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## Effect of diplostomulum infection on endocrine organs of *Heteropneustes fossilis* (Bloch)

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**Abstract:-** Parasites are small organisms distributed ubiquitously and are common cause of illness in aquatic animals including fish. They exist in equilibrium with their host body. *Diplostomulum metacercaria* are the major culprit for large scale death of aquatic animals. They are wide spread in fishes cultivated in oxygen deficient environment. In the present report we studied the effect of infestation of *Diplostomulum* infestation on endocrine organs like head, kidney and thyroid. We observed various symptoms like necrosis, fibrosis and other mechanical damages in the host body.

**Key words:** Parasites, *Diplostomulum*, Necrosis, Fibrosis, Head Kidney, Thyroid Gland.

### INTRODUCTION

Aquatic animals including fishes are one of the most important resource for mankind and a major topic of research in ichthyology in all over the world.<sup>1</sup> India is a country with high fishery resources. Particularly Darbhanga District in Bihar is a rich source of air breathing fishes.<sup>2</sup> Parasites are ubiquitous and are responsible for invasion of fishes in aquatic environment. Fish pathology is major area of interest for researchers. Particularly the effect of helminths parasite on fishes is highly explored and supplemented by various research groups.<sup>3,4</sup> The larval stages of cercaria and *metacercaria* are prime example of digeneans and constitutes an attractive target for fish pathologists. To the best of our knowledge the diagnosis of fish diseases by application of histology is very less

reported in literature and provides an open area of research. Few research groups have worked in the concerned field and published their results. They have demonstrated strigeoids as the fish pathogen for causing diseases.<sup>5,6</sup> Many strigeoids have been reported as pathogen of fish.<sup>7</sup> In some of the reports from our research group we reported that *Diplostomulum* are widely distributed and is very common in many piscivorous birds.<sup>8-14</sup>

The fishes may be cultivated in oxygen deficient water by systematic and scientific culture and are subjected to various parasitic diseases. These diseases not only deplete the fish but also render these diseases to human beings.

Motivated by our previous work in this field and Interesting area of research we hypothesized to investigate the infection of *Diplostomulum* on endocrine organs of *Heteropneustes fossilis*.

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## **MATERIALS & METHODS**

Normal & parasitized *H. fossilis*, averaging 55g each, were collected from the derelict swamps of Keoti fish farm, Darbhanga. On arrival at laboratory, the infected fish were first sorted out on the basis of presence of cysts on the skin at various sites. A part healthy fishes without any sign of metacercarial cysts were kept separate for further examination by opening the body cavity and locating the free metacercariae in the viscera and other organs of the fish host. These fish were maintained in plastic pools & were fed with cleaned pieces of goat liver. Routine examination of fish for locating the strigeoid metacercariae was followed by investigation of skin, muscles, gills, eyes and viscera were made through naked eyes as well as examined in 0.65% saline solution under the dissecting microscope. Tissues measuring 18-22 cm. in total length were analyzed during the study period 2014-2015. The fish were dissected and tissues under study were observed in situ. After post treatment process tissues were though roughly washed with water and dehydrated in graded series of alcohols. The material was cleared in xylol and infiltrated in paraffin wax at 58°C and blocks were prepared. Transverse sections of 3-8  $\mu$  thickness were taken with the aid of microtome. Harris heamatoxyline and eosin stains were applied for histopathological studies.

## **RESULT & DISCUSSION**

In *Heteropneustes fossilis* adrenocortical tissues are located in the haemopoietic head kidney. The adrenocortical tissues are found in the form of lobules of varying thickness which remain packed together, each having follicular lumen. Histologically, the adrenoecortical lobules can be differentiated into chromaffin & interregnal tissues. The interregnal or cortical cells are generally columnar or polygonal with centrally placed nuclei. The chromaffin cells are of irregular shape & large and from dark brown coloured islets which remain scattered in the internal tissues.

Lesions in the adrenocortical tissues were observed along with necrosis at certain points in the infected animals. (Fig 1-2).

Compact thyroid is situated in the subpharyngeal region extending anteriorly in front of first afferent and posteriorly over the fourth afferent branchial artery, although individual or scattered groups of follicles may

also be found away from pharyngeal sinus. Histologically, it is composed of large number of follicles of varying shape & size, bound together by connective tissues. Each follicle is composed of single layer of epithelial cells enclosing a central cavity filled with colloid.

Histological sections of the infected fish showed proliferation of thyroid follicles which had hypertrophic epithelium and hypersecretion of colloid in comparison to that of normal uninfected fish. (Fig 3).

There is little information available on pathological changes in endocrine organs. The fish thyroid, unlike that of the normal, is not an encapsulated structure and ectopic or aberrant thyroid tissue is common.<sup>15,16</sup>

Thyroid tissue is normally found between the first and second gill arches but it may be found in the surrounding musculature and bone lamellae.<sup>17-19</sup> Cases of thyroid hyperplasia are well documented.<sup>19-23</sup> In some instances this hyperplasia was associated with exophthalmos.<sup>24</sup>

In the present study proliferation of thyroid follicles, hypertrophic epithelium & hypersecretion of colloid were observed in the infected fishes in comparison to that of normal fish. Similar observations have also been reported by Stolk (1957)<sup>25</sup> in *Poecilia reticulata* & Dubey (1980)<sup>19</sup> in *H. fossilis*.

Bacterial Granulomata in the adrenal cortex of turbot suffering from corny bacterial kidney disease and chronic furunculosis has been reported.<sup>26</sup> In the present study necrosis was observed in the head kidney of the parasitized *H. fossilis*. The kidney having particularly good blood supply due to its physiological function, Often happens to be an important station for the developing *Diplostomulae* on their way to target organs & in the process, the migrating larvae might cause necrosis as is evident in the present study which is similar to the observations of Dubey (1980)<sup>19</sup>.

## **CONCLUSION**

The present investigation is done to study the effect of diplostomulum infection on endocrine organs of *Heteropneustes fossilis* (bloch). Histopathological examination of skin and muscle revealed that the metacercarial cysts were lying inside the muscle bundle containing groups of parasites. Around the cysts, a marked cellular reaction was observed. After encystment and



Fig. 1 :A Diplostomulum parasite in the Head Kidney section (H & E x 400).



Fig. 2 :A fully extended Diplostomulum in the head kidney (H & E x 280).

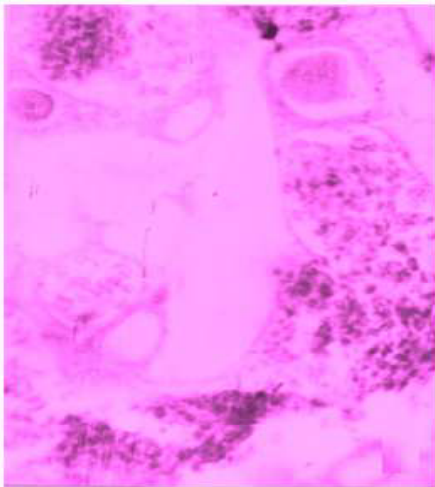


Fig. 3 :T.S. of thyroid of infected fish showing hypertrophic epithelium and hypersecretion of colloid (H & E x 400).

fibrotic encapsulation larval helminths were observed to have produced no obvious damage.

Lesions in the adrenocortical tissues were observed along with necrosis at the points of contact with the parasite in the histological sections.

Histopathological sections of the infected fish showed proliferation of thyroid follicles which had hypertrophic epithelium and hypersecretion of colloid in comparison to that of normal uninfected fish.

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