

# Effect of Induced Red Spot Disease on Haematological Variables in Cultured Fishes of *Channa striatus* and *Channa punctatus* in Warangal, Telangana, India

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**ABSTRACT-** The live freshwater *Channa* fish species were collected from Hasanparthy and Dharmasagar lakes of Warangal district. The present investigation deals with the haematological variables in *C. striatus* and *C. punctatus* infected by different bacterial pathogens such as *A. hydrophila*, *S. aureus*, *P. aeruginosa* and *S. salmonicida*. The considerable variations have been observed in the mean values of blood parameters. Comparison with the control *C. striatus* RBC, HB, values in infected fish were decreased by 63.5%, 23%, increase WBC (21%) had shown and in Differential leucocyte count (DLC) studies in the control fish show that normal percentage range. In case of bacterial infected fishes had shown drastic reduction of (30.7%) in Neutrophils was observed whereas Monocytes (2.3%) and Basophils (0.6%) were not varied much a noticeable increase of (1.5%) in eosinophil and (5.4%) in lymphocytes. And in the *C. punctatus* HB content, RBCs, percentage of Monocytes and Neutrophils were significantly decreased by (9%), (55%), (2.4%) and (18%) respectively. WBCs, Lymphocytes, Esinophils and Basophils were found significantly increased (17%), (5%), (5.8%) and (2.7%) respectively. This was observed in Red spot diseased fishes thus the above results depict the presence of macrocytanaemia in the infected fishes.

**Key-words-** *Channa straitus*, *Staphylococcus aureus*, Hasanparthy, DLC, WBCs

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## INTRODUCTION

*Aeromonas hydrophila*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella salmonicida* is the bacteria is responsible for red spot disease an important bacterial disease that occurs worldwide. It has a large economic impact on fish production because of the extensive and rapid rate of mortality that it causes [1,2]. The clinical signs of the disease begin with corrosion of the dorsal and tail fins, and this progresses to external infection in which gray spots or yellowed areas of erosion appear, generally surrounded by a hyperemic reddened zone, in the cranial region, body surface and gills.

In these locations, there is progressive necrosis involving the epidermis, dermis and musculature [3]. India is a country where the favorable conditions exist for a wide diversity of fish production systems. However the major challenges are faced, particularly in relation to bacterial infections.

The Red spot disease (RSD) endemic to South and Southeast Asia, this is a serious disease of fresh water and estuarine fin fish [4] endemic to South and Southeast Asia. It is a seasonal epizootic condition characterized by infection of *Aeromonas hydrophila*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella salmonicida* and large hemorrhagic necrotizing ulcers typically producing a granulomatous response [5]. As a result of stocking density, ectoparasites, inadequate handling and stressful conditions, out breaks of motile *Aeromonads* associated diseases can reach epidemic proportions among the aquatic animals, leading to massive mortality rates [6]. There are several studies on fish bacterial identification and disease resistance [7-9]. Temperature is an essential and fluctuating environmental factor influencing all life activities which dominate remarkable changes on hematological parameters

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of aquatic animals. Knowledge of haematology is very important since it deals with the morphology, physiology and the biochemistry of fish blood [10]. The haematological parameters are important tools for diagnosis of healthy and infected fishes [11,12]. The blood analysis also reveals the disease status [13,14].

Warangal district of Telangana state has many lakes which include Hasanparthy and Dharmasagar lakes. These lakes are with different fishes with abundant *C. striatus* and *C. punctatus* with high quality of flesh and taste. They also have good market value due to low fat, fewer intramuscular spines and medicinal qualities [15]. The low winter temperature of 20-28°C in this region is ideal for Red spot disease outbreaks which reduced the commercial value of this fish. The present study has taken up to the study of haematological changes in *C. striatus* and *C. punctatus* with bacterial infected fishes were compared with control fishes.

**MATERIALS AND METHODS**

The freshwater *Channa striatus* and *Channa punctatus* infected with different bacterial infection 200 fishes were collected irrespective of age, sex and size from, Hasanparthy and Dharmasagar lakes which are located at a distance of 30 Km from Kakatiya University campus during the rainy and winter seasons between June, 2013 to

April, 2014. Blood samples were collected from caudal peduncle of both control and infected live fishes by dissecting in a tray separately. The collected blood was preserved in Di Potassium EDTA for further analysis as described by [16]. Red blood corpuscles (RBC) and White blood corpuscles (WBC) were counted by haemocytometer crystalline chamber using “Hayeman’s” diluting fluid. Haemoglobin (Hb) estimation was done by haemoglobinometer according to [17] and Differential leucocyte counts (DLC) were calculated by standard formulae, both in the control and bacterial and fungal infected fishes.

