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Histopathological and haematological observation on argulosis in IMC, *Labeo rohita* (Ham.)

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ABSTRACT

In Rohu (*Labeo rohita*), argulosis disease is caused by the crustacean parasite. The parasites were found to attach with skin, fins and gills of fishes. These sites were haemoarrhagic ulcerated and mucus studded, however, kidney, liver and spleen also found infected. The histopathological changes in the skin of affected fish were epidermal desquamation and dermal necrosis inflammatory cell infiltrations also occur. The infected gills showed hyperplasia, hypertrophy, telangiectasis, aneurism and fusion of lamellae. The tubular degeneration, necrosis and haematopoetic tissue proliferation were observed in the kidney of diseased fishes. The infected liver exhibited hypertrophy, cordal disruption of hepatocytes, sincesoidal and blood vessel congestions. While as, spleen showed white pulp necrosis and increase of melanomacrophage centre. At haematological levels various parameters, such as Hb, RBC, WBC decreases while in DLC, Neutrophils, Monocytes and Esnophil values increases while monocytes and Basophil value decreases. The increase or decrease value showed either significant, highly significant or non-significant. It cause various diseases Erythropoesis, anaemia, Leucocytopaemia, Neutropaemia, Lymphopaemia, Esonophilia and Erythropaemia. The histopathological observations on the presence of pathogens in fish also play a vital role in disease control and health management in aquaculture.

Keywords: *Argulus, hyperplasia, Histopathology, haematology, Indian major carp, Rohu*

INTRODUCTION

Argulosis is a disease caused by crustacean parasite (*Argulus* sp.) and commonly known as 'Fish louse'. The larvae and adults of argulus are parasitic to fish. Young fishes are more prone to infection than old ones (Ozturk , 2005). The incidence and intensity of parasite also varied with season (Bichi and BawakI, 2010), parasites typically reach peak abundance during summer and autumn (Moller, 2012). This parasite penetrates the upper layers of the host skin and feeds on blood and body fluids (Van Der *et al.*, 2000). The major fishes affected with this disease are fry, fingerlings and adults of Indian major carps (Sheila *et al.*, 2002). The affected fishes become restless with erratic swimming movements and attachment sites shows sign of ulceration. The argulus can be seen quite clearly with the naked eye. Adult parasites are oval, flat and leaf like in appearance with transparent to whitish in color along with two conspicuous black spots (Sheila *et al*., 2002). Generally, they found to attach with the skin, fins or gills of Indian major carps. But sometime, kidney, liver and spleen are also found to distress with argulus parasites (Hasan, 2005). Histopathology is an important modern tool for quick correct and reliable diagnosis of fish diseases. It helps to identify and extent to damage in the organs of diseased fish and also the etiological agents harbored in target organs of the fish (Sheila *et al.*, 2002). It play significant role for understanding the mechanism of disease processor and the course of diseases ranking from acute and chronic

stages through fish level reactions in host fish by pathogens. Therefore, present study also helps to develop fish quarantine and certification programmes for fish health monitoring.

MATERIALS AND METHODS

The *Labeo rohita* were collected from hatcheries, ponds and tanks of Darbhanga (Bihar) and brought to the laboratory for patho-morphological and anatomical examinations. The patho-morphological examinations were comprised of identifying and locating any visible external lesions emissions haemorrhages, and formations of vista and patches on body surfaces gills and fins. The patho-anatomical examination was thus carried out for finding any viable lesions or inflammations in internal organs.

Methods for Histopathological Experiments:

Small bits of tissues (3-4 mm) from the vital organs like skin, gills, kidney, liver, spleen and intestine of moribund or freshly killed diseased fish samples were collected and fixed in 10% neutral buffered formalin for 18-24 hours. Fixed tissue samples were then processed and paraffin embedded block of all the tissues was prepared using the standard histological methods (Luna, 1968). However, calcified tissues like skin and gills were decalcified with 10% nitric acid, which helped in getting perfect and unbroken serial sections of these tissues during microtome. These blocks were cut into serial sections of 5 to 7µ by a rectory microtome. Thereafter, histological sections were stained with Ehrlich's Haematoxylin (H) and alcoholic Eosin (E) stains as per suggestions of Luna (1968).

Methods for Haematological Experiments:

Heamatological Studies-

Haemoglobin concentration (in gm/100 ml of blood) was estimated by Sahli's Acid-Haematin method. The counting of total number of RBC was made with the help of Thoma-Zeiss haemocytometer with improved Neubauer ruling techniques as described by Darmady & Davenport (1954). Different types of WBC were distinguished by the following characteristics of each cell as described by by Akela *et. al.*(1996). Determination of PCV (Pakced Cell Volume): It was determined by Srivastava *et al.* (1979).

