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ALTERATIONS IN HAEMATOLOG (LINNAEUS), INDUCED BY EXPERIMENTAL *PROCAMALLANUS* INFECTION

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In order to record the effects of experimental *Procamallanus* infection on blood parameters of *Clarias batrachus,* total erythrocyte count (TEC), haemoglobin content (Hb), total leucocyte count (TLC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haeamoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined and calculated on day 15, 30 and 45 post-infection. TEC, Hb and PCV recorded a decline whereas TLC registered an increase during the post-infection period. While MCV and MCH recorded an increase on day 15 and 30 (except on day 45) post-infection, MCHC displayed a significant decrease on day 15 and 45 (except on day 30) post-infection in *C. batrachus*. The haematological alterations due to *Procamallanus* infection appear to cause anaemia in the catfish.

INTRODUCTION

Domesticated animals and fish get affected directly as well as indirectly by parasitic infections (Reid and Armour, 1978; Das and Das, 1997). The parasitic infection disturbs the physiological and metabolic activities of the host inducing changes in blood parameters leading to the disease like anemia and eosinophilia (Satpute and Agrawal, 1974; Roberts, 2001; Madhavi, 2003). The most common disease of fish due to gastrointestinal helminths is macrocytic anaemia (Sinha, 1992; Roberts, 2001; Madhavi, 2003). There exists report on pernicious anaemia in the catfish caused by an enteric nematode parasites belonging to Genus *Procamallanus* (Sinha, 2000). Both adult and larval stages of *Procamallanus* are pathogenic to fish (De and Maity, 2000, Ruhela *et al*., 2006). The need for establishment of standard normal haematological values with a view to aiding in diagnosis the state of health and disease in fishes has been duly emphasized by a number of workers owing to the growing interest in pisciculture (Hesser, 1960; Blaxhall and Daisley, 1973; Roberts, 2001, Ayyappan *et al*., 2006). An attempt has been made to record the alterations in blood parameters of the commercially important freshwater catfish, *Clarias batrachus,* elicited due to experimental *Procamallanus* infection.

MATERIAL AND METHODS

Clarias batrachus used in the present study were collected from local freshwater ponds and also purchased from fish markets of Meerut and adjoining region in western Uttar Pradesh, India. Fishes were acclimatized to laboratory conditions for a week before initiating the experiment. Adult female *Procamallanus* were collected from infected *C. batrachus* by cutting open the intestine. They were kept in watch glass filled with saline solution for natural egg laying at 24-27^{o}C. The eggs were kept in Lock-Lewis solution for healthy embryonation. The solution was changed periodically and 0.1% formalin added to the culture medium to avoid fungal contamination of the eggs. Forty healthy catfish were randomly selected and divided into two equal groups. Catfishes of group 1 were not given any treatment and served as control whereas in catfish of group 2, experimental infection was induced by forcefully pushing 500 embryonated eggs of the nematode into the stomach of each catfish by means of a long-nozzled dropper (De and Maity, 2000; Ruhela *et al*., 2006).

Fresh blood samples from both the groups of catfish were collected from day 15, 30 and 45 by a sharp cut made near the caudal vein and kept in glass vials taking all necessary care to prevent haemolysis and clotting by using anticoagulant. Estimation of total erythrocyte count (TEC), haemoglobin content (Hb), total leucocyte count (TLC) and packed cell volume (PCV) were done by haemoglobinometer, haematocrit tube and haemocytometer. Values of mean corpuscular volume (MCV), mean corpuscular haeamoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as these parameters depend on the corresponding values of Hb, TEC and PCV. Data on haematological values on the control and infected catfishes were evaluated for statistical significance using Students 't` test.

RESULTS AND DISCUSSION

During the present experiment, the infected catfish showed signs of restlessness and came frequently to the surface for gulping air after day 30 of the treatment. The present study demonstrates that the nematode infection in *C. batrachus* induce haematological changes and also elicit a number of systemic disturbances as manifested by changes in the blood parameters. Alterations in blood parameters of the catfish due to experimental *Procamallanus* infection have been summarized in Table 1.

