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Hematological Studies Of Chiloscyllium Plagiosum With Reference To Helminthic Infection

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Abstract
Fish blood parameters can be used as efficient and sensitive indicators to detect the health of the fish. A hematological investigation has been carried out on marine water fish *Chiloscyllium plagiosum* infected with cestode parasite *Phyllobothrium vatsalabaiae n.sp.* Inspite of the fact that the hematological values in fish are clinical significance rather than commercial importance. Red blood parameters include: erythrocyte count (RBC), White blood parameters include Leukocyte count (WBC),Hemoglobin concentration(Hb%), Packed cell volume(PCV), Mean corpuscular hemoglobin (MCH) & Mean corpuscular volume (MCV) were examined and found to be highly significant difference in their parameter of infected fish in comparison to uninfected fish.

Key words: Heamatology, Marine Fish , Helminthic infection

Introduction

Haematological studies of vertebrate groups other than mammals and birds are limited and provided limited haematological data. A number of papers in basic haematology include data on elasmobranches most notably Kish (1951) Larsson *et al*., (1976) and Sunnders (1966 a, b) few includes haemoglobin and haematocrit data or has attempted to differentiate types of erythrocyte and leucocytes.

Parasitized fishes may present significant changes in their haematological and physiological characteristics (Ruane *et al*., 2002). Affecting their development (Singhal *et al.,* 1990; Ranzani., Paiva and Silva Souza, 2004 a,b) in the united states alone parasitic diseases of farmed fish caused in 1989 an economic loss estimated at US $ and 11.5 million (Klesivs and Rogers1995); damage caused on the host depends on parasite species, type of injury in the host tissue, number of parasites and the health status of the host (Tavares –Dias *et al.,*1999 a).

In Asian countries too haematological investigators on freshwater fishes in relation to helminthic infection include Satpute and Agrawal, (1974) and Sinha and Sircar (1974) on *Clarias batrachus* with *Lytocestus indicus* infection. Dubey (1980) and Sinha (1988) on *H. fossilis* with *Procamallanus* infections. Sinha, (2000) worked on haematology of *Clarias batrachus* infected with helminth parasites. Recently Wahid Shah, (2008) showed the impact of helminth parasitism on haematology of fish from Anchar Lake, Kashmir.

However, it is strange that comparable studies on type on the effects of helminth parasites, especially tapeworms and nematodes, in relation to haematological abnormalities so far made in the tropics are only few, whereas much has been written about the '*Bothriocephalus* anemia' due to the tapeworm *Diphyllobothrium latum* in man (Birkeland, 1932; Wardle *et al*., 1937; Totterman, 1944; Bondsorff, 1948; 1956; Nyberg, 1958, 1960; Brante and Emberg, 1958; Scudamore *et al*.,1961 and others).

Material and Methods : Fish sampling sites

From the month June 2018 to May 2019, the marine fish *Chiloscyllium plagiosum* (Anonymous Bennett, 1830) were collected, dissected & investigated for cestode parasites and haematological studies having the body weight 8.60 ± 0.15 gm, length 67 ± 0.14 cm. from Alibag Coast, Dist. Raigad (M.S.) India.

After the preliminary investigation of the length and weight, the fish was then

placed belly upward and blood samples obtained from the caudal circulation with the aid of a heparinised 2cm^3 disposable plastic syringe and a 21-gauge disposable hypodermic needle. The use of plastic syringe is a necessary precaution with fish blood because contact with glass result in decrease coagulation time (Smith *et al*., 1952). The site chosen for puncture was wiped dry with tissue paper to aid contamination with mucus. The needle was inserted at 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 labeled, for estimation of haematological investigation.

Primary and secondary blood indices were determined as per methods described by Houston, (1990). The total red blood cell count (TRBCC) was performed manually on an Improved Neubauer haemocytometer using Hayem's fluid as diluents (Benjamen, 1985), total leukocyte count was estimated by the standard dilution technique using diluting fluid (4% glacial acetic acid and two drops of gelatin) (Talib and Khurana, 1995).