**RESULTS AND DISCUSSION**

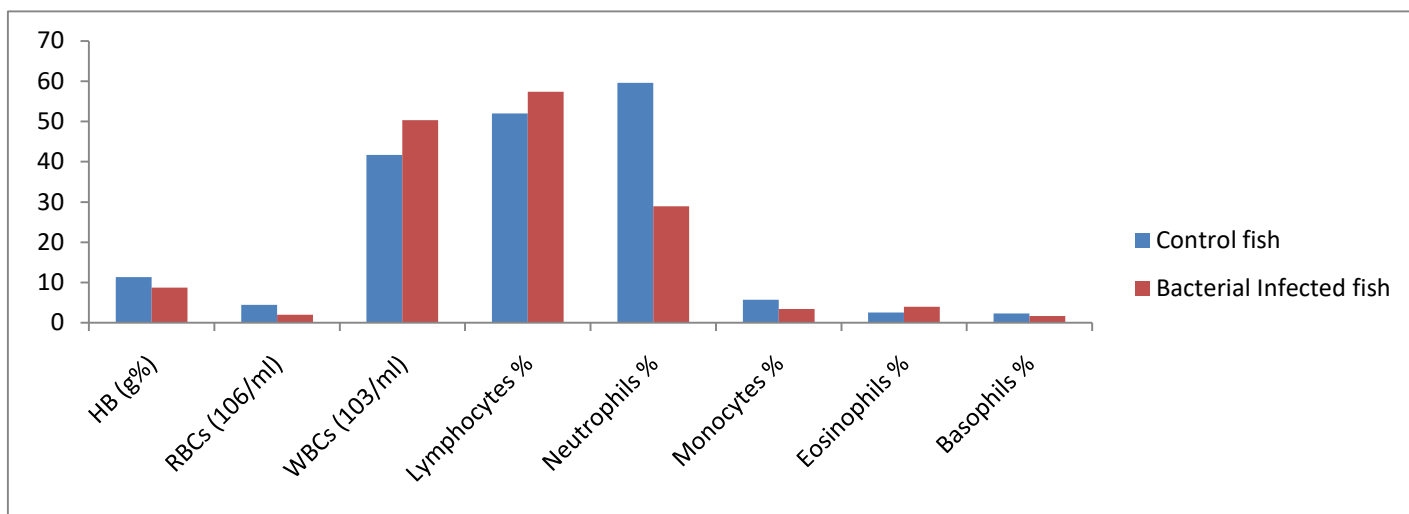
The haematological study has become an essential tool for fishery research. It has been reported that the blood values remarkably vary in different fresh water fishes and this is considered to reflect adaptations to the various environmental conditions [10, 18]. The blood is a patho physiological reflector of whole body and therefore blood parameters are important in diagnosing the functional status of the animal exposed to toxicants. Anemia is also one of the most sensitive pathological situations developed as a result of metals poisoning [19]. (Table 1 & 2; Fig. 1 & 2), shows the results of haematological parameters such as RBC, WBC, HB and Differential Leukocyte Count.

