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HEMATOLOGICAL ASSESSMENT OF PARASITIZED *CHILOSCYLLIUM PLAGIOSUM* FROM THE RAIGAD COAST, MAHARASHTRA, INDIA

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Abstract:

Parasitic infections are known to induce significant physiological and hematological alterations in marine fishes, thereby affecting their health and survival. The present study investigated the effects of cestode parasites, Phyllobothrium vatsalabaiae n. sp. and Nybelinia elongata Shah et al., 1979, on the hematological parameters of Chiloscyllium plagiosum collected from the Raigad Coast, Maharashtra, India. Hematological analysis was carried out by comparing infected and uninfected fish specimens. The results revealed a significant reduction in red blood cell (RBC) count, hemoglobin (Hb) concentration, packed cell volume (PCV), and mean corpuscular hemoglobin (MCH) in infected fish, indicating the development of anemia and impaired oxygen-carrying capacity. In contrast, infected fish exhibited a significant increase in white blood cell (WBC) count and mean corpuscular volume (MCV), suggesting an active immune response to parasitic infection and alterations in erythrocyte morphology. These hematological changes demonstrate that cestode infections adversely affect the physiological condition of C. plagiosum and may serve as reliable indicators of fish health and parasitic stress. The findings highlight the importance of hematological assessment as a diagnostic tool for evaluating the impact of parasitic infections in marine fishes and contribute to understanding host–parasite interactions in coastal ecosystems.

Keywords: *Chiloscyllium plagiosum, Hematology, Cestode Parasites, Phyllobothrium vatsalabaiae, Nybelinia Elongata, Marine Fish.*

Introduction

Haematological studies serve as a valuable tool for assessing the disturbances and diseased conditions caused in fishes. A number of papers in basic haematology include data on elasmobranchs, most notably Kisch (1), Larsson *et al.* (2), and Saunders (3, 4); however, few include haemoglobin and haematocrit data or have attempted to differentiate types of erythrocytes and leucocytes. Parasitized fishes may present significant changes in their haematological and physiological characteristics (5). These parasites affect their development (6, 7). In the United States alone, parasitic diseases of farmed fish caused an economic loss estimated at $11.5 million in 1989 (8). The damage caused to the host depends on the parasite species, the type of injury in the host tissue, the number of parasites, and the health status of the host (9). This work, therefore, aimed at investigating the impact of parasites on the haematological parameters of selected *Chiloscyllium plagiosum* species from the Raigad coastal region of Maharashtra.

Material and Methods

Fish sampling sites

From March 2023 to June 2024, the marine fish *Chiloscyllium plagiosum* (Anonymous Bennett, 1830) were collected, dissected, and investigated for cestode parasites and haematological studies. The sampled fish had a body weight of 8.60 ± 0.15 g and a length of 67 ± 0.14 cm, and were collected from the Alibag Coast, Dist. Raigad (M.S.), India.

After the preliminary measurement of length and weight, the fish was placed belly upward, and blood samples were obtained from the caudal circulation with the aid of a heparinised 2 cm^3 disposable plastic syringe and a 21-gauge disposable hypodermic needle. The use of a plastic syringe is a necessary precaution with fish blood because contact with glass results in a decreased coagulation time (10). The site chosen for puncture was wiped dry with tissue paper to avoid contamination with mucus. The needle was inserted at a right angle to the vertebral column of the fish and was gently aspirated during penetration. It was then pushed gently down until blood started to enter as the needle punctured a caudal blood vessel.

For haematological investigations, blood samples were collected from all fish hosts in glass tubes containing EDTA and were properly labelled for the estimation of haematological parameters.

Primary and secondary blood indices were determined as per the methods described by Houston (11). The total red blood cell count (TRBCC) was performed manually on an Improved Neubauer haemocytometer using Hayem's fluid as a diluent (12). The total leukocyte count was estimated by the standard dilution technique using a diluting fluid consisting of 4% glacial acetic acid and two drops of gelatine (13).

The haemoglobin concentration was estimated by the Sahli method (14). The haemoglobin concentration was converted to acid haematin by the action of 0.1 N HCl using a 0.02 mL pipette. 20 mL of 0.1 N HCl and 0.02 mL of the blood sample were used to fill the graduated tube. The mixture was allowed to stand for 5 minutes before introducing a few drops of distilled water until the colour matched the standard.

