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Hematological changes in *Channa striatus* experimentally infected by *Aeromonas hydrophila.*

Haniffa MA and Abdul Kader Mydeen KP*

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Abstract

Background: This study evaluated the hematological changes in *Channa striatus* (120 ± 3.5 g) intramuscularly administered with *Aeromonas hydrophila.* The experiment consisted of two treatments in triplicates: non-injected control fish; fish injected with 2.4 x 10^8 CFU/mL of *A. hydrophila*. Forty-eight hours after injection, the fish were anesthetized and the blood collected. The hematological parameters included red blood corpuscles (RBCs) count, white blood cells (WBCs) count, packed cell volume (PCV), differential count of WBCs, the derived blood indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were studied in the experimental and control fish.
Results: Fish injected with 2.4 x 10^8 CFU/mL of *A. hydrophila* showed a higher MCV value than control fish. White blood cells and lymphocytes numbers increased significantly in fish injected with *A. hydrophila* when compared to non-injected control. PCV also increased in fish injected with 2.4 x 10^8 CFU/mL of *A. hydrphila.* Hematological data were analyzed with SPSS 7.5 for Windows by using one way analysis of variance.

Keywords: *Channa striatus, Aeromonas hydrophila,* Infection, Haematology.

Haniffa MA and Abdul Kader Mydeen KP*
Centre for Aquaculture Research and Extension (CARE),
St. Xavier's College (Autonomous),
Palayamkottai - 627 002,Tamil Nadu,
INDIA.

***Corresponding E.mail:** abdulmicro2000@yahoo.com

Web address:
http://bioresonline.org/archives/A135.pdf

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INTRODUCTION

Murrels, commonly called snakeheads belonging to the family Channidae (Ophiocephalidae), constitute the most common and dominant group of air breathing freshwater fishes and are highly regarded as food fish in the South and Southeast Asian countries (Wee *et al.,* 1982). It has long been commercially cultured in Thailand, Taiwan, and the Philippines. There are several species of murrels belonging to the genus *Channa* (syn. Ophiocephalus), but only one species, namely *Channa striatus* also called striped murrel, enjoys a good deal of popularity as food fish in many parts of India (Jayaram *et al.,*1981). Besides the high quality of their flesh in terms of taste and texture, they also have good market value due to the low fat, fewer intramuscular spines, and medicinal qualities (Haniffa *et al.,* 2004).

Bacteria of the genus *Aeromonas* are wide spread in fresh, brackish, estuarine and marine water (Carnahan *et al.,* 1996). Motile aeromonads are associated with tail and fin rot hemorrhagic septicemia and epizootic ulcerative syndrome (EUS) in a variety of freshwater and marine fish of the world (Roberts, 1997;). They are frequently isolated from both healthy and diseased fish as well as from other aquatic animals. Under predisposing factors such as poor water quality, high ammonia as a result of high stocking density and feeding, ectoparasites, inadequate handling and stressful conditions, this organism found a portal of entry into the fish host (Moraes and Martins, 2004). Motile aeromonads are considered to be one of the most important bacteria among the etiological agents of fish diseases (Paniagua *et al.,* 1990). The outbreaks of motile *Aeromonas* associated diseases can reach epidemic proportions among the aquatic animals, leading to massive mortality rates (Joseph *et al.,* 1994).

There are several studies on fish bacteria identification, experimental infection or disease resistance (Azad *et al.,* 2001; Al-Harbi and Uddin, 2004; Cai *et al.,* 2004) but little relates the haematological parameters to bacterial experimental infection. The haematological parameters are an important tool of diagnosis that reveals the state of health of fish (Blaxhall, 1972; Rehulka, 2002; *Martins et al.,* 2004a). Blood tissue of fish gives clue about physiology and environmental conditions of fish (Ramaway and Reddy, 1978). Knowledge of hematology is very important since it deals with the morphology, physiology and the biochemistry of blood. By analyzing blood cell characteristics, disease status can be identified (Anderson, 2003). Bruno and Munro (1986) have stated that hematological indices aid in the diagnosis and assessment of disease in fish. In fisheries, it is important to find out illness and parasites as the source of these causes may not be generally detectable in early period of the infection. However it is also possible early diagnosis of illnesses in case of evaluating hematological data, particularly blood parameters (Rimsh and Adamova, 1973).