**Table 1:** Haematological parameters of *C. striatus* (Bloch) infected with bacteria

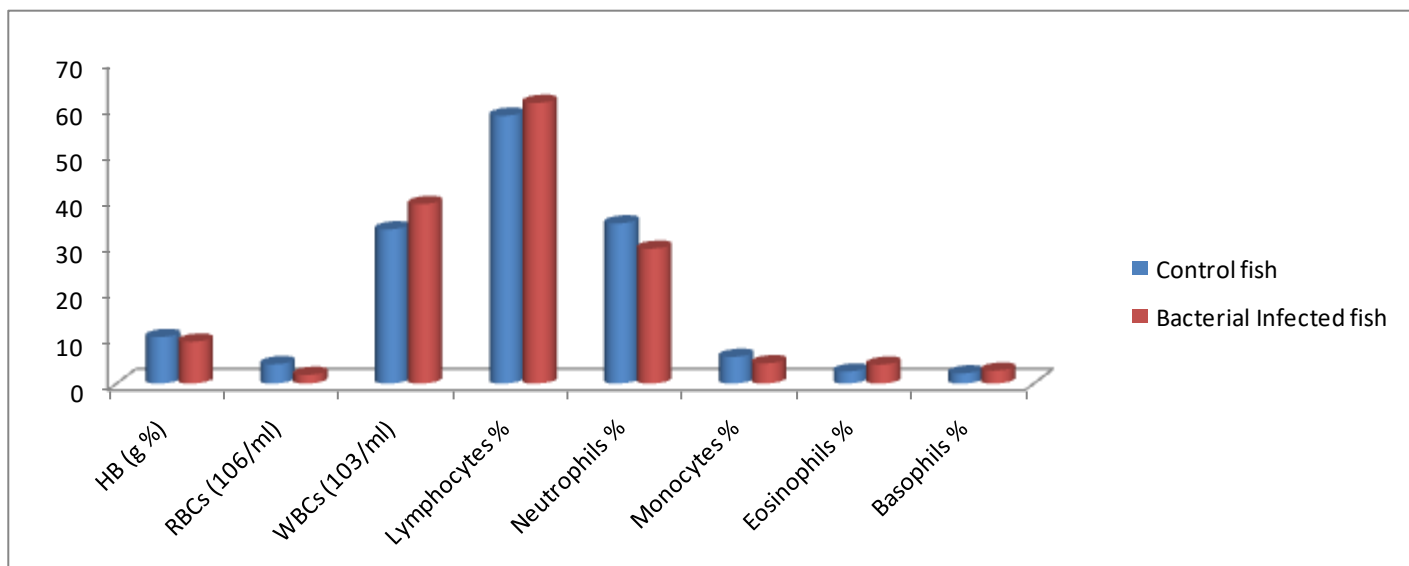
Blood Parameter	Control fish	Bacterial Infected fish	Percentage Change (%)	P value
HB (g%)	11.3±0.34	8.7±0.31	-91.3	0.0001
RBCs (10 <sup>6</sup> /ml)	4.4±0.25	1.96±0.14	-98.04	0.0001
WBCs (10 <sup>3</sup> /ml)	41.67±947.51	50.37±893.85	50.27	0.0001
Lymphocytes %	52.0±0.71	57.4±0.80	-42.6	0.0001
Neutrophils %	59.6±0.62	28.9±0.65	-71.1	0.0001
Monocytes %	5.7±0.34	3.4±0.31	-96.6	0.0001
Eosinophils %	2.5±0.37	4.0±0.36	-96	0.0106
Basophils %	2.3±0.26	1.7±0.35	-98.3	NS

**Table 2:** Haematological parameters of *C. punctatus* (Bloch) infected with bacteria

Blood Parameter	Control fish	Bacterial Infected fish	Percentage Change (%)	P value
HB (g %)	10.1±0.33	9.1±0.29	-90.9	0.0373
RBCs (106/ml)	4.2±0.27	1.89±0.13	-98.11	0.0001
WBCs (103/ml)	33.50±674.13	39.00±756.450	38.90	0.0001
Lymphocytes %	58.2±0.341	61±0.614	-39	0.0009
Neutrophils %	34.8±0.64	29.3±1.67	-70.7	0.0059
Monocytes %	5.8±0.37	4.4±0.74	-95.6	0.0057
Eosinophils %	2.6±0.37	4.1±0.38	-95.9	0.0124
Basophils %	2.2±0.28	2.8±0.38	-97.2	NS



**Fig. 1:** Haematological responses in *C. striatus* showing % increase (+) or decrease (-) During the study period (2013-14) in Hasanparthy and Dharmasgar Lakes



**Fig. 2:** Haematological responses in *C. punctatus* showing % increase (+) or decrease (-) During the study period (2013-14) in Hasanparthy and Dharmasgar Lakes

## STATISTICAL ANALYSIS

The results are obtained from SPSS (12.0) Windows Version. Each assay was replicated 5 times. Values were expressed in Mean  $\pm$  SD of replication at  $p < 0.0001$  for infected (T) and control (C) fishes.