Statistical Analysis:

Mean and standard error (± SE) were calculated for various parameters during this study and the significance of difference was calculated following students' 't' test (Fisher, 1950), 'p' values less than 0.05 were considered significant throughout the study.

Clinical Symptoms and gross pathology:

The skin of *Argulus* affected fishes was pale and sometimes abnormally pigmented. Gills were generally pale in colour. The affected fishes showed weight loss, retarded growth, restlessness, erratic swimming behaviour and loss of appetite. Kidney, liver and spleen of the affected fishes were pale in colour and somewhat abnormal in consistency.

RESULTS AND DISCUSSION

Histopathological Observations:

Skin: *Argulus* parasites caused extensive damage to skin epithelium by insertions of stylets into the epidermal cells. The dermis showed inflammatory reactions and haemorrhages,

Scales fell. The dermis became hypertrophic and showed large vescicles. Some of the blood vessels in the dermis were highly congested and dilated (Figure 1.).

Gills: The most significant lesions in the gills were the secondary lamellar hyperplasia, haemorrhages in the tips of the primary lamellae and fusion of both secondary and primary gill lamellae (Figure 2.). The fusion of secondary lamellae in some places was along the lengths while in other places it was restricted only to the tips of the primary gill lamellae. The pillar cells were found broken at the tips of telangiestatic gills rendering the lamellar epithelium into a balloon-like shape.

Kidney: Kidney showed multifocal enlargement of glomeruli but having the glomerular tufts in many places shrunken, fragmented and necrosed. In some highly affected renal tubules, the epithelial cells showed large vacuoles indicating vacuolar degeneration and basement membrances of these cells were desquamated. The kidney haematopoetic tissue exhibited extreme degree of proliferation and showed infiltration of mostly macrophage cells in the tissue indicating acute inflammatory reactions. In some areas of kidney section, large melanomacrophage centres were seen in the tissue (Figure 3.).

Liver: The haepatocytes were swollen and hypertrophied in many regions of the section (Figure 4). The haepatocyte nuclei in these swollen cells were found pycnotic and dark stained. In other regions of the section some focal necrotic areas were seen in the parenchyma and inflammatory cell infiltration particularly by lymphocytes were noticed. Cord-like arrangement of the hepatocytes was disrupted in many places and there was dilatation of the sinusoidal spaces particularly around the central veins. The sinusoidal spaces were congested with blood cells. Some focal areas of haemorrhages were also seen in the liver.

Spleen: The spleen showed extensive necrosis of both the red and white pulp and there were multifocal areas of haemorrhage in the tissue (Figure 5.). Also there were many large melano-macrophage centres in certain areas of the tissue sections and these centres contained haemosiden pigments (Figure 5.). In some areas there was infiltration of neutrophils in the spleen parenchyma indicating inflammatory reactions.

Diagnostic histopathological lesions identified:

At the sites of parasite attachment the typical skin lesions were epidermal desquamation, dermal necrosis, inflammatory reactions through infiltration of lymphocytes and macrophages, congestion and dilation of blood vessels. Characteristic gill lesions in the disease were gill hyperplasia, hypertrophy, fusion of lamellae and presence of telangiectatic lamellae. Glomerular shrinkage, tubular degeneration and necrosis and proliferation of haematopoetic tissues in the kidney, hypertrophied hepatocytes, disruption of cordal arrangement of hepatocyts, sinusoidal and central vein congestion in the liver and necrosis of red and white pulp, multifocal haemorrhagic areas and presence of many melano-macrophage centres in the spleen were the most important diagnostic lesions in the disease.

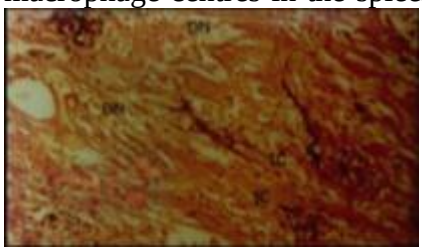

Fig. 1: *Argulus sp.* affected skin of *Labeo rohita* (Ham) showing dermal necrosis (DN), congestion (C) and dilation (D) of dermal blood vessels and infiltrating cells (IC). H. & E., X 100.