This study evaluated the haematological changes in *Channa striatus* intramuscularly administered with 2.4 x 10^8 CFU/ mL of *A.hydrophila* in the caudal region originally isolated from naturally infected *C. striatus.*

MATERIALS AND METHODS

A total of 90 striped murrels (*C. striatus*) of average mean length (15 ± 2 cm) and average weight (180 ± 3.5 g) were collected from fish market, Melapalayam, Tirunelveli (8.44°N, 77.44° E), Tamilnadu, India in the month of February 2010 (**Figure:1**). They were transported to the Centre for Aquaculture Research and Extension (CARE) Aquafarm in live condition with oxygenated water in plastic bags (10 l) and they were acclimatized in cement tanks (3 m × 12 m × 1 m) for 7 days before assay and fed with commercial diet. During this period, the water temperature was maintained at 28 ± 1.5 °C, dissolved oxygen 5.8 mg/L and pH 7.1-7.4.

Pathogenic *A. hydrophila* strain was isolated from infected *C.striatus*, further purified by streaked on selective medium, *Aeromonas* Isolation agar (Hi-media). The isolate was identified by their

Figure 1. Infected *Channa straitus*

reaction to standard test following the Bergey's manual (1998). The pathogenicity of *A. hydrophila* was performed following the method of Lafrentz, *et al*., (2002), it was confirmed by injection of *A. hydrophila* on healthy *C.striatus*, it caused 100% mortality within 72 hours (mean death time was 52.7 hours) with development of clinical symptoms.

This experiment consisted of two treatments in triplicates: non-injected control fish (C) (n=30); fish injected with 2.4 x 10^8 CFU/ mL of *A.hydrophila* (T) (n=60) intramuscularly in the caudal region according to the Matushima and Mariano (1996) and Martins *et al.,* (2004 b) method.

Forty-eight hours after injection, the fish were sacrificed and blood sample was collected by vein puncture using 1ml syringe. Before collecting the blood sample, the needle was treated with 0.5% EDTA to avoid coagulation (Rehulka, 1996). To determine the count of erythrocytes, blood sample was taken with an erythrocytes pipette and diluted (1/200) with the Hayem solution, loaded in haemocytometer and examined in light microscope (Nikon-Eclipse E400 microscope, Germany) with a magnification of 400x (Blaxhall and Daisley, 1973). Leukocytes counting was performed by transporting blood sample (diluted in WBC diluting fluid) with a leukocytes pipette into counting lamella and examined as for erythrocytes (Blaxhall and Daisley, 1973; Blaxhall, 1981; De Wilde and Houston, 1961).

The amount of hemoglobin was determined according to cyanomethemoglobin procedure (Blaxhall and Daisley, 1973). Non-clotted blood (20microlitre) was diluted with Drabkin solution (5mL) and left stand for 10 min. The absorbency of the mixture was read at 540 nm and the amount for hemoglobin was calculated using hemoglobin standard solution (Azizoglu and Cengizler, 1996). Non-clotted blood was transferred into PCV tube and centrifuged at 12,500 rpm for 5 min and the ratio of blood components in plasma was determined (Jewet *et al.,* 1991; Amlacher, 1970). For differential leukocyte count, six blood smears per fish were prepared from fresh blood, air-dried, stained with Leishman-Giemsa's stain and fixed in methanol. In each sample, three visual fields at 1,000 X were identified for the leukocyte count (Harikrishnan *et al.,* 2003).The percentage of neutrophil (NEU), eosinophil (EOS), lymphocyte (LYM) and monocyte (MON) tissues was determined. The derived blood indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae. Hematological data were analyzed with SPSS 7.5 for Windows by using one way analysis of variance.

