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PARASITE INDUCED ALTERATIONS ON SOME HAEMATOLOGICAL PARAMETERS OF A FRESHWATER FISH, *CHANNA PUNCTATUS* COLLECTED FROM PONDS OF MORIGAON DISTRICT, ASSAM

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***Abstract*: Blood parameters are useful for the measurement of haematological disturbances in parasitized fishes and provide information about the disorders in the host. So, the present investigation was carried out to study the effects of parasite on the blood or blood cells of a freshwater fish, *Channa punctatus*; collected from ponds of Morigaon district, Assam, India. Twenty five fishes (ten fishes collected from unpolluted fish pond and fifteen fishes collected from a domestic sewage-fed pond) were examined for parasites and their effects on blood. Two trypanosoma species (*Trypanosoma elongatus and Trypanosoma sauli*) were found in the fishes collected from sewage-fed pond. The haematological manifestation of the infected *Channa punctatus* (host) showed remarkable decrease in haemoglobin content by -19.402%, red blood cells by -20.883% and white blood cells by -8.042%. Differential leucocytes count was also performed and found that neutrophils, lymphocytes, monocytes, eosinophils and basophils of infected fishes were remarkably reduced to 33%, 55%, 1%, 0% and 0% respectively, compared to uninfected fishes. Haemoglobin content significantly reduced to 7.02±3.24g/dl, red blood cells to 1.97±2.62(10^6mm^3), neutrophils to 33.00±2.56(%) and lymphocytes to 55.00±3.11(%). Morphological changes in the blood cells caused by parasitic invasion observed in the infected fishes reflected tissue damages like deformation of blood cells and nucleus, distorted shape of cell, ameboid nucleus, condense cytoplasm, vacuolated cells, blood cell membrane breakage, elongated cell and nucleus and displaced nucleus in WBC. Infected fishes showed weak body, dull body colour and anemia. Micronuclei were not observed in the present study. In our experiment no changes were observed in different blood parameters of uninfected fishes.**

***Keywords*: Blood parasites, Haematological alterations, *Channa punctatus*.**

I. INTRODUCTION

Fishes are very important sources of protein for human health. So, it is important that they should be kept healthy and free from infection. But due to disorders caused by parasites, the rate of growth as well as reproduction rate is decreased, resulting in loss of potential food and economic loss to the fish farmers. The major group of parasites in freshwater fishes are trematodes, cestodes, nematodes and acanthocephalons which caused infections. Parasites of fishes can either be internal or external. Fish may be infected by the parasites as final or intermediate host in the life cycle of parasites. Every parasite of fish extends some degree of harmful infection or shown changes either on morphology or physiology of its host. These changes can cause haematological disorders (Klontz, 1994; Martins *et al.*, 2004). Haematological parameters

are used as indicators of fish health in relation to diseases and surrounding water alteration (Garcia-Navarro, 1994; Cataldi *et al.*, 1998; Serpunin and Likhatchyova, 1998; Ranzani-Paiva *et al.*, 2000). The snake headed fish, *C.punctatus* is a carnivorous freshwater fish widely distributed in the ponds and beels of Morigaon district, Assam. The water quality of sewage-fed ponds is poor and may be the cause of emergence of parasites in the fish, *C.punctatus*. Blood parameters are used as a tool for monitoring the health or physiology of fishes and responses to pathogens. However, no such studies have informed on the impact of parasites on blood parameters of a freshwater fish, *C.punctatus*. Keeping in view the increasing importance of fishes and parasitic infections in freshwater fishes, the present study was choosen to study on the haematological changes due to parasitic infection.