The haemoglobin concentration is estimated by Sahli method (Sahli, 1962). The haemoglobin concentration was converted to acid haematin by the action of 0.1 N HCl using 0.02 ml pipette. 20 ml of 0.1N HCl and 0.02 ml of blood sample were used to fill the graduated tube. The mixture was allowed to stand for 5 min before introducing few drops of distilled water until colour match the standard. The determination of packed cell volume was done using method described by Wintrobe (1934). The 1 ml volume haematocrit tube sealed at one end was filled with fish blood and set in centrifuge for 30 min at 4000 rpm. Erythrocyte values such as Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) Mean Corpuscular Haemoglobin Concentration (MCHC), and were calculated as:

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

$$MCH = \frac{Hb\ Value}{RBC\ count}$$ Express in Picogramm's

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

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

Result & Discussion

Table I: Mean haematological parameters of *Chiloscyllium Plagiosum* (Anonymous Bennett, 1830) infected with *Phyllobothrium vatsalabaiae n.sp*

Haematological parameters		**Uninfected fish**	**Infected fish**
Total erythrocyte count - RBC (x 10/ mm^3)		3.18 ± 0.08	2.9 ± 0.187**
Total leucocyte count – WBC (x 10^4/ 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 (µ^3)	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 %	5.2 ± 0.61	17.3 ± 1.208 NS
	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

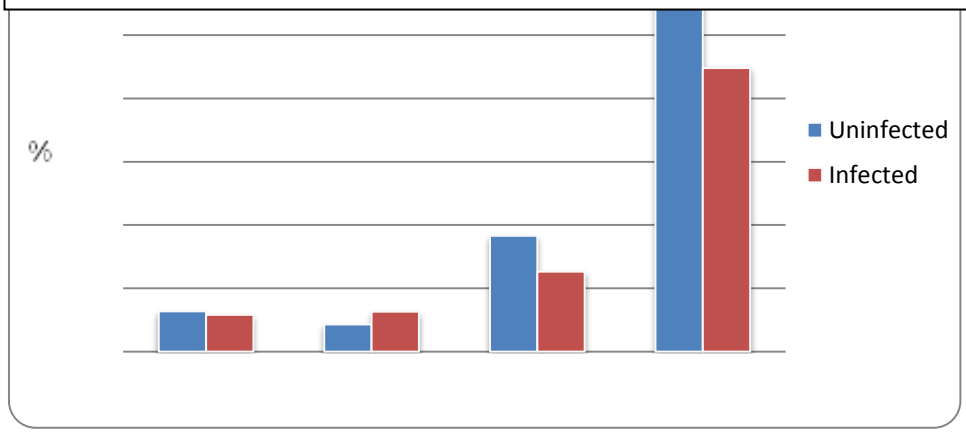
NS= non-significant * = significant, ** = highly significant, ***very highly significant

The above Haematological result obtained in Chiloscyllium plagiosum (Anonymous Bennett, 1830), infected with *Phyllobothrium vatsalabaiae n.sp.* show either increase or decrease in the infected as compare to the uninfected (healthy fish).

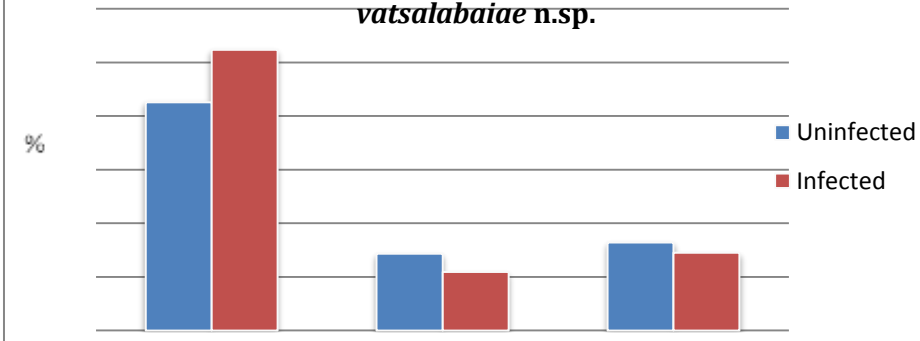
Table no. 1 Haematological investigations have also been carried out by take blood samples from uninfected and infected Chiloscyllium plagiosum and significant decline in the TEC, HB% and PCV was recorded TEC was lower in the infected fish ie. Chiloscyllium plagiosum as compared to normal fish It was verified that TEC count [uninfected (3.18 ± 0.08) infected (2.9 ± 0.18)], Haemoglobin percent [uninfected (9.14 ± 0. 41), infected (6.32 ± 0. 20)], Packed cell volume percent [uninfected 27. 8 ± 0. 83, infected (22.4 ± 2.30)], where the number of total leucocytes was [uninfected (2.16 ± 0.13), and infected (3.15 ± 0.01)], MCV [uninfected (85. 20 ± 1.10), infected (104.69 ± 2.10)], MCH [uninfected (28. 73 ± 0.98), infected (21.85 ± 1.35)], and MCHC [uninfected (32.88 ± 1.50)., infected (29.01 ± 2.53)] were significantly decreased ($P < 0.05$), then those observed in the uninfected fish.