RESULTS

The hematological parameters of the experimental fish (T) were compared with control fish (C) are presented in **Table: 1,** during this assay, no mortality was observed after experimental infection. Fish injected with 2.4 x 10^8 CFU/mL of *A. hydrophila* (T) showed a gradual decrease in Haemoglobin (HB,g/dl), Haematocrit (PCV,%), Mean Corpuscular Haemoglobin (MCH, pg), Mean Corpuscular Haemoglobin Concentration (MCHC,g/dl), Red blood cell (RBC,10^3/mL) which were significantly lower ($P < 0.05$) from values of the control fish (C) which indicated poor physiological blood production. Mean Corpusular Volume (MCV, fl), White Blood Cells (WBCs, 10^3/ mL), Lymphocytes (LYM, 10^3/mL), Monocytes (MON, 10^3/mL) and Eosinophils (EOS, 10^3/mL) of A.hydrophila injected fish (T) which were increased significantly ($P < 0.05$) than values of the control fish (C). The number of lymphocytes in fish injected with 2.4 x 10^8 CFU/mL of *A.hydrophila* (T) was significantly ($P < 0.05$) higher than that of the control fish (C).

DISCUSSION

The predominance of *A. hydrophila* in epizootic ulcerative syndrome (EUS) affected fish has also been reported previously by Kumar *et al*., (1990) in India, Tonguthai (1985) in Thailand, Wong and Leong, (1987) in Malaysia, Dana (1987) in Indonesia, Roberts *et al*., (1986) in Myanmar and Balasurya (1987) in Srilanka. Lio-Po *et al*., (1992) reported that several species of bacteria and fungi were found to be associated with EUS affected snakehead *C. straiatus* and that 89% of the total isolates were *A. hydrophila*. *A. hydrophila* can often be isolated from ulcers or internal organs of EUS-affected fish (Llobrera and Gacutan, 1987; Pal and Pradhan, 1990). Some of these *A. hydrophila* strains have been characterised as virulent (Torres *et al*., 1990; Suthi, 1991; Karunasagar *et al.,* 1995) or cytotoxic (Yadav *et al*., 1992). Sabina *et al*., (2004) have reported that *A. hydrophila* is one of the important pathogens of fish in freshwater and brackish water.

Table: 1. Changes in the hematological parameters of *Channa striatus* injected with 2.4 x 10^8 CFU/mL of *A. hydrophila*. (*Values* expressed in Mean ± S.D of 3 replicates) (P<0.05).

BLOOD PARAMETERS	*Channa striatus*	
	Control fish (C)	*A.hydrophila* Injected fish (T)
Hb (g/dl)	11.2 ± 0.32	9.6 ±0.42
Hematocrit(PCV) %	42.8 ± 0.67	38.5±0.34
MCV (fl)	154.4± 0.95	242.1± 0.92
MCH (pg)	35.5±0.12	24.9±0.65
MCHC (g/dl)	31.6±0.13	23.4±0.12
RBCs (10^3/ml)	31.21± 0.16	22.51±0.23
WBCs (10^3/ml)	24.0 ± 0.25	37.5±0.35
Lymphocytes (10^3 /ml)	17.8±0.14	21.43±0.24
Neutrophils (10^3 /ml)	8.21± 0.73	6.43±0.67
Monocytes (10^3 /ml)	1.9±0.20	3.36±0.43
Eosinophils (10^3 /ml)	1.1±0.22	1.89± 0.25
Lymphocytes %	41.66± 0.74	62.3±0.54
Neutrophils %	53.24±0.25	26.1 ±0.15
Monocytes %	3.6±0.26	7.51±0.98
Eosinophils %	1.5 ± 0.21	4.0±0.23