### Haemoglobin (HB)

The hemoglobin content of *Channa striatus* and *Channa punctatus* were  $11.3 \pm 0.34$ , grams and  $10.1 \pm 0.33$ , grams percent in control fishes. Where as in fish infected with bacteria exhibited. It is  $8.7 \pm 0.31$  grms and  $9.1 \pm 0.29$ , grms. In comparison with the control the HB % in infected fishes was found decreased by (23%) and (9%). The Hb variation in infected lakes of fishes was due to seasonal pollution, low oxygen, heavy native fishes and chemical stress [20-21]. Have also reported a decrease of Hb content in fish *Heteroneustes fossils* exposed to paper mill effluents. The bacterial infection influences the malfunctioning of hematopoietic system [22]. Corresponding to the decrease of RBC count due to the bacterial infection, the hemoglobin percentage was also exhibited a similar decrease in fishes.

### Red Blood Corpuscles (RBC)

The results have showed a significant decrease in RBC number. The Total Erythrocyte count was observed to be decreased, in Red spot disease (RSD) infectious *Channa striatus* and *Channa punctatus* by (63.5%), (55%) in relation to control. A decrease in RBC was also reported [23]. Which is sedentary habit of these fishes, the bacterial infection induced extravasation of blood and reduction of haemo-synthesis which in turn fails the hematopoietic tissue to release the blood cells [22]. Erythrocytes or the red blood corpuscles are the major cellular elements found in the blood. The observations revealed that the structure of normal red cell or normocyte varies in shape from being oval or elliptical, biconcave disc or round (Fig. 3 & 4).

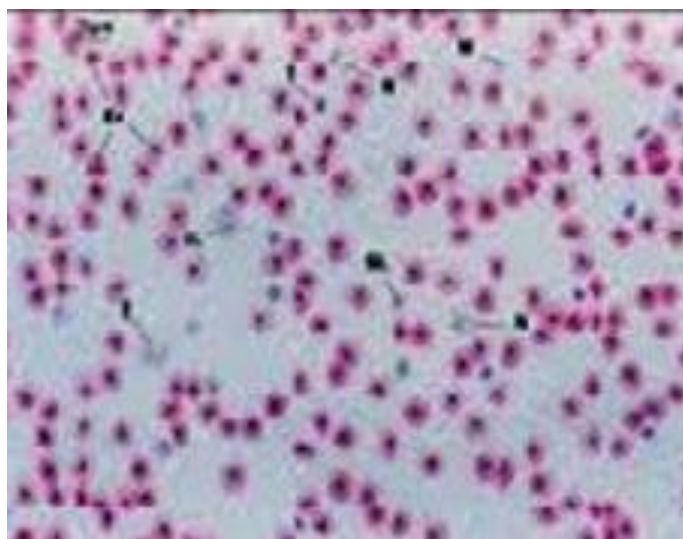


Fig. 3: Erythrocytes (Control)

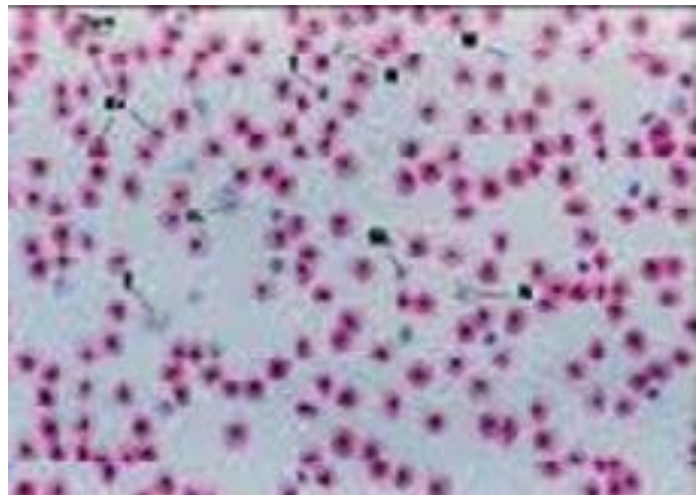


Fig. 4: Erythrocytes (Infected)

Fishes infected by bacteria did not reveal any significant changes either in the shape or in the cytological inclusions of the erythrocytes. The total erythrocyte count observed in control *C. striatus* and *C. punctatus* is  $4.4 \pm 0.25 \times 10^6$ /cu. mm,  $4.2 \pm 0.27 \times 10^6$ /cu. mm of diluted blood. The fish infected with bacteria exhibited decrease in the erythrocyte counts are  $1.96 \pm 0.14 \times 10^6$ /cu. mm,  $1.89 \pm 0.13 \times 10^6$ /cu. mm. Indicating a decrease of about (63.5%) and (55%) of red blood cells. But a significant fall down of erythrocyte count is noticed in bacterial infection. However it is still 36.5% and 45% is less than the control count.