The total erythrocyte counts (TEC) of the control *C. batrachus* were observed to be 3.58±0.29, 3.48±1.44 and 3.41±0.24 million/mm^3, respectively on day 15, 30 and 45. A significant decrease in TEC of the catfish to 2.47±0.32, 2.33±0.24 and 2.22±0.24 million/mm^3, respectively were recorded on the corresponding post-infection period. Haemoglobin (Hb) content of the control *C. batrachus* was 12.76±0.46, 12.52±0.53 and

11.97±0.52 mg/dl on day 15, 30 and 45. A significant decrease in Hb content to 11.10±0.21, 11.05±0.15 and 9.42±0.29 mg/dl, respectively were recorded during the corresponding post-infection period. TLC values of uninfected catfish were found to be 32.50±0.57, 31.10±0.46 and 33.00±0.29 $x10^3/mm^3$, respectively on day 15, 30 and 45. A significant increase in the TLC value to 38.00±0.29, 37.50±0.58 and 36.40±0.15 $x10^3/mm^3$, respectively were observed on the corresponding post-infection period. PCV values of control catfish were 40.14±0.69, 39.94±0.54 and 32.67±0.33% on day 15, 30 and 45. *Procamallanus* infection induced a significant decrease in PCV to a level of 36.86±0.80, 36.00±0.29 and 28.00±0.58%, respectively on the corresponding post-infection period.

MCV values of the control *C. batrachus* were 114.68±0.51, 122.89±1.26 and 112.26±1.00 μ^3, respectively on day 15, 30 and 45. A significant increase in MCV value to the level of 143.42±1.43 and 136.88±0.99 μ^3 were observed on day 15 and 30 post-infection. However, on day 45 of the treatment, this value decreased to the level of 108.10±1.33 μ^3. The values of MCH of control catfish were 36.45±0.59, 36.98±0.34 and 37.69±0.67 pg, respectively on day 15, 30 and 45. A significant increase in the level of MCH to 43.19±1.63 and 40.01±0.0.33 pg were recorded on day 15 and 30 post-infection. However, this value decreased to the level of 32.5±1.04 pg on day 45 of infection. MCHC values of control catfish were 32.98±0.59, 30.99±1.15 and 33.58±0.94%, respectively on day 15, 30 and 45. The MCHC values registered a significant decrease to the tune of 28.21±0.58 and 30.07±1.51% on day 15 and 45 post-infection, however, this value was almost normal (30.69±1.10%) on day 30 of the infection.

Table 1: Effect of experimental *Procamallanus* infection on haematological parameters of *Clarias batrachus*

Parameter	15 Days		30 Days		45 Days	
	Control	Infected	Control	Infected	Control	Infected
TEC (million/ mm^3)	3.58 ±0.29	2.47 ±0.32[c]	3.48 ±0.44	2.33 ±0.24 [b]	3.41 ±0.20	2.22 ±0.24[d]
Hb (mg/dl)	12.76 ±0.46	11.10 ±0.21[b]	12.52 ±0.53	11.05 ±0.15[a]	11.97 ±0.52	9.42 ±0.29[c]
TLC ($10^3/mm^3$)	32.50 ±0.57	38.00 ±0.29[d]	31.10 ±0.46	37.50 ±0.58[d]	33.00 ±0.29	36.40 ±0.15[d]
PCV (%)	40.14 ±0.69	36.86 ±0.80[b]	39.94 ±0.54	36.00 ±0.29[d]	32.67 ±0.33	28.00 ±0.58[d]
MCV (μ^3)	114.68 ±0.51	143.42 ±1.43[d]	122.89 ±1.26	136.88 ±0.99[d]	112.26 ±1.00	108.10 ±1.33[b]
MCH (pg)	36.45 ±0.59	43.19 ±1.63[d]	36.98 ±0.34	40.01 ±0.33[d]	37.69 ±0.67	32.5 ±1.04[d]
MCHC (%)	32.98 ±0.59	28.21 ±0.58[a]	30.99 ±1.15	30.69 ±1.10	33.58 ±0.94	30.07 ±1.51[a]

Values are mean ± standard error (SE) of five specimens. Significant response: [a] $P < 0.05$, [b] $P < 0.02$, [c] $P < 0.01$, [d] $P < 0.001$.

The present investigation reveals that the infected catfish showed restlessness and frequent visit to the surface for gulping air. This observation confirms the report of Hartman and Lessler (1964) that fishes having RBC with reduced surface area/volume coupled with reduction in RBC number remain in very disadvantageous position in respect to respiratory efficiency. Totterman (1944) observed decrease in erythrocyte count and haemoglobin value to less than 20% due to *Diphllobothrium latum* infection in man. Reduction in the number of red blood cells may be due to decreased rate of erythropoeisis (Satpute and Agrawal, 1974). Decrease in haemoglobin content and haematocrit value under stressful condition would be an expected consequence of less number of erythrocytes.

A significant decline in the values of PCV, TEC and Hb in fishes after infection may leads to the development of anemia. Similar view has also been expressed by Blaxhall (1972), Blaxhall and Daisley (1973) and Sinha (2000). A significant decrease in MCHC (except on day 30 post-infection) and increase in MCV (except on day 45 post-infection) supports the suggestion that iron deficiency might contribute to the development of anemia in *C. batrachus* as parasites harbouring the intestine might be utilizing the metabolite (Sinha, 2000).