II. MATERIALS AND METHODS

Collection of specimens and investigation of endoparasites: Ten matured *C.punctatus* (8 to 10cm length, 70 to 80 g weight) were captured using nets from a unpolluted pond and another fifteen species of *C.punctatus* (7.7 to 9.5cm length, 60.5 to 78g weight) were collected from a domestic sewage-fed pond located at Morigaon district, Assam. After capturing, fishes were transferred alive to the laboratory for endoparasite investigations. Endoparasites were not found in fishes collected from unpolluted pond but two species of Trypanosoma (*Trypanosoma elongatus* and *Trypanosoma sauli*) were found in the fishes collected from sewage-fed pond. To investigate haematological parameters, the blood samples of uninfected and infected fishes were taken from the caudal vein of fishes by using a heparinized syringe and collected in glass vials containing EDTA as an anticoagulant. Immediately after blood sampling, red blood cells (RBC) count, white blood cells (WBC) count, haemoglobin content (Hb) and blood smear for differential leucocyte count (DLC) were performed from both the groups (infected and uninfected) following the methodology of Blaxhall and Daisley (1973). Determination of RBC and WBC counts were performed with Neubauer's chambers. Differential leucocytes count was performed with blood smears stained with Giemsa solution.

Estimation of haemoglobin: N/10 HCL is taken in hemoglobin tube (has two graduations-one side g/dl and other side shows the Hb %) up to the mark 20. Wipe out the surface of the pipette with the help of tissue paper or cotton so that excess blood may not be added to the hemoglobin tube. Dispense the blood into N/10 HCL taken in the hemoglobin tube, rinse the pipette with the same solution and mix properly with the help of stirrer. Place the tube at room temperature for 10 minutes for complete conversion of hemoglobin into acid haematin. After the reaction completes, place the hemoglobin tube in the column in Sahli's comparator box and start diluting the dark brown coloured compound (acid haematin) formed in the haemoglobin tube using the N/10 HCL or distilled water by adding drop by drop of it in to the solution and mix with the help of stirrer after each addition. This process is done until the end point comes matching the colour of standard with the colour of the test. If the colour is matched note down the reading indicating in Sahli's hemoglobin tube.

Total red blood cell count: RBCs of *C.punctatus* were counted using an improved Neubaur haemocytometer. Blood was diluted to 1:200 with Hayem's diluting fluid. RBC was counted in 5 loaded haemocytometer chambers and total numbers were recorded as $10^6 mm^3$.

Total white blood cell count: WBCs of *C.punctatus* were counted using an improved Neubaur haemocytometer. Blood was diluted to 1:20 with Turk's diluting fluid and placed in haemocytometer. 4 large square chambers of the haemocytometer were counted under the microscope. The total number of WBC was counted in the haemocytometer chamber and total numbers were reported as $10^3 mm^3$.

Study on blood cell morphology: Blood was collected from the heart by puncturing it with a needle and thin smears were prepared on clean slides. Slides ware fixed in methanol for 10 minutes, then left it for air dry at room temperature and stained with 6% Giemsa stain in Sorenson buffer (pH 6.9) for 20 minutes. After air dried, the slides were washed with water again and kept it for some times to dry then mount the slides. The smears were examined by light microscopy (Olympus) under oil immersion at 100× magnification.

III. RESULTS

The results of haematological indices indicated that Hb content, RBC and WBC were decreased in the parasite infected fishes than in the uninfected fish, *C.punctatus*. Analysis on parasite infected fishes revealed remarkable differences (%) between the haematological indices of the infected and uninfected species (Table-1).

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Table-1: Haematological alterations in parasite infected and uninfected *C.punctatus*, Mean±SD of 5 replicates, - indicates % decreased over uninfected blood parameters.

Parameters	Experiment-I on uninfected fish (*C.punctatus*)	Experiment on parasite infected fishes (*C.punctatus*)		
		Experiment-II	Experiment-III	Experiment-IV
Hb content (g/dl)	8.71±0.94	8.45±1.53 -2.985%	7.99±2.11 -8.266%	7.02±3.24* -19.402%
Total count of RBC (10^6mm^3)	2.49±0.76	2.33±0.33 -6.425%	2.15±0.78 -13.655%	1.97±2.62* -20.883%
Total count of WBC (10^3mm^3)	9.7±0.23	9.18±1.72 -5.361%	9.45±1.55 -2.577%	8.92±1.91 -8.042%
Neutrophils (%)	35.00±0.44	34.00±1.15 -2.857%	34.00±2.31 -2.857%	33.00±2.56* -5.714%
Lymphocytes (%)	58.00±0.89	57.00±2.23 -1.724%	54.00±3.75* -6.896%	55.00±3.11* -5.172%
Monocytes (%)	2.00±0.21	1.00±0.03 -50.00%	0.00±0.00 -100%	1.00±0.001 -50.00%
Eosinophils (%)	3.00±0.11	2.00±0.41 -33.3%	1.00±0.001 -66.6%	1.00±0.002 -66.6%
Basophils (%)	2.00±0.09	1.00±0.02 -50.00%	0.00±0.00 -100%	0.00±0.00 -100%

