Trypanosomiasis among fishes is known since the discovery of first report in the fish Salmo trutta by Valentini from Europe and Lingard from India, besides those during seventies & eighties of 20th century from India by Joshi and Gupta. However, not much work is available on the effects of trypanosome infection on fishes made systematically during past century except for some original contributions of Tandon & Joshi on two freshwater teleost from India, viz Clarias batrachus & Mystus vittatus, along with reporting two new species of trypanosomes. It was towards end of the twentieth century that some very good contributions came from all over the world as reviewed & cited by Woo, in his book, as well from India mainly from the school of Joshi and Gupta. All these reports offer highly varying and some times contradictory effects of trypanosomiasis on a variety of clinical blood / haematological parameters like TEC, TLC, DLC, cytological features to haemoglobin, packed cell volume (haematocrit) to ESR and blood glucose, calcium, lactic acid contents to cholesterol. This paper presents results of trypanosomiasis, found in a group of the catfish Heteropneustes fossilis, found infected with Trypanosome magur.

MATERIALS AND METHODS

A batch of 32 live freshwater catfishes Heteropneustes fossilis was purchased from the Peeth Bazar of BHEL Haridwar, brought alive to our laboratory of the department in the plastic buckets, transferred to the aquarium and maintained under laboratory condition for the purpose. The fish were rested for acclimation of about one week, before initiating these to clinical observations. To make various estimations from blood samples fishes were handled, anaesthetized, blood drawn, and processed for making studies on different selected parameters including slide preparations and staining these by Giemsa stain, were followed as followed earlier by Tandon & Joshi. The values given here are for equal number of male and female specimens, in each group. It was only after observing the stained slides under high power of microscope it came to our attention that out of a lot of these 32 fishes as many as 16 specimens were...
harbouring the said species of trypanosomes. At least three stained preparations were made for each fish.

**RESULTS AND DISCUSSION**

At the beginning it must be mentioned that during their acclimatization period to laboratory conditions 8-9 fishes died on different days viz. 3, 2, 3 & 1 fishes, on first, second, third and fourth day, respectively, at a stretch, within one week of arrival, in our laboratory. The intensity of infection widely varied as found during scanning of the Giemsa stained blood smears. The stained blood smears for few fishes, among the infected ones showed presence between 1 to 10 trypanosomes per field of scanning. On an average about 3-5 trypanosomes were found per thousand red blood cells of scanned area, under 10 x 40 x powers lenses of microscope, exhibiting the differences in the intensities of parasitemia. There were conspicuous variations in the differential counts of both types of blood cells i.e. white and red ones in their numerical as well as cyto-morphological makeup. The values given here are mean and ranges for 06 specimens of the twin groups.

The behavioral parameters related to swimming, feeding and respiratory behavior were also closely monitored of each of these fishes, from the time of their arrival in our laboratory till the last day of their being used, and later on co-related to their clinical haematological values. In brief, smaller sized fishes found infected with trypanosomes named *Trypanosoma maguri* were sluggish in comparison to the larger sized infected fishes and healthy ones. Even at times such smaller sized fishes were not active in their feeding modes.

The values given in Tables 1 & 2 show that range of variations among the infected ones showed presence between 1 to 10 trypanosomes per field of scanning. On an average about 3-5 trypanosomes were found per thousand red blood cells of scanned area, under 10 x 40 X powers lenses of microscope, exhibiting the differences in the intensities of parasitemia. There were conspicuous variations in the differential counts of both types of blood cells i.e. white and red ones in their numerical as well as cyto-morphological makeup. The values given here are mean and ranges for 06 specimens of the twin groups.

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The values given in Tables 1 & 2 show that range of variations for each parameter are quite wide. The mean values for six parameters showed conspicuous fall among diseased fishes as compared to healthy fishes. The numerical strength of large lymphocytes, small lymphocytes, basophils, eosinophils, thrombocytes and erythrocytes had fallen by 20.8%, 21.6%, 12.5%, 64.5%, 12.0%, and 28.2%, respectively. On the other hand values of large haemoblasts, small haemoblasts, monocytes, neutrophils, macrophages, erythroblasts (Plate: Pic-7) and microcytes had increased by 78.4%, 31.2%, 56.0%, 50.0%, 700%, 149.1%, and 232.0%, respectively, among the diseased fishes compared to healthy ones (Tables 1 & 2).

**Cyto-morphology of Blood Cells:** The blood pictures of diseased fishes were very interesting and there was hardly any uniformity of structural and cyto-chromatic behaviours (Plate: A: 1-8) and responses. There were polychromasias among all types of cells, anisocytosis (Plate: A:2), poikilo-cytosis and dislocation of nuclei (Plate: A: 2 & Plate: B: 15) and disfiguration among erythrocytes (Plate: B: 10) as well as among the cells of leucocytic series. Again anisocytosis was most conspicuous along with vacuolization (Plate: A: 2) and pinching off phenomenon among the nuclei of all sorts of cells.

