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Research paper

Haematological and Biochemical Changes in Freshwater Zig-Zag Eel *Mastacembelus armatus* (Lacepede) Infected with Trypanosomes

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ABSTRACT

Trypanosome is widespread parasite in freshwater fishes causing disease Trypanosomiasis. During the present investigation, 20% of sample population *Mastacembelus armatus* from song river system of Dehradun (Uttarakhand) showed trypanosome infection. The presence of trypanosomes in the blood caused conspicuous changes in TEC, TLC, Hb, ESR, PCV, MCV, MCH, MCHC and DLC correlated with the intensity of infection. In general, anaemia accompanied with erthropenia, microcytosis, macrocytosis and basophilia. The severe infection resulted decrease is alkaline phosphate, acid phosphate, serum cholesterol, blood urea, serum, iron and lactate dehydrogenase, aldose, and S-nuclectidase increased.

1. Introduction

Fish is considered as an important valuable and highly nutritious source of animal protein. Several researches have been earned out on the effect of the parasitic diseases on wide range of fish species all over the world (Aly et al., 2005), but little is known about blood parasitic diseases, particularly in cold water fishes in Doon Valley, Uttarakhand. Fish trypnosome causes changes ranged from changes in somatic indices and condition factors, anemia and pale gills to general weakness, loss of escape reflex, emaciation and ascitis in infected fish (Tandon, 1986; Kobata, 1985; Lom & Dykova, 1992; Joshi & Sharma, 1992; Joshi, 1989; Smith et al., 2004; Kharat and Kothavade, 2012; Gupta and Gupta, 2012). Haemofla-gellate parasites disease including lysozyme, haemolysin reactive protein, lectins and the epidermal migration of inflammatory cells (Gupta and Gupta, 2012).

Trypanosoma, a flagellate protozoan is found in the blood of many vertebrates as parasite and causes a disease called trypanosomiasis in fishes and humans. Chronic infection with trypnosomes dwelling extra-cellularly in blood and tissues of their host and recorded in all vertebrates classes (Hoag et al., 1998). In fish blood, the parasites have trypamastigotes with different morphology upon transfer to vector, the blood sucking leech, the parasites transform into proliferating epimastigotes.

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Thereafter, non-dividing metacyclic tryptomastigotes form move to the proboscis of leech can and be transferred to another fish during the second meal (Loam, 1979; Woo, 1987). Trypnosoma infects variety of freshwater cyprinid and non-cyprinid fish like carp, gold fish and cold water fishes. Natural infection tends to be long lasting immunity has been suggested to be antibodies mediated (Khurat & Kothacade, 2012). The principle replicating stages of trypanosomes are tryptomastigotes in blood of fish and epimastigotes in digestive tract of leeches. Number of studies have recorded on trypanosome infection in blood of fresh water fishes in India. Nonetheless the haematological studies with reference to trypanosomiasis are few and include Tandon and Chandra (1978), Joshi and Tandon (1980), Joshi and Dabral (1981), Joshi et al. (1983, 1985, 1989), Rauthan et al. (1995), Aly et al. (2005), Gupta and Jairajpuri (1981), Gupta et al. (2003).

2. Materials And Methods

A total number of 50 samples (*Mastacembelus armatus*) were collected from river Song near Gularghati and Lacchiwala during summer season with average body length 60 cm and weight 300 gms. These fish were transported alive to laboratory of Zoology Department in prepared large plastic bags and kept in prepared full glass. The fish were fed commercial pellets and they were subjected to clinical and post-mortem examinations according to the method described by (Joshi, 1989). Fish blood samples were stained with diluted Qualigen's Giemsa and were screened for the blood parasites with light microscopy at 1000X magnification. Parasites were measured using morphometry on random selection according to terminology of morphometric values and standard commonly adapted by Hoare and Wallace (1966). Morphometric distances including posterior end to Kinetoplast (PK), Posterior end to mid nucleus (PN), total length including flagellum (TL) and free flagegellum (F) were measured using micrometry. Blood of infected fishes was analysed for alterations in the biochemistry of the host. Serum samples were colorimetrically analyzed for aspartate alkaline phosphatase, acid phosphatase, 5 nuclectidase, Aldolase, Lactate dehydrogenase (King & Wooton, 1959), Zlatkis et al. (1953) for cholesterol, Natelson (1957) for blood urea, Ramsay (1958) for serum iron.