In the present study Hb content showed significant reduction 7.02±3.24 g/dl in infected fish blood as compared to uninfected fish Hb content (8.71±0.94 g/dl). The haemoglobin level was found to be decreased by -19.402% in parasite infected fish blood, as compared to uninfected fish blood. The RBC of healthy fish (free from parasites) was recorded as 2.49±0.76,10^6mm^3 which significantly dropped down to 1.97±2.62,10^6mm^3 in infected *C.punctatus*. The RBCs were decreased by -20.883% in the parasite infected fishes. The total count of WBC in uninfected fish was 9.7±0.23,10^3mm^3 which decreased to 8.92±1.91,10^3mm^3 in the infected blood of *C.punctatus*. The level of neutrophils, lymphocytes, monocytes, eosinophils and basophils in uninfected fish was observed as 35±0.44%, 58±0.89%, 2±0.21%, 3±0.11% and 2±0.09%, which decreased to 33±2.56 %, 55±3.11%, 1±0.001%, 0±0.00 % and 0±0.00 % in infected fishes respectively. Neutrophils and lymphocytes were significantly decreased in parasite infected fish blood (Table-1).

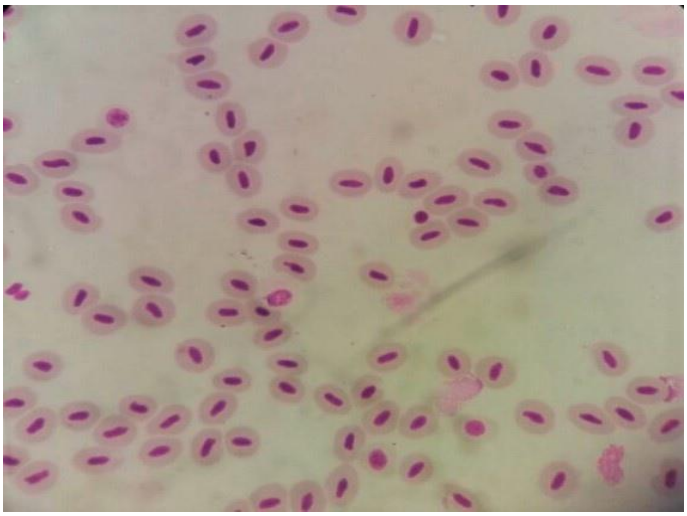

Fig.-1: Normal blood cells: RBC oval to elliptical, centrally located oval-elliptical nucleus.

Morphology of uninfected blood cells: The erythrocytes are oval to elliptical in shape with abundant pale, eosinophilic cytoplasm and centrally located oval-elliptical nucleus. Lymphocytes are more common, small cells with densely basophilic nucleus. Cells contain a small amount of distinctly blue cytoplasm. Cells are usually rounded but often irregular in shape. Monocytes are largest cells, irregular in shape with an eccentric, large, heterochromatic nucleus

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(kidney shaped). Neutrophils are rounded cells that have nuclei that may or may not be segmented and their cytoplasm is slightly blue. Cells are more or less similar to erythrocytes or larger than erythrocytes. Eosinophils are granular, more distinct than those of neutrophils. Usually they are round or rod shaped. Basophils are granular, stain so darkly as to obscure the nucleus (Fig.-1). It was observed that Hb, RBC and WBC are normally present in healthy fishes.