The determination of packed cell volume was done using the method described by Wintrobe (15). The 1 mL volume haematocrit tube, sealed at one end, was filled with fish blood and set in a centrifuge for 30 minutes at 4000 rpm.

Erythrocyte values, such as Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC), were calculated as:

$$\mathrm{MCV} = \frac{\mathrm{PCV} \times 1000}{\mathrm{RBC\ count}}$$

$$\mathrm{MCH} = \frac{\mathrm{Hb\ Value}}{\mathrm{RBC\ count}} \quad \text{(Expressed in picograms)}$$

$$\mathrm{MCHC} = \frac{\mathrm{Hb\ value}}{\mathrm{PCV} \times 1000}$$

Differential leucocyte count was done by preparing a blood smear and staining it with "Leishman stain blue" by the Mender method.

Results

Table 1: Mean haematological parameters of *Chiloscyllium plagiosum* infected with *Phyllobothrium vatsalabaiae* n.sp.

Haematological Parameters	Uninfected Fish	Infected Fish
Total erythrocyte count – RBC (x 10/ mm3)	3.18 ± 0.08	2.9 ± 0.187**
Total leucocyte count – WBC (x 104/ mm)	2.16 ± 0.31	3.15 ± 0.01*
Haemoglobin content – Hb (g %)	9.14 ± 0.41	6.32 ± 0.20**
Packed cell volume – Ht (%)	27.8 ± 0.83	22.4 ± 2.30*
Erythrocyte Constant		
Mean Corpuscular Volume – M.C.V (μ3)	85.20. ±.1.20	104.69.±. 2.10*
Mean corpuscular Haemoglobin M.C.H(μg)	28.73 ± 0.98	21.85 ±1.35**
Mean Corpuscular Haemoglobin Concentration – M.C.H.C (%)	32.88 ± 1.50	29.01 ± 2.53*
Differential leucocyte Count (DLC)		
Lymphocyte %	50.4 ± 7.56	27.4 ± 4.39**
Neutrophil %	49.6 ± 7.56	21.4 ± 2.07*
Basophil %	6.38 ± 0.31	17.3 ± 1.208NS
Monocyte %	5.72 ± 0.16	9.71 ± 0.16 NS
Eosinophil %	3.41 ± 0.11	2.36 ± 0.06*

Significant difference between infected and uninfected fish

NS= non-significant * = significant, = highly significant, ***very highly significant

The haematological results obtained in *Chiloscyllium plagiosum* infected with *Phyllobothrium vatsalabaiae* n.sp. show either an increase or a decrease in infected fish compared to uninfected (healthy) fish.

As shown in Table 1, haematological investigations carried out by taking blood samples from uninfected and infected *Chiloscyllium plagiosum* recorded a significant decline in TEC, Hb%, and PCV. TEC was lower in infected fish compared to normal fish. It was verified that the TEC count [uninfected (3.18 ± 0.08), infected (2.9 ± 0.18)], Haemoglobin percent [uninfected (9.14 ± 0.41), infected (6.32 ± 0.20)], and Packed cell volume percent [uninfected 27.8 ± 0.83, infected (22.4 ± 2.30)] were significantly decreased ($P < 0.05$) compared to those observed in uninfected fish. Conversely, the number of total leucocytes [uninfected (2.16 ± 0.13), infected (3.15 ± 0.01)] and MCV [uninfected (85.20 ± 1.10), infected (104.69 ± 2.10)] showed changes, while MCH [uninfected (28.73 ± 0.98), infected (21.85 ± 1.35)] and MCHC [uninfected (32.88 ± 1.50), infected (29.01 ± 2.53)] significantly decreased compared to healthy controls.

The percentage of differential leucocyte cell counts showed an increase ($P < 0.05$), particularly in lymphocytes [uninfected (50.4 ± 7.56), infected (27.4 ± 4.39)], basophils [uninfected (6.38 ± 0.31), infected (17.3 ± 1.20)], and monocytes [uninfected (5.72 ± 0.16), infected (9.71 ± 0.16)]. A significant decrease was observed in neutrophils [uninfected (49.6 ± 7.56), infected (21.4 ± 2.07)] and eosinophils [uninfected (3.41 ± 0.11), infected (2.36 ± 0.06)] in infected fish relative to uninfected fish.

Table 2: Mean haematological parameters of *Chiloscyllium plagiosum* infected with *Nybelinia elongata* Shah *et al.* (1979).