### Total Leucocyte Count (TLC)

A significant increase (21%) and (17%) of total leucocyte count were found in the infected fishes. The enormous increase of leucocyte count in infected *C. striatus* and *C. punctatus* could be correlated to leucocytosis due to anoxic stress, low temperature and lack of food, high organic and inorganic compounds ( $\text{NH}_3$ ) and heavy native fishes [24]. While studying on *C. striatus* and *C. punctatus* have reported that leucocytes constitute second important category of the circulating blood cells and will show increased proliferation on exposure to mitogen, concanavalin, *Aeromonas hydrophila*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella salmonicida* infections indicates the leucocytes are polyclonally activated (Fig. 5 & 6).

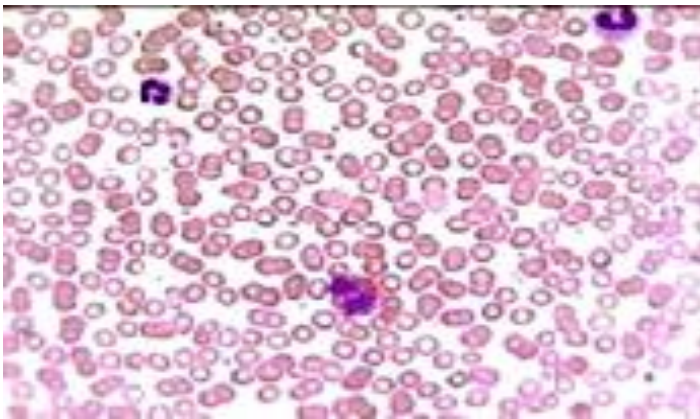


Fig. 5: Leucocytes (Control)

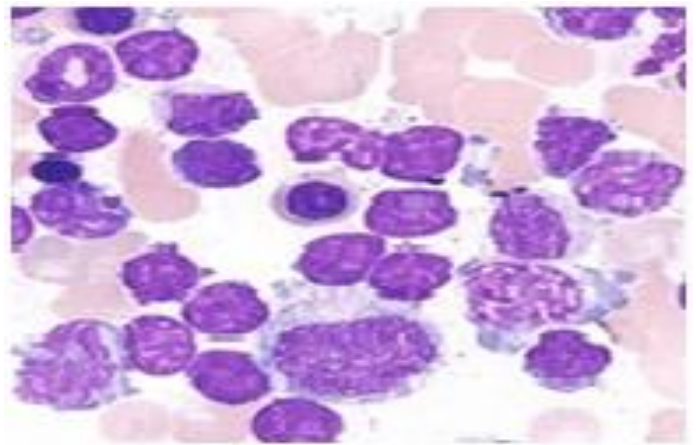


Fig. 8: Lymphocytes (Infected)

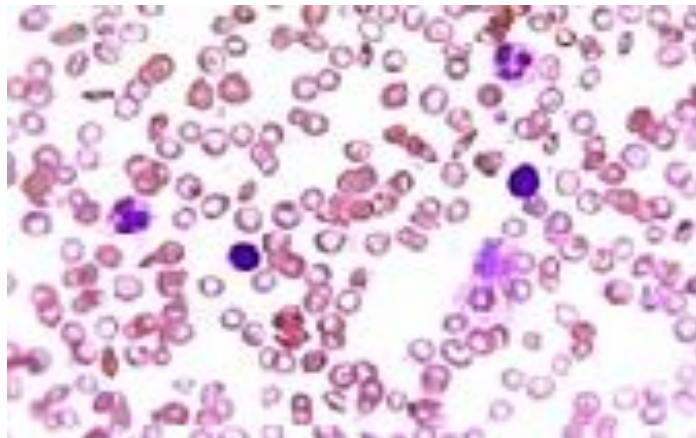


Fig. 6: Leucocytes (Infected)

### Neutrophils

In the present study the Neutrophils constitutes high percentage of leucocyte count in the control fishes. The neutrophil percentage in the control fishes of *C. striatus* and *C. punctatus* recorded values were  $59.6 \pm 0.62\%$  and  $34.8 \pm 0.64\%$ . While in the bacterial infected fishes got decreased significantly by (30.7%) and (18%). This is commonly a condition called neutropenia. All the physiological causes of leucopenia, results in neutropenia. Haematological values fluctuate in accordance with the variations in the environment, according to [27] (Fig. 9 & 10).