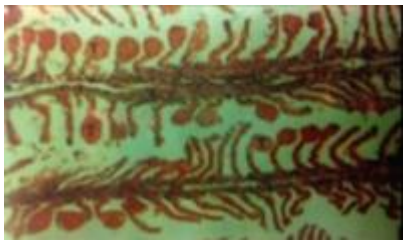

Fig. 2: Gill of *Argulus* affected *Labeo rohita* (Ham) showing telangiectatic (T) secondary gill lamellae. H. & E., X 200.

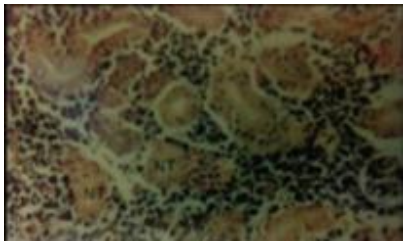

Fig. 3: Kidney of *Argulus* affected *Labeo rohita* (Ham) showing glomerular shrinkage (GS), necrotic tubules (NT) and proliferation of haematopoetic tissue (HT). H. & E., X200.

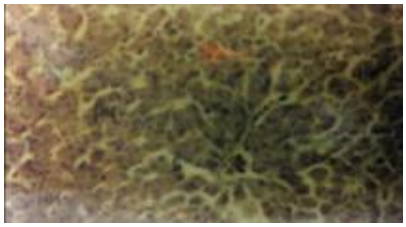

Fig. 4: Liver of *Argulus* affected *Labeo rohita* (Ham) showing, hypertrophied hepatocytes (HH), congestion (C) of blood vessels and dilation of sinusoids (S). H. & E. X 200.

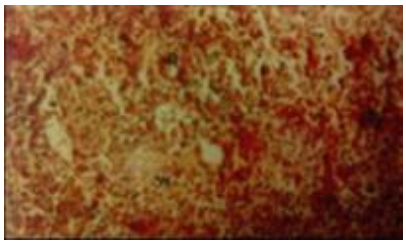

Fig. 5: Spleen of *Argulus* affected *Labeo rohita* (Ham) showing necrosis of red (R), haemorrhagic areas (HA) and white pulps (W). H. & E., X 200.

Haematological Observations:

The present study was effects of crustacean parasites *Argulus sp.* on haematological parameters to the fish Indian major carps, *Labeo rohita*. During present study the Hb, RBC, WBC, DLC and PCV has been explained under *Argulus sp.* to the fish *Labeo rohita.* Under Argulosis the haematological parameters either increases or decreases to cause various disorders or abnoruadition such as Hb decreases to cause erythropoiesis, RBC decreases to cause anaemia, WBC decreases to causes leucocytopenia. Hb decreases to cause erythropoeisis, RBC decreases to cause anaemia, Lymphocytes decreases to cause lymphopaenia, PCV decreases to cause erythropaenia, while Eosinophil increases to cause Eosinophilia and neutrophil increases to cause Neutropaenia.

From Table 1, it is quite clear that Hb in control value is 12.50 ±0.06 gm/dl which is decreases under Argulosis 5.86 ±0.11 gm/dl and showed highly significant ($P<0.001$). Similarly the value of RBC decreases under Argulosis at 2.56 ± 0.08 in contrast to control value 6.37± 0.01. the decreased value in diseased fish showed highly significant ($P < 0.001$) (Table –1, Figure-6). Values of Neutrophils, Monocytes, Esnophil is increasing under treatment groups such as 16.2 ± 0.04, 8 ± 0.03 and 1.4± 0.02 in compare to control value such as 7.82 ± 2.01, 5 ± 0.05, 1.1 ± 0.03. Neutrophils are highly significant ($P< 0.001$), while Esnophil showed significant ($P < 0.01$) and Basophil showed non-significant ($P < 0.05$) (Figure 7). In DLC the values of Lymphocytes and Basophils are decreases under treatment

groups. In control to values are 68.33± 242 and 1.1± 0,02 while under treatment the values decreases as 47 ± 0.02 and 1.3 ± 0.02. the Lymphocytes showed significant (P < 0.01) while Basophil showed non significant (P < 0.05). Similarly PCV also decreases under treatment group as 12.03 ± 0.03 compared to control group as 36.98 ± 0.06. It showed significant (P < 0.01) (Figure 8).

Table 1

Showing the parasitic effects of *Argulus sp*. on Hb, RBC, WBC, DLC, PCV of *Labeo rohita*.