Erythrocytes in various stages of degeneration were quite frequently seen (Plate: B: 11-13) in a few specimens, but in some other specimens of almost equal intensities of infection (parasitemia) bizarre stages of erythrocytic necrosis were almost nil. Smirnova and Laird & Bullock have mentioned such structures under lysis of erythrocytes as piscine erythrocytic necrosis. Could it be due to some sort of immune response developed by the infected fishes or the trypanocidal antibodies in fish, as reported by Barrow, remains to be studied at micro-immunological level. Destruction & distortions in cytomorphological/histomorphological status due to trypanosomiasis has been shown in detail in the fish *Channa (Ophiocephalus) punctatus* earlier by Joshi. But during this study we did not find abnormally increased numbers of

**Table 1. DLC (%) of healthy and diseased fish *Heteropneustes fossilis* (Bloch). All values are mean & range 06 observations each. [LL - Large lymphocyte, SL- Small lymphocyte, H- Haemoblast, SH- Small haemoblast, M- Monocytes, N- Neutrophil, B- Basophil, E- Eosinophil, MP- Macrophage and T- Thrombocyte]**

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<th>LL</th>
<th>SL</th>
<th>H</th>
<th>SH</th>
<th>M</th>
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<th>B</th>
<th>E</th>
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<tr>
<td><strong>Healthy Fishes</strong></td>
<td><strong>Standard body length : 15.4 (12.1-17.5) cm</strong></td>
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<td>25.5</td>
<td>(19.0-5.0)</td>
<td>25.5</td>
<td>(15.0-29.0)</td>
<td>5.1</td>
<td>(2.0-9.0)</td>
<td>3.2</td>
<td>(0.9-0.0)</td>
<td>5.0</td>
<td>(2.0-8.0)</td>
<td>8.0</td>
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<tr>
<td><strong>Infected Fishes</strong></td>
<td><strong>Standard body length : 15.7 (13.1-18.0) cm</strong></td>
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<tr>
<td>20.2</td>
<td>(15.0-5.0)</td>
<td>20.0</td>
<td>(12.0-40.0)</td>
<td>9.1</td>
<td>(3.0-14.0)</td>
<td>4.2</td>
<td>(2.0-6.0)</td>
<td>7.8</td>
<td>(4.0-12.0)</td>
<td>12.0</td>
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hypersegmented granulocytes (mainly the neutrophils) as have been reported by Joshi for *Channa punctatus*. Now such situations pose questions before us as why there should be such cyto-morphological differences due to the same parasite, of course not the same species. It leads to be suggestive that fishes are (being the first vertebrates are perhaps) in a stage of intra-immunological evolution of antigen-antibody related defense mechanism, to develop cellular and humoral sorts of defense mechanism in their haemodynamic system. The red blood corpuscles show all sorts of distortions has been again found in this case too. Not only there occurs fall in the numbers of mature erythrocytes, but that the number of immature erythrocytes, i.e. the erythroblasts at different stages of maturation enhanced manifold, besides the number of microcytes (Table 2). This is due to stress on haemopoisis on account of trypanosomal antibodies, which cause lysis of erythrocytes (as also pointed out by Gupta in a comprehensive review on the related subject), on one hand leading to fall in their population, thus increasing their demand in circulation to struggle against the parasites, which is competing for oxygen as well as for nutrients like glucose in the circulation and lead to the depletion of serum glucose, cholesterol, besides concomitant fall in the stock of liver glycogen, as reported by Joshi in the fish *Heteropneustes fossilis*, besides the immunological requirements, which may be there. In one specimen of *Heteropneustes fossilis* having trypanosomes the echinus-erythrocytes, termed as echinocytes here, were found in abundance, (Plate-A: 8).

At times presence of micronuclei among erythrocytes (Plate-A: 5,6) as well as among few leucocytes (Plate-B:12,14) were also found to the extent of 1-5 % erythrocytes among diseased fishes which was around 0.001 % among healthy fishes. Since there were wide range of variations in cellular behavior of all types of cells, that simply means the degree of response would be impacted by various variables like age and nutritional status of fishes, duration of infection, stage of infection, may be sex of the fish, ambient temperature and like that. Tandon & Joshi in *Clarias batrachus & Mystus vittatus* and Joshi & Dabral in *Heteropneustes fossilis* reported stimulation of general hemopoisis when the intensity of trypanosome infection was high among *Clarias batrachus* and *Mystus vittatus*. They also found hyper-segmentation of nuclei of the neutrophilic cells and relatively large numbers of erythroblasts besides high degree of poikilocytosis. These authors also reported an high order of hypochromasia, which was co-related with the anemic status of diseased fishes, as also documented by Gupta. Similar studies with variations in different selected haematological parameters are for *Cyprinus carpio* by Smirnova, due to Haemogregarina. Kawatsu reported anisocytosis, nuclear as well as cellular fragmentation of RBCs, macrocytic anemia and structural abnormalities of leucocytes in *Salvelinus fontinalis* suffering from an undiagnosed disease. Similarly Enomoto in four species of fishes observed immature leucocytes, [identical to lymphoid haemoblasts of *Catton*] increased only when infectious disease was of inflammatory nature. Such lymphoid haemoblasts were frequently encountered in present study too, in diseased fishes, but there was no inflammation of any sort.

