Research Article

Haematological studies on *Trichogaster fasciatus* fish infected with *Clinostomum complanatum* metacercariae

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**ABSTRACT**

Parasitic infections in general and helminth infections in particular may potentially reduce the quality and quantity of yield in aquaculture industry. Considering the significant role of various blood components in the diagnosis of diseases, we collected blood sample from the *Trichogaster fasciatus* fish infected with excysted progenetic metacercariae of *Clinostomum complanatum*. A number of blood parameters were analysed and compared with non-infected blood sample of the fish under study. Since, the haematological parameters provide a valuable tool for monitoring the fish health, the present findings may be exploited as a pilot study to understand the fish health and disease condition. In the present study, we observed significant reduction in the level of blood parameters such as RBC, Hb concentration, HCT, MCV, MCH, PLT, MPV, PDW and RDW in the infected fish as compared to the non-infected fish. It is suggested that the level of infection can elicit a stress response in the host which can be correlated with changes in the haematological parameters and may be helpful in detecting diseases and serious illness conditions in the infected fishes.

Key words: *Clinostomum complanatum*; Haematology; Parasitic infection; *Trichogaster fasciatus*

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**Introduction**

India is basically an agricultural country where fish and fish related aquaculture research activities plays a major role in shaping the economic prosperity. In India, a major part of the population is dependent on agriculture and farm based products for their livelihood. To carry out a fruitful aquaculture practice, maintenance of fish health is of prime concern as it has direct effect on the annual yield. Among various other factors governing fish well-being, parasitic infections have a detrimental effect on fish health and production. Infestation and infection of farmed and wild fish by several species of helminth parasites including trematodes are a common incidence. Haematological studies serve as a valuable tool for assessing the disturbances and diseased condition caused in fishes. The parasite under study is known to possess zoonotic potential in human beings (Chung et al. 1995). The infection of *Clinostomum complanatum* in human beings or birds may be due to the consumption of improperly cooked or raw infected fish. Fishes in general are vulnerable to various parasitic infections which have adverse effects on their health (Sinderman 1990). Parasitic infections may potentially reduce the quality and quantity of yield in aquaculture programme by direct or indirect ways.

Considering the significant role of various blood parameters in the diagnosis of several
diseases, we studied a number of blood parameters to compare normal and infected *Trimeresurus fasciatus* fish infected with excysted progenetic metacercariae of *C. complanatum* in order to investigate and establish the effect of parasitic infection on piscine haematological parameters as a reliable indicator of the health status of fish under study. The available literature revealed that parasitic infections have considerable harmful effects on fish health and alters the various haematological parameters to furnish the overall damage. It can be summarized that the helminthic infections are not only a menace to fish health, but they also have potential zoonotic factor in many cases, affecting human health. Comprehensive analysis of the available literature in recent times on alterations of piscine haematological parameters due to *Clinostomum* sp. from the Indian subcontinent highlights two independent studies in freshwater ecosystem. Shah et al. (2009) and Kaur et al. (2012) observed an evident alteration in the various parameters of whole blood in infected and non-infected fishes in Kashmir and Bhopal regions, respectively. Investigation on the changes in haematological parameters due to infection by excysted progenetic metacercariae of *C. complanatum* in *T. fasciatus*, in this part of the country is the first attempt to the best of our knowledge as no information is available on various haematological parameters of the fish under study.

**Materials and Methods**

**Collection of fish**

Live *Trichogaster fasciatus* fishes, 3.7-6.1 cm in length, were purchased from the local fish market of Aligarh. Fishes were thoroughly washed and maintained in an aquarium filled with tap water. The water was changed every alternate day with an appropriate aeration. Since the fishes under study come under the category of ‘food fishes’, the current experimental work has no ethical issues. However, we followed standard protocols to perform the haematological studies.

**Collection of Blood**

The blood was collected from both infected and non-infected *T. Fasciatus* fishes by cardiac puncture with the help of a 1 ml sterile hypodermic syringe in sterilized vials coated with EDTA. The sample was used either fresh or stored immediately at 4°C until used.

**Analysis of Blood**

Whole blood was used for the analysis of red blood cells (RBC), haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet (PLT), mean platelet volume (MPV), red blood cell distribution width (RDW), platelet distribution width (PDW) and the ratio of platelets-large cell ratio (P-LCR). All these parameters were evaluated by an Autoanalyser (Sysmex, KX-21, Japan).

**Statistical analysis**

The values obtained during the present study are expressed as mean ±SEM (Standard error mean). Student’s t-test was applied for comparing the means using SPSS 16.0 version for windows software. The level of significance was considered at p<0.05.