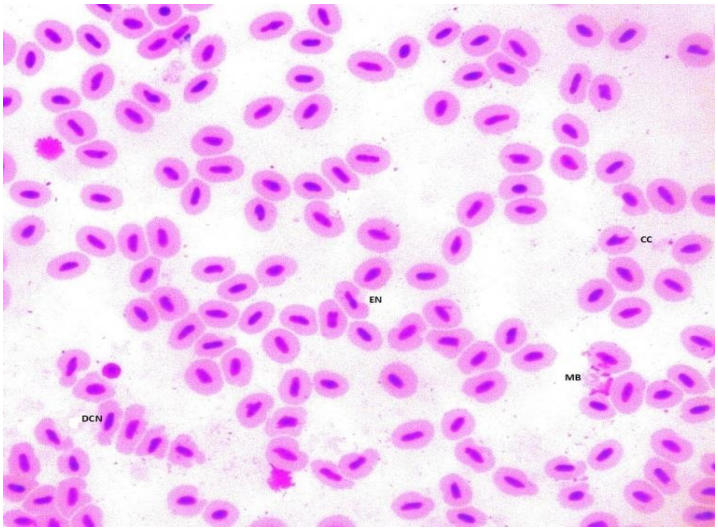

Fig.-2: Cytoplasmic condensation (CC), Membrane breakage (MB), Elongated nucleus (EN), Deformed cell and nucleus (DCN).

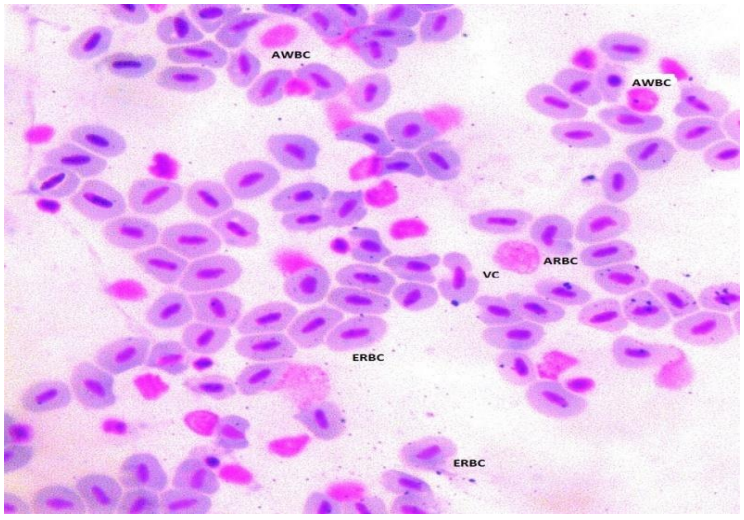

Fig.-3: Enlarged RBC (ERBC), Vacuolated cells (VC), Abnormal WBC (AWBC), Abnormal RBC (ARBC).

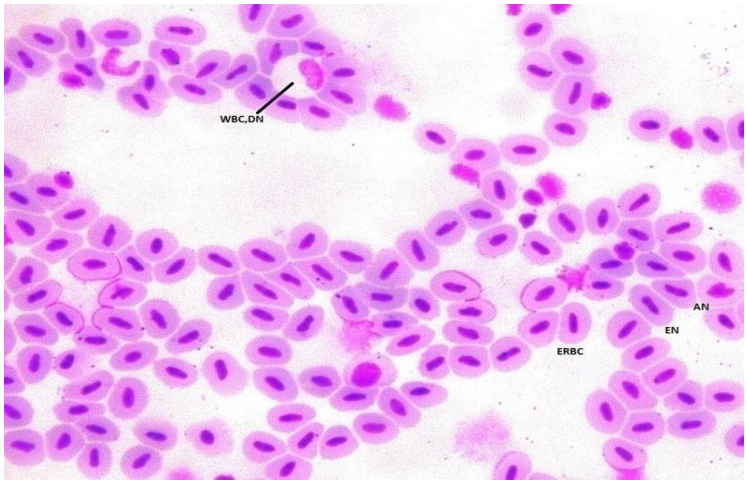

Fig.-4: Elongated RBC (ERBC) with elongated nucleus (EN), WBC with displaced nucleus (WBC, DN), Ameboid nucleus (AN).

Morphological changes of infected blood cells: The blood cells of parasite infected *C.punctatus* showed deformation in their shape (Fig.-2). The RBC showed distorted shape, membrane breakage, elongated nucleus, cytoplasmic condensation, deformed cells and nucleus (Fig.-2), the cytoplasm was cleaved and vacuolated, some with lobed nucleus and formed bleb like structure (Fig.-3). Enlarged RBC, vacuolated cells, abnormal RBC and WBC (Fig.-3), WBC with irregular and displaced nucleus, elongated RBC with elongated nucleus and ameboid nucleus (Fig.-4) were also observed in the parasite infected fish blood. Micronuclei were not observed in the present study.