Haematological Parameters	Uninfected Fish	Infected Fish
Total erythrocyte count - RBC (x 10/ mm3)	3.16 ± 0.01	2.4 ± 0.18**
Total leucocyte count – WBC (x 104/ mm)	2.31 ± 0.04	4.62 ± 0.59*
Haemoglobin content – Hb (g %)	9.22 ± 0.2	6.14 ± 0.01**
Packed cell volume – Ht (%)	26.08 ± 0.01	23.6 ± 0.08*
Erythrocyte Constant		
Mean Corpuscular Volume – M.C.V (μ3)	82.53 ± 0.02	98.77± 0.03*
Mean corpuscular Haemoglobin M.C.H(μg)	27.79 ± 0.04	25.85 ±0.04**
Mean Corpuscular Haemoglobin Concentration – M.C.H.C (%)	30.24 ± 0.04	23.31 ± 0.73*
Differential leucocyte Count (DLC)		
Lymphocyte %	47.87 ± 4.77	25.56 ± 0.97**
Neutrophil %	47.41 ± 0.89	21. ± 1.13 *
Basophil %	5.2 ± 0.61	15.56 ± 0.71NS
Monocyte %	5.3 ± 0.43	9.25 ± 0.31 NS
Eosinophil	3.39 ± 0.42	2.63 ± 0.36

Significant difference between infected and uninfected fish

NS= non-significant * = significant, = highly significant, ***very highly significant

The haematological results obtained in *Chiloscyllium plagiosum* infected with *Nybelinia elongata* Shah *et al.,* 1979 show either an increase or a decrease in infected fish compared to uninfected (healthy) fish.

As shown in Table 2, haematological investigations carried out by taking blood samples from uninfected and infected *Chiloscyllium plagiosum* recorded a significant decline in TEC, Hb%, and PCV. TEC was lower in infected fish compared to uninfected fish. It was verified that the TEC count [uninfected (3.16 ± 0.01), infected (2.4 ± 0.18)], Haemoglobin percent [uninfected (9.22 ± 0.2), infected (6.14 ± 0.01)], and Packed cell volume percent [uninfected 26.08 ± 0.01, infected (23.6 ± 0.08)] were significantly decreased ($P < 0.05$) compared to those observed in uninfected fish. Conversely, the number of total leucocytes [uninfected (2.31 ± 0.04) and infected (4.62 ± 0.59)], MCV [uninfected (82.53 ± 0.02), infected (98.77 ± 0.03)], MCH [uninfected (27.79 ± 0.04), infected (25.85 ± 0.04)], and MCHC [uninfected (30.24 ± 0.04), infected (23.31 ± 0.73)] were altered significantly compared to healthy uninfected fish.

The percentage of differential leucocyte cell counts showed an increase ($P < 0.05$), particularly in lymphocytes [uninfected (47.87 ± 4.77), infected (25.56 ± 0.97)], basophils [uninfected (5.2 ± 0.61), infected (15.56 ± 0.71)], and monocytes [uninfected (5.3 ± 0.43), infected (9.25 ± 0.31)]. A significant decrease was observed in neutrophils [uninfected (47.41 ± 0.89), infected (21.0 ± 1.13)] and eosinophils [uninfected (3.39 ± 0.42), infected (2.63 ± 0.36)] in infected fish in relation to uninfected fish.

Discussion

A major part of the world's food is supplied from fish sources, making it essential to secure the health of fishes (16). Haematological parameters reflect the health status of fish (17) and are highly affected by abiotic and biotic factors (18). For practical diagnostic purposes, different blood parameters are meaningful to compare alterations in the fundamental characteristics of symptomatic fish to those of asymptomatic fish.

The present study of the marine fish *Chiloscyllium plagiosum* parasitized with *Phyllobothrium vatsalabaiae* n. sp. and *Nybelinia elongata* Shah *et al.,* 1979 revealed a significant reduction ($P < 0.05$) in RBC count, haemoglobin concentration, and packed cell volume. Similar findings have been reported by Radhakrishnan *et al.* (19), who observed a significant decrease in RBC count, haemoglobin content, and haematocrit in *Saurida tumbil* (Bloch) infected with *P. ganapatii*. Engelhardt *et al.* (20) observed a decrease in RBCs in rainbow trout infected with *Proteocephalus neglectus*. A reduction in haemoglobin concentration under infected conditions has also been observed by Yoshinaga *et al.* (21) in Japanese flounder infected with *Neoheterobothrium hirame*. Shah *et al.* (22) also found a reduction in RBC count and haemoglobin percentage in *Cyprinus* infections with *Bothriocephalus*. Blanner *et al.* (23) confirmed that PCV was significantly lower in Arctic Charr (*Salvelinus alpinus*) infected by larval *D. dendriticum*. PCV can serve as a measure of anemia and oxygen-carrying capacity, acting as an indicator of physiological condition (24). These findings corroborate data reported by Sopinska (25) for carp parasitized by cestodes.