**Results**

The haematological parameters act as a valuable tool for monitoring the fish health. Therefore, in the present study a comparative analysis of changes in normal and *C. complanatum* infected fish blood, (*T. fasciatus*) has been done. The values of RBC count, haemoglobin concentration, HCT, MCV, MCH, MCHC, PLT, MPV, RDW, PDW and P-LCR are summarized in Table 1.

a) **RBC count:** The RBC count showed significant reduction in infected fish blood as compared to the non-infected blood.

b) **Haemoglobin concentration:** Similarly, haemoglobin concentration showed significant reduction in infected fish blood (7.0±0.08 g/dL) as compared to non-infected blood (8.66±0.21 g/dL).
c) **Haematocrit (HCT):** The mean value of HCT in healthy fish was recorded as 23.36±0.14% which dropped down to 20.83±0.06% in infected *T. fasciatus*.

d) **Mean corpuscular volume (MCV):** The mean value of MCV in non-infected fish was 103.3±0.25 fL which decreased to 96.76±0.08 fL (1.07 folds) in blood of infected *T. fasciatus*.

e) **Mean corpuscular haemoglobin (MCH):** The MCH value showed non-significant reduction in infected *T. fasciatus* as compared to healthy fish.

f) **Mean corpuscular haemoglobin conc. (MCHC):** The mean corpuscular haemoglobin level was found to be raised by 4.7% in infected blood, as compared to non-infected fish blood.

g) **Thrombocytes (PLT):** The level of PLT in non-infected fish was observed to be 90.33±0.88 x10^3/μL which decreased to 77.66±0.88 x10^3/μL in infected fish (14.1% decrease).

h) **Red cell distribution width (RDW):** Similarly, RDW value significantly decreased from mean value of 75.1±0.03 fL (healthy fish) to 54.2±0.05 fL in infected fish (27.9% reduction).

i) **Platelet distribution width (PDW):** Platelet distribution width dropped down in infected fish, showing 3.2 % insignificant decrease in its level as compared to the control fish.

j) **Mean platelet volume (MPV):** The level of mean platelet volume showed 16.1% reduction in infected fish when compared with non-infected *T. fasciatus*.

k) **P-LCR (Platelets-large cell ratio):** The P-LCR was found to be reduced to 46.4% in infected fish as compared to the non-infected fish.

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**Discussion**

Haematological or blood variables are important in understanding the impact of disease on an individual as well as on a population to assess the health of individual animals. The blood variables are frequently used as diagnostic technique to establish the health status of the fish exposed to various pollutants in water bodies. Fishes are also known to harbour a number of helminth parasites which in turn affect their health. Therefore, the evaluation of haematological characteristics serves as an important tool, which can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes (Kori-Siakpere et al. 2005).

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**Table 1:** The Level of hematological parameters in non-infected and infected *Trichogaster fasciatus* fish

<table>
<thead>
<tr>
<th>Blood Parameters</th>
<th>Units</th>
<th>Non-infected</th>
<th>Infected</th>
<th>Percent change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Blood Cells (RBC)</td>
<td>x 10^6μL⁻¹</td>
<td>3.72±0.23</td>
<td>2.14±0.008*</td>
<td>-42.5%</td>
</tr>
<tr>
<td>Haemoglobin (Hb)</td>
<td>g dL⁻¹</td>
<td>8.66±0.21</td>
<td>7.0±0.08*</td>
<td>-19.17%</td>
</tr>
<tr>
<td>Haematocrit (HCT)</td>
<td>%</td>
<td>23.36±0.14</td>
<td>20.83±0.06*</td>
<td>-10.84%</td>
</tr>
<tr>
<td>Mean Corpuscular Volume (MCV)</td>
<td>fL</td>
<td>103.3±0.25</td>
<td>96.76±0.08*</td>
<td>-6.4%</td>
</tr>
<tr>
<td>Mean Corpuscular Haemoglobin (MCH)</td>
<td>pg</td>
<td>33.2±0.06</td>
<td>32.8±0.05</td>
<td>-2.1%</td>
</tr>
<tr>
<td>Mean Corpuscular Haemoglobin Conc. (MCHC)</td>
<td>g dL⁻¹</td>
<td>32.46±0.08</td>
<td>34.06±0.06*</td>
<td>+4.7%</td>
</tr>
<tr>
<td>Platelet Count (PLT)</td>
<td>x 10^3μL⁻¹</td>
<td>90.33±0.88</td>
<td>77.66±0.88*</td>
<td>-14.1%</td>
</tr>
<tr>
<td>Red Cell Distribution Width (RDW)</td>
<td>fL</td>
<td>75.1±0.033</td>
<td>54.2±0.05*</td>
<td>-27.9%</td>
</tr>
<tr>
<td>Platelet Distribution Width (PDW)</td>
<td>fL</td>
<td>10.8±0.057</td>
<td>10.46±0.03</td>
<td>-3.2%</td>
</tr>
<tr>
<td>Mean Platelet Volume (MPV)</td>
<td>fL</td>
<td>8.7±0.057</td>
<td>7.3±0.05*</td>
<td>-16.1%</td>
</tr>
<tr>
<td>Platelets-Large Cell Ratio (P-LCR)</td>
<td>%</td>
<td>23.3±0.25</td>
<td>12.5±0.20*</td>
<td>-46.4%</td>
</tr>
</tbody>
</table>