3. Results and Discussion

Parasitological examination for trypanosoma in collected fish (50) revealed that 10 fish (20%) were trypanosome positive and concurrently were tested for all parameters in comparison to apparently healthy 10 fish. Examination of stained film reveled presence of trypanosomas between blood cells as seen. The clinical picture showed that infected fish with trypanosomes were dull and suffered from respiratory distress, loss of escape reflex, mild ascitis and paleness of the skin and gills. The observed clinical signs were nearly similar to those observed by Tandon (1986), Joshi (1989), Lom and Dykova (1992), Aly et al. (2005), and Gupta and Gupta (2012). Post-mortem examination revealed paleness of the internal organs (liver, kidney, spleen) as result of dysfunction of the haemopiotic organs especially the kidney. This observation was nearly similar to those obtained by Brown and Gratzek (1980), Joshi (1982), Joshi and Sharma (1992), Joshi (1989), Smith et al. (2004), Aly et al. (2005) and Shahil et al. (2013).

Changes in some blood parameters were found related to trypanosome infection as compared to those of the healthy fishes. Total erythrocyte count was found lowered in the infected fish as compared to the healthy ones. The fall in decreased fish was nearly 20% against the normal healthy fish value of $2.90 \pm 0.10 \times 10^6$/cmm. The TLC value was found to have increased under disease by about 32% against the healthy fish value of 12,660 ± 242/cmm. The MCV value had also risen in the infected fish by about 9% and the ESR value rose by about 54% as compared to the healthy fish values. The PCV value also showed a fall of about 10% though the MCH value showed a rise of about 4.2% in the diseased fish *Mastacembelus armatus*. The mean corpuscular value tends to show that there is an increase in the release of premature erythrocytes in circulation. This increase is directly supported from the relative abundance of erythrocytes versus erythroblast, where the number of the erythroblasts has increased by about 63% in deceased fish. The rise in erythroblasts in circulation is a symptom of stained erythroporisis generally associated with anameia or and asphyxic stress in fishes (Joshi & Sharma, 1992). The anaemia is further confirmed by MCHC value which showed almost a concomitant fall with haemoglobin value in the infected fish. Thus the results obtained from the present investigation are well accordance to the Tandon and Chandra (1978), Joshi and Dabral (1981), Joshi et al. (1983), Joshi (1985), Joshi and Sharma (1992), Joshi (1989), Saha et al. (1997), Sharma and Joshi (1991), Kharat and Kothavade (2012) and Gupta and Gupta (2012).

However, it is interesting to note that the fish under infection showed macrocytic-hypochromic anaemia as exhibited by the increased MCV, erythroblast and depleted Hb and MCHC as well TEC values in the infected fish. Similarly, a recent report by Joshi and Sharma (1992), Gupta and Gupta (1990), Joshi (1989, 2000), Gupta and Gupta (2012). The DLC also revealed noticeable changes. The number of large and small lymphocytes, haemoblasts, basophils and monocytes rose in the infected fish (Table 1), while the number of natrophils, eosinophils tends to fall, due to infection. The trypanosomiasis causes noticeable changes in various blood values in fishes has been pointed out by Joshi and Dabral (1981), Joshi (1981, 1989, 2000), Aly et al. (2005), Gupta et al. (2003) and Gupta and Gupta (2012).

Thus the present studies further strengthen our observations that these haematozoans causes diagnostic alterations in a number of clinical haematological parameters in higher vertebrates. It is clear that the degree of anaemia vary from species to species (Joshi, 2000). However, it is still uncertain whether the haemaflagellate present in the blood produces some sort of toxic substances (Lome et al., 1989) which in turn either inhibits the haeme synthesis or increase red cells fragility thereby the red cell life span.

The reduction in RBC count, haemoglobin value and packed cell volume in the infected fishes occurred as a result of the parasitic infestation that often leads to anemia (Martins et al., 2004). Furthermore, the parasites simply act as stressor and during primary stages of stress the packed cell volume is altered due to the release of catecholamines which can mobilize RBC from spleen (Wells & Weber, 1990) or induces RBCs to swell, as a result if fluid entry into intracellular compartment. Similar result was recorded by Joshi (1989). Decreased total leucocytes count in response to pollutant exposure has been noticed by Singh and Srivastava (1992) and Pandey and Pandey (2001).