Parameter	Variable	
	Control	Diseased fish (0.1 g/l)
Blood Hb (gm/l)	12.50 ±0.06	5.86 ±0.12 ***
TEC(RBC) (x 10^6µl)	6.37±0.01	2.56±0.08 ***
DLC (WBC) (% values)	3.6	2.3
Neutrophils	7.82 ±2.04	16.2 ±0.04 ***
Lymphocytes	68.33±2.42	47.0±0.02 **
Monocytes	5.0±0.03	8.0±0.03 *
Eosinophil	2.0±0.03	3.0±0.02 **
Basophil	1.1 ±0.02	1.3 ±0.02 *
PC (%values)	36.98 ±0.06	12.03 ±0.03 **

Values are mean ± SE of 5 individual observations:-

* P<0.5 Non Significant
** P<0.01 Significant
*** P<0.001 Highly Significant

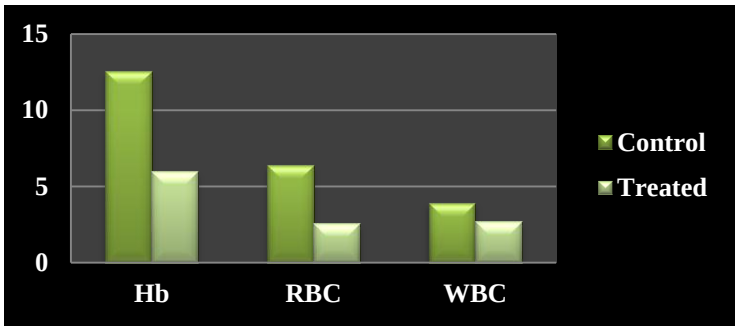

Fig. 6: Showing the parasitic effect of *Argulosis sp.* on Hb, RBC, WBC in *Labeo rohita* ***P < 0.001.

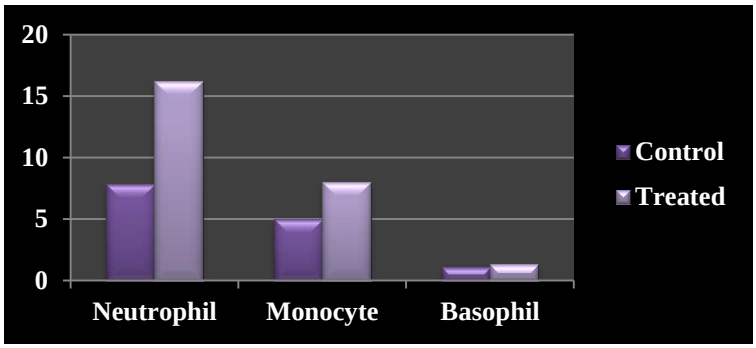

Fig. 7: Showing the effect of *Argulus sp*. on Neutrophil, Monocyte, Basophil in *Labeo rohita* * P<0.05, *** P<0.001.

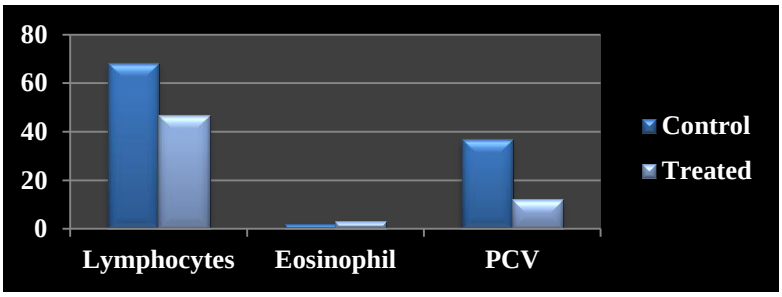

Fig. 8: Showing the parasitic effect of *Argulus sp.* on Lymphocyte, Eosinophil, PCV, in *Labeo rohita* ** P<0.01.

The histopathological changes recorded in the liver, kidney and spleen of the diseased samples are in accordance to Kabata (1970), Schaperclaus (1986), Dey (1989), Singh and Srivastava (1992) and Hasan (2005), and they reported hyperplasia of dense connective tissue Kidneys which Exhibited marked glomerular changes and tubular degeneration and necrosis. According to Kabata (1970) *Argulus* causes extensive pathological changes in the skin such as a circular depression, haemorrhages and marginal welt of raised epithelium. The observed histopathological changes in the skin and gills of the infected fishes probably might have been due to the combined effected of mechanical damages and release of toxins by the parasites at the site of attachment (Feist and Longshaw, 2008). Schaperclaus (1986) reported that degenerations and lymphocyte aggregations can be observed histologically on the places where *Argulus* parasites infect.

