabling changing conditions to be diagnosed early, which in turn would facilitate the implementation of remedial measures during culture operations. Statistical analysis of haematological findings revealed significant differences ($P<0.05$) using one way ANOVA. In the current study, haematological assays manifested significant differences in infected samples in comparison with control group. Hence it could be concluded that haematological studies could be a valuable and available tool for prognosis and primary diagnosis in some infectious diseases in first stages of new probably outbreaks.

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