The neutrophils increased along with those of eosinophils & basophils. But in the present case eosinophils (Plate-B:9,14) and basophils (Plate-A:6) have fallen in number in most of the diseased fishes (Table 1). Tandon & Joshi also reported a left shift in the arneth values of neutrophils, which was not found in present study. Joshi in case of *Clarias batrachus* reported sharp rise in haemoblast cells under all categories of trypanosomiasis, attributed to extra requirements / pressure, may be due to immunological requirements or due to response from fish to increase their number in circulation, on account of ongoing haemolytic processes within the diseased fishes. Fall of thrombocytes (Plate-B:10) appears to be a common feature among these fishes as also reported earlier by Joshi in *Clarias batrachus*. In the present study an increase in degenerating forms like cell casts/basket cells (Plate-B:9,15), nuclear casts (Plate-9,10), and cells at various stages of cellular lysis (Plate-B:11- 13) were noticed in 1 or 2 infected fishes and not among all.

On the other hand haemolysis can be caused due to haemolysins secreted by the live trypanosome, has been suggested by Woo, besides the status parasitemia to cause a variety of pathological changes has its own influence (Woo & Islam & Woo) on the blood picture.

Our experience Joshi permits us to indicate that seasonal ambient temperature should also impact such immunological and haematological responses among fishes under such types of diseases, where both the parasite and host are struggling to acclimate or acclimatize to the seasonal variables, around. Smirnova has also suggested such possibilities. In this regard
The role of different types of leucocytes viz. lymphocyte, granulocytes and monocytes remains to be studied in isolation, because each type of cell behaves differently even in the same species of fish. Such variations, then should be impacted / guided by the tenure and status of infection, appears the most probable cause of variations.

The role of smaller lymphocytes( Plate-B:11,14 ) is still not well understood. Do these cells play role those like the B-lymphocytes(?) offers scope for further studies. These observations become more critical when we review the reports of Joshi & Dabral11, who found that larger sized fishes were anaemic but not showing leucocytosis despite heavy infection as compared to the smaller sized cat fishes. However, trypanosomiasis leads to hyper-variations-distortions in cellular structures as reported earlier5 appears a certainty among fishes, but why, when & how it happens remains to be found out.

Table 2. Comparative differential counts(%) of erythrocytic cells of healthy and infected Fish Heteropneustes fossilis (Bloch). All values are for 06 specimens each.[EC -Mature erythrocytes, EB- Immature erythrocyte or Erythroblast and MC- Microcytes].

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<thead>
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<th>Status</th>
<th>Erythrocytes</th>
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<tr>
<td></td>
<td>EC</td>
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<tr>
<td>Healthy</td>
<td>93.3 (90.0-95.6)</td>
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<tr>
<td>Infected</td>
<td>82.5 (77.0-84.3)</td>
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ACKNOWLEDGEMENTS

Senior author extends thanks to the UGC, New Delhi for financial support with sanctioning BSR Faculty Fellowship to him. Thanks are due to HOD Department of Zoology & Environmental Sciences, Gurukula Kangri university, Haridwar (India) for laboratory facilities etc. and also to the authorities of GKV, permitting to carry out the research work.

REFERENCES

Plate A: 1. Showing normal erythrocytes of an healthy *H. fossilis*. 2. Showing a large lymphocyte, a monocyte and a neutrophil. A few cells also showing vacuolization. A few erythrocytes also showing micronuclei. 3. Showing a Trypanosome, normocytes and erythroblasts with relatively larger nucleus besides a senile RBC. 4. Showing three trypanosomes under one field and a hypochromic RBC. 5. Picture showing a trypanosome(T), small lymphocytes (SL), cell casts of an RBC, anisocyte (a), large lymphocyte and micronuclei within few RBCs. 6. Showing a basophil & few erythroblasts. 7. Showing a trypanosome, a few erythroblasts (EB), a degenerating RBC & 8. Showing echinocyes & two trypanosomes with light hypochromasia. Except microphotograph no.1, rest all are for fishes found infected with trypanosomes.
Plate B: 9. Showing an eosinophil (E) and cellular (CC) & nuclear debris (ND) in a trypanosomic *H. fossilis*. 10. Showing a group of Thrombocytes (TH) and erythrocytes in various stages of deformities. 11. Showing polychromatic coloration, preponderance of erythroblasts, degenerating erythrocytes (DE-1), haemoblast (H) large (LL) and small lymphocytes (SL) & microcytes. 12. Showing various stages of erythrocytic degeneration / disintegration (DE-1-4). 13. Showing a macrophage (MP), a degenerating erythrocyte (DE), 14. Showing group of small lymphocytes (SL), eosinophil (E) and a large lymphocyte with micronucleus besides erythrocytes showing 1-2 micronuclei (m) on their periphery. 15. Showing a large haemoblast (H), cell casts (CC) & nuclear deformities & displaced nuclei within RBCs in a fish due to trypanosomiasis.