IV. DISCUSSIONS

In the present investigation, some haematological indices such as Hb content, RBC count, WBC count and DLC have been used as markers for evaluating fish health due to parasite infection. The adverse effects of parasites (*Trypanosoma elongatus* and *Trypanosoma sauli*) on its host species (*C.punctatus*) were recorded. The haematological results showed that the host was affected severely by the parasite. Parasites provoke a remarkable morbidity to human and cause serious damage to aquaculture, which is valuable source of food and employment in developing countries (WHO, 2002; Chai *et al.*, 2005). On the other hand, parasites are acquired through the eating of fishes that contain parasites or parasite larvae and are an unrecognized food safety risk in communities with tradition of eating raw fishes.

In the light of present study, reduction of Hb, RBC and WBC of fishes collected from sewage-fed, polluted pond in comparison to those collected from unpolluted fish pond which leads to fluctuation in blood parameters. Similar results have also been reported by Ghani and Bhuyan (2011), Shahi *et al.* (2013). Blood parameters were affected due to parasitic infection also reported by Saxena and Chauhan (1993) in the blood of *H.fossilis*. The Hb content was remarkably decreased in the present investigation, because Hb molecules may be oxidized and unable to bind or carry oxygen molecule, which is in agreement with the works of Brown and McLeay (1975). Khan (1985) observed low level of Hb content in the infected *Gadus murhua* with *Trypanosoma muramenensis*, which is similar to that of present investigation. The reduction of Hb, RBC and WBC in the infected fishes occurred as a result of the parasite infection that often leads to anemia. Martin *et al.* (2004) in his investigation also reported similar results in *L.macrocephalus* collected from pond. Decrease of TLC in response to pollutant exposure has been observed by Singh and Srivastava (1994) and Pandey and Pandey (2001). A similar trend in the fishes of present investigation was observed by Shah *et al.* (2009) in Anchar Lake and river Jhelum. The blood cell morphology was observed and found that it was distorted in shape, cytoplasm was cleaved, lobed and blebed formed in nucleus, abnormal RBC and WBC, WBC with irregular and displaced nucleus, elongated RBC with elongated nucleus, ameboid nucleus and vacuolation in cytoplasm. Earlier studies of Jayaprakash and Shettu (2013) showed similar abnormalities in fish (*C.punctatus*) due to parasitic infection. Swelling of blood cells was also recorded in the present study, is in agreement with the works of Gupta and Saraswat (1991) in *C.punctatus*; Gupta *et al.* (1998) in *C.fasciatus;* Hassen (2002) and Ismail (2003) in *C.garipineus;* that are naturally infected with parasite, *Trypanosoma mukasai.*

The parasites found in the blood of *C.punctatus* showed many deleterious effects. Alterations due to effects of parasites in haematological parameters have been reported to play important roles in the assessment of the state of fish health. The affect of parasitism on the fish (host) is of considerable importance because fishes are one of the important table items for human. Effects of blood parasites is harmful for fish population and may cause high mortality of marine or freshwater fishes.

REFERENCES

[1] Blaxhall P.C. and Daisley K.W. (1973): Routine haematological methods for use fish with blood. J. Fish Biol., 5:771–781.

[2] Brown D.A. and Mc Leay D. (1975): Effect of nitrate on methemoglobin and total haemoglobin of juvenile Rainbow Trout. Pro. Fish Cult., 3:36-43.

[3] Cataldi E, Di Marco P, Mandich A, Cataudella S. (1998): Serum parameters of Adriatic sturgeon *Acipenser naccarii* (Pisces: Acipenseriformes): effects of temperature and stress. Comp. Biochem. Physiol., 121(A):351–354.

[4] Chai J.Y., Murrell K.D. and Lymbery A.J. (2005): Fish borne parasitic zoonoses: status and issues. Int. J. Parasitol., 35:1233-1254.

[5] Garcia-Navarro CEK.(1994): Hematologia dos animais domésticos. In: Garcia-Navarro CEK, Pachaly JR, editors. Manual de Hematologia Veterinária. Primeira edição.Varela: São Paulo; 1994. pp. 11–119.