At haematological levels various parameters, such as Hb, RBC, WBC decreases while in DLC, Neutrophils, Monocytes and Esnophil values increases while monocytes and Basophil value decreases. The increase or decrease value showed either significant, highly significant or non-significant. It cause various diseases Erythropoesis, anaemia, Leucocytopaemia, Neutropaemia, Lymphopaemia, Esonophilia and Erythropaemia. Khurshid and Ahmed (2012) speculated that mechanical damage caused by Acanthocephala to the host intestine could cause vitamin B-12 and folic acid deficiency which are otherwise responsible for RBC maturation. The mechanical injury may also lead to side tracking of iron to affected tissues which is otherwise responsible for erythropoiesis. Increased number of TLC values may be associated with the defense mechanism and immunological responses against infectious diseases causes by helminth parasites. In the present investigation on IMC suffering from the disease Argulosis the haematocrit, decreased significantly. The same result was recorded by Silva-Souzaetal (2004) who found that increasing the monocytes percentage value occurred in copepode Lernea infested fish. The primary function of monocyte is phagocytosis and digestion of large particulate matter such as large micro-Organisms (Savari *et al*., 2011).

REFERENCES

1. Bichi, A. H. and Bawak, I. S. S. 2010. A survey of ectoparasites on the gi ll, skin, and fins of Oreochromis niloticus at Bagauda Fis h Farm, Kano, Nigeria, *Bayero Journal of Pure and Applied Science*, 3(1):83.
2. Dey, R. K. 1989**.** Histopathology of Argulosis in the Indian major carps, *Lab eo rohita* (Ham.). In: Nat Sem Forty Years of Freshwater Aquaculture in India, CIFA, pp: 16. Dulin M. 1979. Fish diseases. TFH publications, Neptune City, N. J.

3. Feist, S. W. and Longshaw, M. 2008. Histopathology of fish parasite infection–importance for populations. *Journal of Fish Biology* **73,** 2143–2160.

4. Fisher, R. A. 1950. Stastical Methods for Research Workers, IIth edition. Oliver and Boyd, London. freshwater fishes. *Nat Env Poll Technol*, 1:201-206.

5. Hassan, N. 2005. Study on some aspects of parasitic diseases of some fresh water fishes. PhD thesis, L.N.M. Univ., Darbhanga.

6. Kabata, Z. 1970. Crustaceans as enemies of fish. In: Diseases of fish (Eds. Snieszko SF and Axelord HR). TFH publications, Neptune City, N.J.

7. Khurshid, I. and Ahmed F., 2012. Impact of helminth parasitism on haematolgoical profile of fishes of Shallabugh wetland, Kashmir. J. TER, DAMA. Vol. No. 1. ISSN : 2319-474X (Print); 2319-4758 (Online).

8. Luna, G. 1968. Manual of histologic staining methods of the armed forces institute of pathology. (3rd Edition), Mc row-Hill Book Company, New York.

9. Moller, O. S., 2012. Argulus foliaceus. In Fish Parasites: Pathobiology and Protection, Oxfordshire, UK, CABI, 337–346

10. Nandp, N. C. and DAS, S. R. 1991. Argulosis causing juvenile mortality in some fishes at Kakdwip, *West Bengal. Indian J Fish*, 38(2): 132-133.

11. Ozturk, M. O. 2005. An investigation on metazoan parasites of co mmon carp (Cyprinus carpio) in Lake Eber. Turkiye Para zitol Derg, 29(3):204-210.

12. Savari, A., Hedayati, A., Safahieh, A. and Movahedinia, A. 2011. Characterization of blood cells and hematological parameters of Yellow fin sea bream (Acanthopagrus latus) on some creeks of Persian f. *Worked J. Zoology*, 6 (1):26-32.

13. Schaperclaus, W. 1986. Fish disease. (Vol 2, Eds: Chari MSR and Kothekar), Oxonium Press Pvt Ltd, New Delhi.

14. Sheila FAA, Sivakumar and Chandran R. 2002.Infestation and prevalence of copepod parasite, *Argulus indicus* on some

15. Silvasouza, A. T., Almeida, A. and Machado, P. M. 2004. Effect of the infestation by Lernaea cyprinacea on the leucocytes of Schizodon intermedius. Revista Brazileira De Biologia, 60(2):217-220.

16. Singh, J. P. and Srivastava, S. K. 1992. Observations on occurrence of Argulosis in pond fishes in Patna area. Fishing Chimes, 12(7): 30-31.

17. Van Der, Salm, A. L., Nolan, D. T., Spanings, F. T. A. and Wendelaar, Bonga S. E. 2000. Effects of infection with the ectoparasite *Argulusjaponicus* (Thiele) and administration of cortisol on cellular proliferation and apoptosis in the epidermis of common carp, *Cyprinus carpio* L., skin. J Fish Diseas, 23(3)