(-) indicates decrease, (+) indicates increase, values marked with * are significant at p≤0.05
In order to investigate the extent of damage due to *Clinostomum complanatum* infection in the blood of its second intermediate host, *Trichogaster fasciatus* fish, some haematological parameters were considered during the present study. We found that the values of RBC count, Hb concentration, HCT, MCV, MCHC, PLT, MPV, RDW and P-LCR of infected fish were significantly different from non-infected fish whereas MCH and PDW showed insignificant differences. The level of RBC count, Hb content and PCV has been reported to be reduced in catfish infected with helminth parasites (Martins et al. 2004). Study by Kaur et al. (2012), also recorded decrease in the amount of RBC and haemoglobin concentration. Further, reduced level of RBC and Hb content in *S. niger, S. labiatus, C. carpiocommunis* and in *C. carpiospecularis* has been reported due to either single parasite species or with mixed infection of cestode, trematode and acanthocephalans (Khurshid and Ahmad 2012). The data pertaining to the blood parameters is scarce in case of trematode infection in general and there is no report on haematological studies in *C. complanatum* infected *T. fasciatus* fish. Therefore, the parameters analyzed in the current haematological study are used for the first time in *T. fasciatus* infected with *C. complanatum* metacercariae. It can be suggested that the reduced levels of Hb, RBC, HCT, MCV, MCH, PLT, RDW, PDW, and P-LCR in case of infected fish blood in our study may be due to some harmful secretions released by the metacercariae which might be interfering with these parameters and thus reducing the level. Further, the lower value of HCT in infected fish blood might be a consequence of accelerated lysis of erythrocyte due to parasitic stress. The blood HCT drop was also reported in fish infected with *Trypanosoma murmanensis* (Khan 1987), *Myxobolus artus* (Yokoyama et al. 1996) and *Rhipidocotyle fennica* infection (Jeney et al. 2002). Zaragoza et al. (2011) observed higher levels of HCT, WBC and eosinophils in *Lutjanus guttatus* fish infected with dactylogyrid monogenean parasite.

Chronic parasitism can cause anaemia as a result of blood loss or impaired erythropoiesis which can be produced by a variety of reasons including mineral and specific amino acid deficiency from disturbed protein metabolism and toxic marrow (Jain 1986). The decreased amount of RBC, Hb, PCV was recorded in the blood of *Gallus gallus domesticus* infected with the cestode, *Cotugnia digonopora* (Bhure et al. 2011) is in agreement with the present study, but MCH and MCV levels were higher in case of infected host which is not in accordance with our results. The decreased count of RBC, Hb, and PCV in the blood of *Carassius carassius* and *Schizothorax curvifrons* was reported due to the infection by *Trypanosoma* (Shahi et al. 2013) which is in accordance with our findings. The parasite can simply act as a stressor and during primary stages of stress, the PCV is altered due to the release of catecholamines that can mobilize RBCs from spleen (Wells and Weber 1990) or induce the RBCs to swell as a result of fluid entry into the intracellular compartment (Chiocchia and Motaïs 1989). Therefore, in the light of above information and on the basis of present findings it could be suggested that the level of infection can elicit a stress response in the host, which is seen in terms of these important haematological parameters.

**Conclusion**

To the best of our knowledge, this is the first attempt to investigate the effect of excysted metacercariae infection on haematological parameters of *T. fasciatus*. From the present findings, it can be concluded that the parasite infestation cause variable degree of physiological adversities in the host fish and the resulting effect can be evaluated in the blood components of the infected fish. The assessment of these blood parameters has always been helpful in detecting infection and related diseases. Similar kind of approaches could also be employed for the assessment of parasitic infections in the fish fauna. This in turn would improve the health of
fishes and a parasite free aquaculture system could be established for sustainable development.

Conflict of interest

The authors declare that there is no conflict of interest.

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