The results presented in this study have revealed an interesting pattern showing that the level of HB, values of PCV, MCH and MCHC and the number of RBCs were significantly decreased in fish injected with *A.hydrophila* when compared to the control fish. The decreased HB trend may be a result of the swelling of the RBC as well as poor mobilization of HB from the spleen to other hemopoeitic organs (Scott *et al.,* 1981). These data support the present finding that the significant decrease in RBC and HB content is possibly due to hypochromic microcytic anemia caused by *A. hydrophila*. Similarly, decreased red blood corpuscles and PCV were found in coho salmon (*Oncorhynchus kisutch*) infected with *Vibrio anguillarum* (Harbell et al., 1979); in Asian cichlid fish (*Etroplus suratensis*) with epizootic ulcerative syndrome (Pathiratne *et al.,* 1998); in rainbow trout (*Oncorhynchus mykiss*) with ulcerous dermatitis (Rehulka, 1998); in rainbow trout experimentally infected with *Aeromonas sobria* and *A. caviae* (Rehulka, 2002); in carp (*Cyprinus carpio*) experimentally infected with *A. hydrophila* (Harikrishnan *et al.,* 2003) and in Nile tilapia experimentally infected with *Streptococcus iniae* (Chen *et al.,* 2004).

In this experiment, the PCV level significantly decreased in fish injected with *A.hydrophila.* For instance, the pearl spot fish *Etroplus suratensis* when infected with EUS becomes anaemic and then suffers a significant reduction in RBC, HB, and PCV levels (Pathiratne *et al.,* 1998).

In this present study, fish injected with 2.4 x 10^8 CFU/mL of *A.hydrophila* showed increased MCV, WBCs, LYM, MON and EOS. Pathiratne and Rajapakshe (1998) have reported that increased WBCs were found in Asian cichlid fish (*Etroplus suratensis*) with epizootic ulcerative syndrome. Total leucocytes count suggested severe leucocytosis of 24.0 ±0.25x 10^3 WBC/ mL in control to 37.5 ±0.35 x 10^3 WBC/ mL in fish injected with 2.3 x 10^8 CFU/mL of *A.hydrophila*. This fact shows more production of leucocytes in *A.hydrophila* injected fish enhancing the fish defense mechanisms.

In this present study, an increase in MCV were observed in fish injected with 2.4 x 10^8 CFU/ mL of *A.hydrophila*, it may be attributed to the swelling of the erythrocytes, resulting in a macrocytic anaemia. An increase in MCV is also linked to the swelling of the RBC as a result of a hypoxic condition or impaired water balance (osmotic stress) or macrocytic anaemia in fishes exposed to stress (Tort *et al.,* 1988); this would

increase the affinity for oxygen in the blood (Soivio *et al.,* 1981). The decreased level of MCH and MCHC were observed in fish injected with 2.4 x 10^8 CFU/mL of *A.hydrophila* in the present study clearly indicates that the concentration of HB in the RBC was much lower in the infected fishes than in the control fishes, thereby indicating an anaemic condition. The MCHC, as a good indicator of RBC swelling is neither influenced by the blood volume nor by the number of cells in the blood, so can be interpreted incorrectly when new cells with different HB concentration are released into the blood circulation (Soivio *et al.,* 1981). A significant decrease in the MCHC after *A. hydrophila* infection is probably an indication of RBC swelling and/or a decrease in HB synthesis. The higher lymphocytes count observed in fish injected with *A.hydrophila* in this study has also been recorded in infected brown trout and rainbow trout.

As the aquaculture industry expands, tools to monitor the health status of fish using standardized and inexpensive methods will be needed. Evaluation of hematological analyses will enhance the culture of fish by facilitating early detection of infectious disease and identification of sub-lethal conditions affecting production performance. This will contribute to more specific, timely and effective disease treatments in the future**.**

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