Biochemical parameters assessments of blood are important in evaluating the fish health (Joshi, 1989) in captivity or wild species. In Intensive Culture, osmoregulatory disturbances can occur due to disease and several other factors (Woo, 1987). Hence aquaculture needs constant accompaniment of fish health status to avoid comprising performance parameters and the financial aspects of fish farming. Biochemical baselines values established may allow important clinical decisions about about fish species.

It was noticed that freshwater fish *Mastacembelus armatus* were parasitized by trypanosomes in Song river. The percentage of infection varied from 15-20% in fish is common zig-zag eel *Mastacembelus armatus* beside the leeches attached to the fish carried the larval stages of the trypanosomes. The result presented in Table 2 show that the parasites lowered the levels of alkaline phosphate (4.10 ± 2.0 ka unit/100 mg), Acid phosphate (6.70 ka unit/100 mg), Serum cholesterol (250.62 ± 8.12 mg/100 ml), Blood glucose (50.2 mg/100 ml), Blood protein (2.42 ± 0.10 mg/'100 ml), Serum iron (40.25 ± 5.80 g/100ml), Serum cholinesterase (3.80 ± 1.30 unit/ml). On the other hand elevation in 5'-Nucleotidase (9.20 ± 2.50 m:IU/l), Aldose (42.30 ± 7.50 Nm/ml/min) and Lactase dehydrogenase (390.2 ± 90.1 IU/l).

Table 1 Haematological changes in freshwater zigzag eel *Mastacembelus armatus* infected with Tryanosomes

Blood Parameters	Non-infected Fish	Infected Fish
TEC ($\times 10^6$ / mm^3)	2.90 ± 0.10	2.02 ± 0.20
TLC ($\times 10^3$ / mm^3)	12660 ± 242	14820 ± 560
PCV (%)	42.4 ± 0.70	30.40 ± 1.80
Hb (g%)	10.40 ± 0.80	8.60 ± 0.60
ESR (mm/h)	4.00 ± 0.03	6.28 ± 0.10
MCV (μm)	140.2 ± 3.21	152.4 ± 2.70
MCHC (%)	30.69 ± 0.32	20.60 ± 0.80
MCH (pg)	32.40 ± 1.04	34.20 ± 0.69
DLC (%)		
Haemoblast	10.6 ± 0.8	14.2 ± 1.4
Lymphocyte Large	8.0 ± 1.0	5.4 ± 1.2
Lymphocyte Small	14.6 ± 1.6	20.4 ± 1.2
Monocyte	1.2 ± 0.4	2.2 ± 0.6
Neutrophil	24.2 ± 2.3	20.4 ± 1.7
Eosinophil	1.4 ± 0.2	1.1 ± 0.7
Basophil	1.3 ± 0.1	1.7 ± 0.4
Thrombocyte	34.3 ± 4.0	29.6 ± 2.5

Table 2 Biochemical changes in freshwater zigzag eel *Mastacembelus armatus* (Lecepede) infected with Tryanosomes

Blood Parameters	Non-infected Fish	Infected Fish
Alkaline Phosphate (ka units/100 ml)	430.0 ± 2.22	4.10 ± 2.00
Acid Phosphate (ka units/100 ml)	8.12 ± 4.64	6.70 ± 3.42
Blood Glucose (mg/100ml)	64.0 ± 3.50	50.2 ± 4.25
Serum cholesterol (mg/100 ml)	281.10 ± 110.41	250.62 ± 80.12
Blood Protein (mg/100ml)	3.00 ± 0.10	2.42 ± 0.10
Blood Urea (mg/100ml)	7.50 ± 4.0	3.40 ± 0.80

Serum Iron (g/100ml)	60.10 ± 16.25	40.25 ± 5.80
Serum Cholinesterase	7.50 ± 1.52	3.80 ± 1.50
Lactate dehydrogenase	340.0 ± 60.0	390.2 ± 90.1
Aldose (µm/ml/min)	30.18 ± 7.62	42.30 ± 7.50
5'-Nuclectidase (m IU/)	5.10 ± 1.30	9.20 ± 2.50

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