After the exposure to infection for 45 days, haemoglobin level and PCV decreased. The reason may be release of immature cells from haemopoietic tissues into the blood stream (Wepener *et al*., 1992; Kumar *et al*., 1999a, b). On day 30 and 45 post-infection, the decreased values of Hb, PCV and TEC appear to be due to the toxic effects of the secretions/excretions by the parasite. Release of cellular Hb into plasma may be a reason (Narain and Srivastava, 1979; Kumar *et al*., 2004). In plasma Hb increases when haem from lysed or damaged erythrocytes is released under pathological conditions (Eastman, 1977).

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REFERENCES

Ayyappan, S., J. K. Jena, A. Gopalkrishnan and A. K. Pandey, 2006. Handbook of Fisheries and Aquaculture. Directorate of Publications and Information in Agriculture (ICAR), New Delhi.

Blaxhall, P. C. and K. W. Daisley, 1973. Routine haematological methods for use with fish blood. *J. Fish Biol.*, **5**: 771-781.

Blaxhall, P. C., 1972. The haematological assessment of the health of freshwater fish: a review of selected literature. *J. Fish. Biol*., **4**: 593-604.

Das, M. K. and R. K. Das, 1997. Fish and prawn diseases in India: Diagnosis and control. Inland Fisheries Society of India, Barrackpore.

De, N. C. and R. N. Maity, 2000. Development of *Procamallanus saccobranchi* (Nematoda: Camallanidae), a parasite of a freshwater fish in India. *Folia.Parasitol*., **47**: 216-226.

Eastman, R. D., 1977. Clinical haematology. John Wright & Sons, Sutton, United Kingdom.

Hartman F. A. and M. A. Lessler, 1964. Erythrocyte measurements in fishes, Amphibia and reptiles. *Biol. Bull. Mar. Biol. Lab Woods Hole*, **126**: 83-88.

Hesser, E. F., 1960. Methods for routine fish haematology. *Progr. Fish-Cult*., **122**: 164-171.

Kumar, K., P. Patri and A. K. Pandey, 2004. Haematological and biochemical responses in the freshwater air-breathing teleost, *Anabas testudineus* (Bloch), exposed to mercury. *J. Ecophysiol. Occup. Hlth.,* **4**: 97-108.

Kumar, K., Y. K. P. Sinha and A. K. Pandey, 1999a. Endosulfan induced haematotogical alterations in the climbing perch, *Anabas testudinenus* (Bloch). *J. Natcon.,* **11**: 125-134.

Kumar, K., Y. K. P. Sinha and A. K. Pandey, 1999b. Effect of mercuric chloride toxicity on the blood of climbing perch, *Anabas testudinenus* (Bloch). *J. Natcon.,* **11**: 275-283.

Madhavi, R., 2003. Metazoan parasites in fishes. *In:* Aquaculture Medicine (Eds. I. S. Bright Singh, S. S. Pai, R. Philip and A. Mohandas). School of Environmental Sciences, Cochin University of Science & Technology, Cochin. pp. 64-88.

Narain, A. S. and P. N. Srivastava, 1979. Haemato-histological responses of the Indian freshwater catfish, *Heteropneustes fossilis,* to environmental pollution by sewage, fertilizers and insecticides. *Arch. Biol. (Bruxelles),* **90**: 141-159.

Reid, J. F. S. and J. Armour, 1978. An economic appraisal of helminth parasites in sheep. *Vet. Rec.*, **1**: 4-7.

Roberts, R. J., 2001. Fish Pathology. 2nd Edn. W. B. Saunder, Philadelphia & London.

Ruhela, S., A. K. Pandey and A. K. Khare, 2006. Effect of experimental *Procamallanus* infection on certain blood parameters of the freshwater catfish, *Clarias batrachus* (Linnaeus). *J. Ecophysiol. Occup. Hlth.*, **6**: 73-76.

Satpute, L. R. and S. M. Agrawal, 1974. Parasitic effects on its haematology and histopathology. *Indian J. Exp. Biol*., **12**: 584-586.

Sinha, K. P. 1992. Macrocytic anaemia in the catfish, *Clarias batrachus*. *Biojournal,* **4**: 229-230.

Sinha, K. P., 2000. Haematological manifestation in *Clarias batrachus* carrying helminth infections. *J. Parasit. Dis*., **24**: 167-170.

Totterman, G., 1944. On the occurrence of pernicious tapeworm anemia in *Diphyllobothrium* carriers. *Acta Med. Scand*., **118**: 410-416.

Wepener, V., J. H. J. van Varen and H. H. Dupreez, 1992. Effect of manganese and iron at a neutral and acidic pH on the haematology of the banded tilapia. *Bull. Environ. Contam. Toxicol*., 49: 613-619.