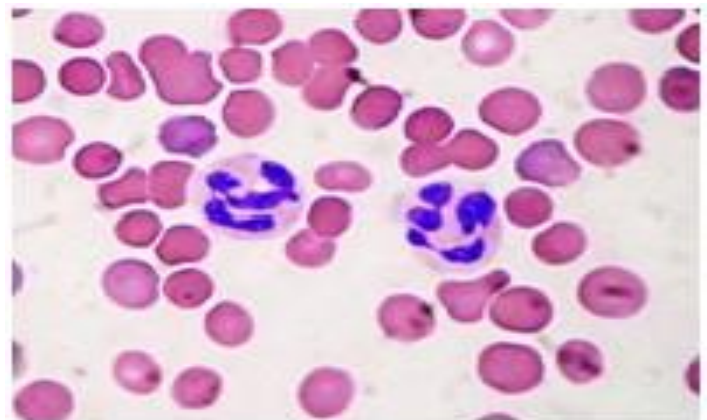


Fig. 9: Neutrophils (Control)

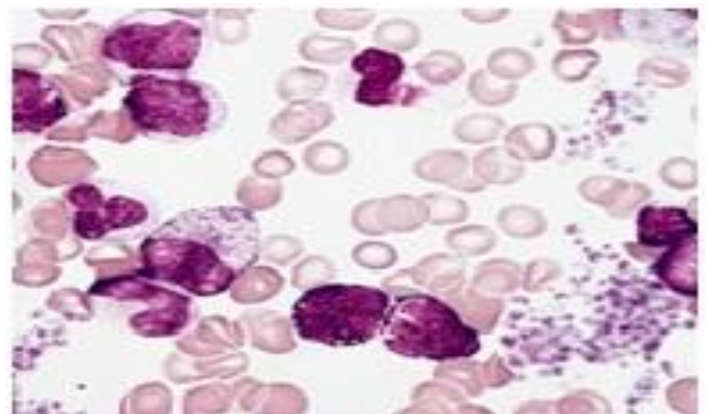


Fig. 10: Neutrophils (Infected)

### Differential Leukocyte Count (DLC)

#### Lymphocytes

Present study reveals that the lymphocyte percentage in the control *C. striatus* and *C. punctatus* fishes were  $52.0 \pm 0.71\%$  and  $58.2 \pm 0.341\%$ , whereas a significant increase of (5.4%) and (5%) was recorded in the infected fishes. This condition is called as the lymphocytosis which is an immunological response against the stress induced by the polluted environment. An increase of lymphocytes indicates the pathological condition due to bacteria and stress full toxic substances in the infected fish [25]. An enhanced lymphocyte proliferation was also observed in Atlantic Manhadevan with ulcer disease syndrome [26] (Fig.7 & 8).

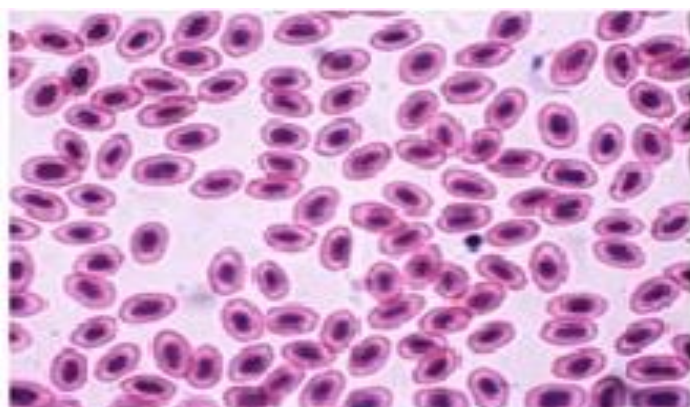


Fig. 7: Lymphocytes (Control)

### Monocytes

The percentages of monocytes were  $5.7 \pm 0.34\%$  and  $5.8 \pm 0.37\%$ . In the control *C. striatus* and *C. punctatus* fishes. Whereas it got decreased to (2.3%) and (2.4%) in the bacterial and fungal infected fishes. Similarly of these results were analyzed significant increases in monocytes are also reported [28] in infected an air breathing fish, of *Clarias batrachus* (Fig. 11 &12).