Similar findings conform to those of Sinha and Sircar (26), Sinha (27), and Satpute and Agrawal (28) in *C. batrachus* due to helminth infection. A decrease in RBC count was also observed in *Rita rita* infected with trematodes (29). In fishes where there is a reduction below the normal range of erythrocyte numbers, there is a corresponding reduction in haemoglobin values per cell (30).

When the worm burden is high, there is increased blood loss caused by hemorrhage and consumption by the worms, leading to an overall low Hb%. This can affect the productivity of the fish through mortality, decreased growth rates, reduced meat quality, and increased susceptibility to other pathogenic parasites and bacteria.

The present study has a negative correlation with the results of Mlay *et al.* (31), where no significant difference ($P > 0.05$) in Hb percentage was found in Catfish and Tilapia infected with *Anisakis* spp. It also shares a negative correlation with the results of Martins *et al.* (32), who found no significant difference ($P > 0.05$) in erythrocytes and haemoglobin in *Leporinus macrocephalus* infected with *Goezia leporini*, though their results were associated with haematocrit reduction ($P < 0.05$).

On the other hand, haematocrit values provide an indication of the hemopoetic activity of the animal. Abnormal haematocrit can indicate nutritional deficiencies, the presence of disease-causing microorganisms, and other health aberrations (33, 34). Therefore, haematology may provide important information on the general physiology and health status of organisms living under environmental stress.

In the present study, leucocytes were monitored in the marine fish *Chiloscyllium plagiosum* infected with the cestode parasites *Phyllobothrium vatsalabaiae* n.sp. and *Nybelinia elongata* Shah *et al.,* 1979. Leucocytes showed a significant increase ($P < 0.05$) in infected fish compared to normal fish. This matches the findings reported by Radhakrishnan *et al.* (19), who recorded a highly significant increase in leucocytes in *Saurida tumbil* infected with *Penetrocephalus ganapatii*.

This result was also similar to Shah *et al.* (35), who reported a significant increase in leucocyte count ($P < 0.05$). The cell numbers under infection conditions are in total agreement with Denisov (36), who observed an increase in TLC by 44% in silver carp infected with *Posthodiplostomum cuticola*. Similarly, Saxena and Chauhan (37) found a TLC increase of 2.77% in *Heteropneustes fossilis* infected with *Lucknowia indica*. This is consistent with the findings of Sinha (27), who recorded a highly significant increase in leucocyte count (21%) in *Clarias batrachus* infected with *Lytocestus*. However, the present study has a negative correlation with the results of Akmirza (38), where no significant difference ($P > 0.05$) was found in the leucocyte count of *Rutilus rutilus* infected with *Cryptobia tincae*.

Even though MCV is higher in heavy infections than in uninfected fish, the observed increase is not always statistically significant, while MCH and MCHC show some changes that significantly decrease in heavy infections.

Regarding the differential count of WBCs, the results show that there is a progressive reduction in the percentage of lymphocytes with increasing severity of infection, concurrent with a progressive increase in the percentage of neutrophils, basophils, and monocytes, while eosinophils decrease in heavy infections.

Conclusion

The complete study reveals that the intensity of cestode parasite infections is responsible for haematological changes in the fish host. The mechanical damage caused by cestodes to the host intestine could cause vitamin B12 and folic acid deficiencies, which are otherwise responsible for RBC maturation. Furthermore, host health status influences haematological parameters in fish, and the extent of worm infection observed in this study had a significant impact on the levels of erythrocytes, haemoglobin, and packed cell volume.

The present investigation concludes that infections with the cestode parasite *Nybelinia elongata* show more distinct fluctuations in different haematological parameters compared to *Phyllobothrium vatsalabaiae* n.sp. within the host *Chiloscyllium plagiosum*.

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