[6] Ghani M.O. and Bhuyan A.I. (2011): Community structures of endoparasitic helminths of *C.punctatus* from a freshwater river and a polluted lagoon of Bangladesh. Bangladesh J. Zool., 39:173-185.

[7] Gupta N. and Saraswat H. (1991): *Trypanosoma rohilkandae* species from a freshwater Teleost fish, *C.punctatus*. Him. J. Environ. Zool., 5:29-33.

[8] Gupta N., Saraswat H. and Gupta D.K. (1998): A new variety of Trypanosoma from the blood of *C.fasciatus* and its role in inducing haematological changes. J. Parasitol. Appl. Anim. Biol., 7:33-40.

[9] Hassen F.E.Z.M. (2002): Studies on diseases of fish caused by Henneguya infestation, Ph. D Thesis, Faculty of Veterinary Medicine, Suez Canal University, Egypt.

[10] Ismail G.A.E. (2003): Histopathological and physiological studies on naturally infected *C.garipieneus* with Trypanosoma. M.Sc. Thesis, Cairo University, Faculty of Science, Egypt.

[11] Jayprakash C. and Shettu N. (2013): Changes in haematology of the freshwater fish, C.punctatus exposed to the toxicity of deltamethrin. J. Chem. and Pharmaceutical Res., 5(6):178-183.

[12] Khan R.A. (1985): Pathogenesis of *T.murmanensis* in marine fish of North-Western Atlantic following experimental transmission. Can. J. Zool., 63:2141-2144.

[13] Klontz G.W. (1994): Fish hematology. In: Stolen JS, Fletcher TC, Rowley AF, Kelikoff TC, Kaattari SL, Smith SA (eds) Techniques in fish immunology, 3. SOS Publications, pp. 121–132.

[14] Martins M.L., Tavares-Dias M., Fujimoto R.Y., Onaka E.M. and Nomura D.T. (2004): Haematological alterations of *Leporinus macrocephalus* (Osteichthyes: Anostomidae) naturally infected by *Goezia leporine* (Nematoda: Anisakidae) in fish pond. Arquivo Brasileiro de Medicina Veterinaria Zootecnia, 56: 640 – 646.

[15] Pandey A.K. and Pandey G.C. (2001): Thiram and Ziram fungicide induced alterations on some haematological parameters of freshwater catfish, *Heteropneustus fossilis*. Indian J. Environ. Ecoplan., 5(3):437-442.

[16] Ranzani-paviva M.J.T., Silva-Souza A.T., Pavanelli G.C. and Takemoto R.M. (2000): Hematological characteristics and relative condition factor (Kn) associated with parasitism in *Schizodon borellii* (Osteichthyes, Anostomidae) and *Prochilodus lineatus* (Osteichthyes, Prochilodontidae) of the Paraná River, Porto Rico region, Paraná, Brazil. Acta Scientiarum, Maringá, 22(2): 515-521.

[17] Saxena K.K. and Chauhan R.R.S. (1993): Effects of parasitic infection on the blood of *Heteropneustus fossilis* (Bloch). Bioved, 4: 41-43.

[18] Serpunin G.G. and Likhatchyova O.A. (1998): Use of the ichthyo-haematological studies in ecological monitoring of the reservoirs. Acta Vet. Brno, 67:339–345. doi: 10.2754/avb199867040339.

[19] Shah A.W., Parveen M., Mir S.H. Sarwar S.G. and Yousuf A.R. (2009): Impact of Helminthes parasitism on fish haematology of Anchar Lake, Kashmir. Pakistan Journal of Nutrition, 8(1): 42-45.

[20] Shahi N., A.R.Yousuf and T. Yaseen (2013): First report of blood parasites in fishes from Kashmir and their effect on the haematological profile. Veterinary Journal, 3(2):89-95.

[21] Sing N.N. and Srivastava A.K. (1994): Formothion induced haematological changes in the freshwater catfish, *Heteropneustus fossilis*. Ecotoxicol. Environ. Monit., 4:137-140.

[22] WHO (1995): World Health Organisation, Control of food borne Trematodes infection, Geneva, WHO 1-1.

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