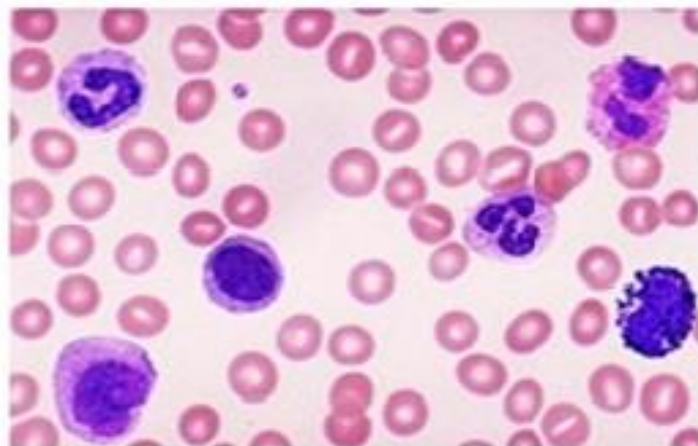


Fig. 11: Monocytes (Control)

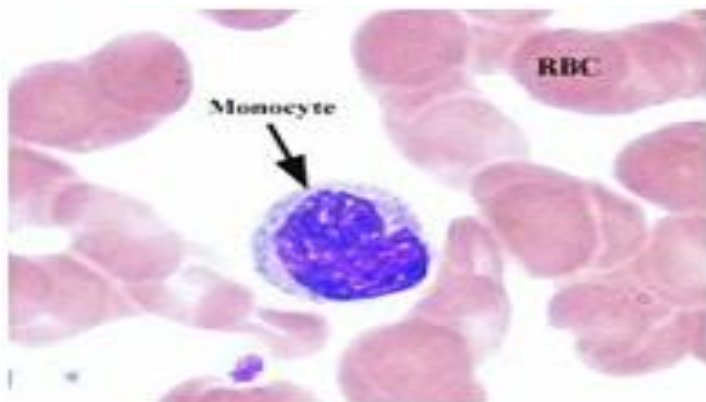


Fig. 12: Monocytes (Infected)

### Eosinophils

The eosinophils are less in number compared to neutrophils. Present results show that, average eosinophil count was  $2.5 \pm 0.37\%$  and  $2.6 \pm 0.37\%$ . In the control fishes of *C. striatus* and *C. punctatus* but the significant increase of (1.5%) and (5.8%), in bacterial infected fishes. The increase in the eosinophils percentage may be cause of red spot disease. Water pollution has stress effect on the blood parameters, which in turn increases the eosinophil count by causing allergies, similar results were observed in infected *Clarias batrachus* an air breathing fish [28] (Fig. 13 &14).

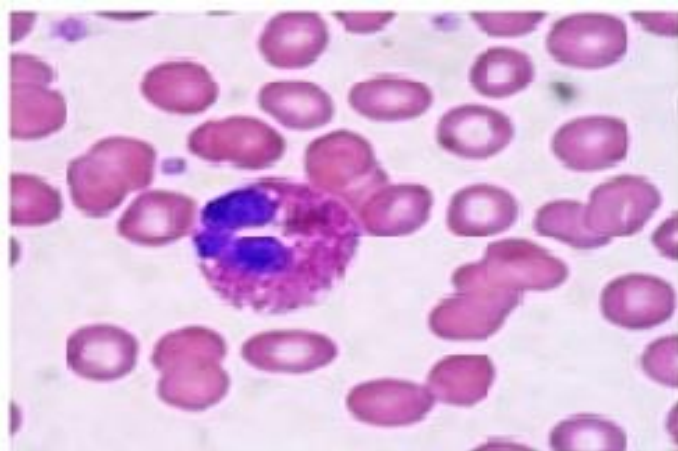


Fig. 13: Esinophils (Control)