The percentage of differential leucocyte cell count showed an increase ($P < 0.05$), particularly in lymphocyte [uninfected (50. 4 ± 7.56), infected (27. 4 ± 4.39)], basophil [uninfected (6.38 ± 0.31), infected (17.3 ± 1.20)], monocyte [uninfected (5.72 ± 0.16), infected (9.71 ± 0.16)], and significant decrease in neutrophil [uninfected (49.6 ± 7.56), infected (21. 4 ± 2. 07)], and eosinophil [uninfected (3.41 ± 0.11), infected (2.36 ± 0.06)]; in infected fish, in relation to that observed in uninfected fish.

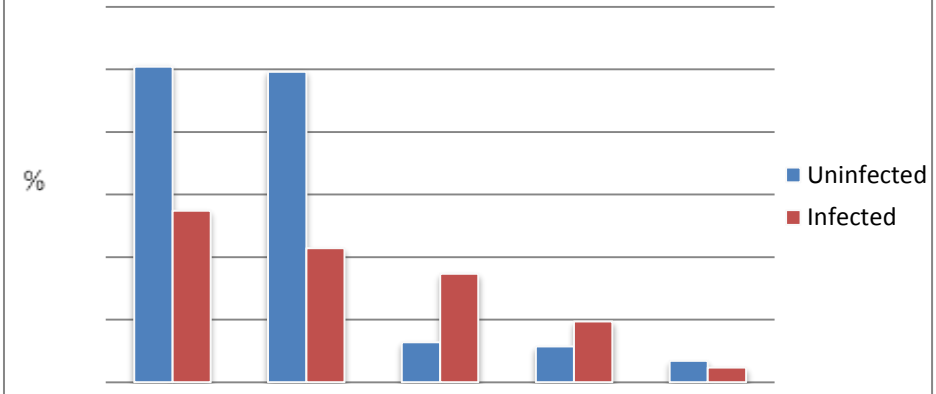
Graph No-1. Showing RBC, WBC, HB, PCV in *Chiloscyllium plagiosium* infected with *Phyllobothrium vatsalabaiae* n.sp



Graph No-2 . Showing MCV, MCH, MCHC in *Chiloscyllium plagiosium* infected with *Phyllobothrium vatsalabaiae* n.sp.



Graph No-3 Showing DLC % in *Chiloscyllium plagiosum* infected with *Phyllobothrium vatsalabaiae* n.sp.



Discussion

A major part of the world's food is being supplied from fish source, so it is essential to secure the health of fishes (Tripathi et al., 2002). Haematological parameters reflect the health status of fish (Conroy, 1972) and are highly affected by abiotic and biotic factors (Iwama et al., 1986) for practical diagnostic purpose the different blood parameters are meaningful to compare the alterations in fundamental characteristics of symptomatic to that of asymptomatic fish.

Additionally, these changes in blood parameters depend on several factors, such as species, temperature, age, light, nutrition, sexual maturity, cyclical changes in health, water quality, dissolved oxygen, gender, static water slopes and handling, stress, transportation, size, feeding and breeding density as well as microbial infections and parasitic infections (Ranzani-Paiva et al., 2003; Tavares-Dias and Moraes, 2004; Val et al., 2016; Fazio, 2019).

The present study of marine fish Chiloscyllium plagiosum Anonymous (Bennett,1830); parasitized with *Phyllobothrium vatsalabaiae n. sp*., had a significant reduction ($P < 0.05$) in RBC count haemoglobin concentration and packed cell volume (Table no.1) similar finding has been reported by S. Radhakrishanan et al., (1983) were they observed significant decrease in RBC count, haemoglobin content and haematocrit in Saurida tumbil (Bloch) infected with P. ganpatii. In 1989 Engelherdt et al., observed decrease in Rainbow trout infected with Proteocephalus neglectus, the RBC, reduction in haemoglobin concentration under infected condition has been observed by Yoshinaga et al., (2001) in Japanese flounder infect with Neohetrobothrium hirame. Abdul Wahid Shah et al., (2008); also found reduction in RBC count & haemoglobin percentage in Cyprinus infection with Bothriocephalus. Blanner et al., (2005) confirmed PCV was significantly lower in Arctic Charr (Salvelinus Alpinus) fish infected by larval D. dentriticum. PCV can serve, as a measure of anemia and oxygen carrying capacity and thus an indicator of physiologic condition (Honston 1997). It is corroborated data reported by Sopinska (1985); for carp parasitized by cestodes. Similar findings also confirm to that of Sinha and Sircar, Sinha (2000), Satpute and Agrawal (1974), in C. batrachus due to helminth infection. The decrease in RBC count was also observed in Rita rita infected with trematode (Agarwal, 1989); In fishes where there is reduction below the normal range of value of the erythrocyte numbers there is crosspondent reduction in haemoglobin values per cell (Larsson et al., 1984);

When the worm number is high there is increased blood loss caused by hemorrhage and consumption by worms. Leading to an overall low Hb %. Thus, can affect the productivity of the fish through mortalities, by decreasing growth rate, reducing the quality of the meat and making the hosts more susceptible to pathogenic parasites and bacteria.