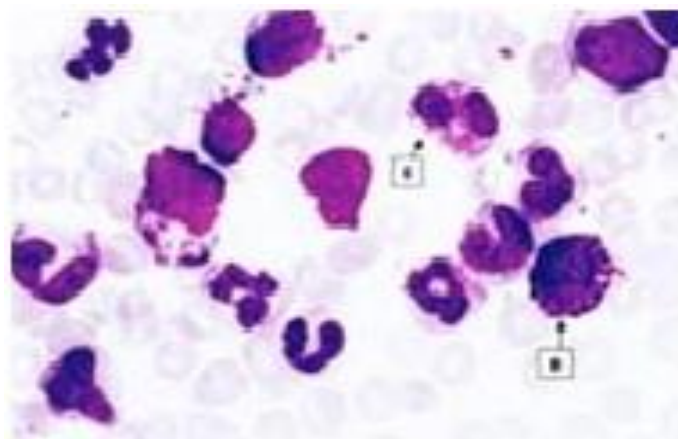


Fig. 14: Esinophils (Infected)

### Basophils

The normal recorded basophil percentages for the control *C. striatus* and *C. punctatus* fishes were  $2.3 \pm 0.26\%$  and  $2.2 \pm 0.28\%$ , while in the bacterial infected fishes the values have reduced to (0.6%) and (0.8%). Present results are in correlation with [28] with the work on infected *Clarias batrachus* (Fig. 15 & 16).

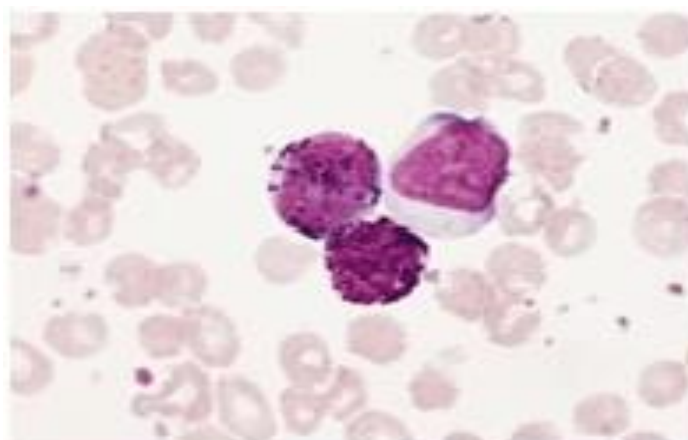
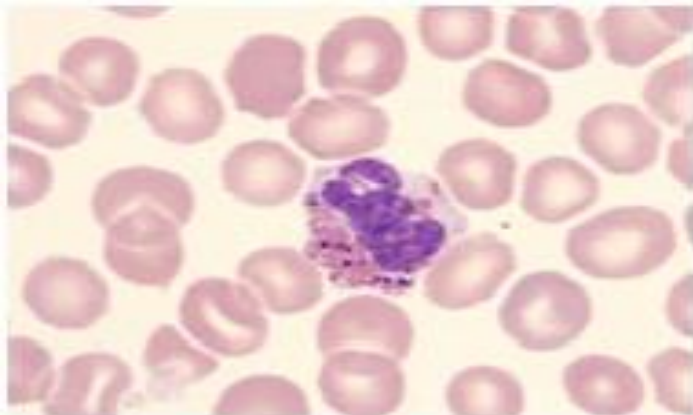


Fig. 15: Basophils (Control)



**Fig. 16:** Basophils (Infected)

## CONCLUSIONS

The present investigation it was concluded that the *A. hydrophila*, *S. aureus*, *P. aeruginosa* and *S. salmonicida* were pathogenic bacteria of both *C. striatus* and *C. punctatus* causes for development of red spot disease (RSD) characteristics on freshwater fishes of Hasanparthy and Dharmasagar lakes. The fluctuations in haematological parameters may be due to the seasonal variation, habitat of the fish biology. And the infection was observed epidermis, wounds and lesions were also observed on epidermal surface of the body. The study also reveals that fishes are most sensitive to change in their ecosystem which reflects their physiological conditions. Above the species of bacteria may be effects by fish even under the low temperature (20-25°C) and stressed environmental and chemical conditions.

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