The present study has a negative co-relation with result of Mlay et al., (2007) where there was no difference ($P>0.05$) in Hb percentage in Anisakis spp infected with Catfish and Tilapia. It is also similar negative co-relation with result of Martin et al., (2004) where there was no difference ($P>0.05$) in erythrocyte and haemoglobin in Leporinus macrocephalus infected with Goeria leporine, but this result was found associated with haematocrit reduction ($P>0.05$).

On the other hand, the haematocrit gives an indication of the hemopoietic activity of the animal abnormal haematocrit also can indicate nutritional deficiencies, the presence of disease-causing microorganism and other health aberrations (Blaxhall, 1972 and Anderson, 1990); therefore, haematology may provide important information on the general physiology and health status of the organism living under environmental stress.

The present study leucocyte was noted in the marine fish i.e Chiloscyllium plagiosum Anonymous (Bennett, 1830) infected with cestode 198 parasite *Phyllobothrium vatsalabaiae n.sp*. (table no.1), the leucocyte had a significant increase ($P < 0.05$) in infected fish as a compare to the normal fish. It is similar finding reported by Radhakrishnan et al., (1983); were recorded high significant increase in leucocyte in Saurida tumbil infected with Penetrocephalus ganapatii.

This result was similar to Abdul Wahid Shah et al., (2009); there was significant increase in leucocyte count ($P< 0.05$). The numbers under infections conditions are in total agreement with Denisov, (1979); who observed in increase of TLC by 44% in silver carp infection with Posthodisplostomum cuticola. It is also similar result showed that saxena and Chauhan, (1993) found TLC increase by 2.77 % in Hetrophentus fossil infected Lucknewia indica. This is similar findings to Sinha (2000) were he recorded high significant increase in leucocyte count (21 %) in Clarius batrachus infected with Lytocestus. Present study has a negative corelation with result of A. Akmirza et al., (2007) where there was no difference ($P<0.05$), in leucocyte count in *Rutilus rutilus* with *Cryptobia tincae*. It is

difficult to comment on changes in number of blood immune cells in parasitized fish, because the exact function of each cell can confound the interpretation of results when attempting to make conclusions about the degree of stress experienced by parasitized fish. When fish were parasitized by different parasites in unlike intensity levels, has been reported lymphocytosis accompanied by neutrophilia and monocytosis (Tavares-Dias *et al*., 2002), lymphocytopenia accompanied by monocytosis and neutrophilia (Sopinska, 1985; Silva-Souza *et al.,* 2000); and lymphocytopenia accompanied by neutrophilia and basophilia (Martin *et al.,* 2004), as well as lymphocytosis and neutrophilia (Rahkonen and Pasternack, 1998). In parasitized fish, increase in levels circulating of monocytes has been attributed to an improvement of cell defence system (Sopinska, 1985). Neutrophils possessing phagocytic capability are often the first leukocytes to migrate to the site of infection (Rahkonen and Pasternack, 1998; Silva Souza *et al.,* 2000), therefore increase or decrease of granulocytes may be found, on the dependence of the infection. Even though MCV is higher in heavy infection, than in the uninfected fish. The observed increase is not statistically significant MCH. MCHC shows some changes significantly decrease in heavy infection are not statstically significant.Regarding differential count of WBC, the result presented in table 1: Show that there is progressive reduction in percentage of lymphocytes with increasing severity of infection. Con current with progressive increase in the percentage of neutrophil, basophil & monocyte, eosinophil decreases in heavy infection. However, significant differences in the hematological parameter were observed between values of the infected and uninfected in this study.

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37. Hematological parameters constitute an important tool that reveals the health state of fish. Parasitism may induce lowered growth and hematological alteration

Hematological parameters constitute an important tool that reveals the health state of fish. Parasitism may induce lowered growth and hematological alteration; this alteration may affect the natural resistance of fish to parasites. . However, significant differences in the hematological parameter were observed between values of the infected and uninfected in this study, suggesting that in the intensive helminth infection elevated haematological and biochemical parameters will be observed in the fish.