ABSTRACT

The present investigation was carried out to study the effect of digenetic trematode, Clinostomum complanatum on histopathology and haematology of Nandus nandus. The body of the fluke is elongated with rounded ends measuring 5.13 – 8.40 mm in length and 2.20 – 3.24 mm in width. It was recovered from the branchial chamber, body cavity, liver, intestine and ovaries of Nandus nandus, showing the total prevalence of 42.8% and mean intensity of 2.13. Histopathological changes were observed mainly in the liver which were represented by loosening of hepatic tissue, eccentrically situated nuclei of hepatocytes and necrosis. The haematological manifestation of the infected N. nandus showed marked decrease in the content of haemoglobin and RBC count, and increase in the count of lymphocytes and granulocytes.

Keywords: Haematology, Nandus nandus, Clinostomum complanatum, Metacercariae, Histopathology

1. Introduction

Disease aetiology is a complex of three major components which includes the host (fish), the parasite (stressor) and the environment. Fish does not only serve as the host for different parasites but some parasitic forms cause serious damage to the tissue and also alters the normal physiology, histology and haematology of the host. The histological study of liver is a direct evidence of robust effect on the fish health as it is considered as the principal site for digestion, filtration and storage of glucose in fishes. Liver also functions to detoxify the blood impurities coming from the intestine. Therefore, the histological study of liver is helpful in knowing the possible effect of parasitism on the functions of different body systems.

Blood is a good indicator to determine the health of an organism (Joshi et al., 2002). It also acts as a pathological reflector of the whole body. Hence, the haematological parameters are important in diagnosing the functional status of the fish (host) infested by helminth parasites (Joshi et al., 2002) and also to evaluate the physiological condition and nutritional state of fish (Chagas and Val, 2003). Several species of the genus Clinostomum (Digenea: Clinostomidae) are frequent parasites of fishes which are the second intermediate hosts harbouring the encysted metacercariae in several organs (Lo et al., 1980, 1981, 1982). Clinostomum complanatum (Rudolphi, 1819) is known to causes yellow grubs in the fish and make the unsuitable for human consumption. Digenean metacercariae which occur in liver and other visceral organs cause severe melanosis of the liver and visceral fibrosis (Mitchell et
al., 1988). Since the larvae of *C. complanatum* have been observed in the liver of *N. nandus*, histopathological and haematological investigations were carried out for the first time in Bhopal region. For the purpose of present investigation, *Nandus nandus* was chosen since it is a predatory, piscivorous and amply available fish at Bhopal.

### 2. Materials and Method

#### 2.1 Collection of fish specimens

Living specimens of this fish were collected from the Lower Lake of Bhopal as well as from local fish markets. They were brought to the laboratory and examined morphologically and internally for the occurrence of helminth parasites.

#### 2.2 Collection of parasite

Fish specimens were dissected out in physiological saline (0.75% NaCl) for collecting digenetic parasites. Encysted and excysted metacercariae of *Clinostomum complanatum* were collected from the body cavity, branchial chamber and liver. Collected parasites were fixed in hot alcohol-formol-acetate (AFA) solution and stained with aceto-carmine to prepare permanent slides. The parasites were identified according to the keys given by Gibson *et al.* (1992) and Yamaguti (1958). Ecological analysis of parasites was done according to the method of Margolis *et al.* (1982).

#### 2.3 Histopathological study

The infected tissue of *N. nandus* was taken out and fixed in alcoholic Bouin’s fluid for 72 hours. After the complete removal of picric acid, the tissue was processed for preparation of paraffin wax blocks. The tissue was then cut at 6 µm thick sections which after proper processing prepared in permanent slides.

#### 2.4 Haematological studies

For purpose of haematological parameters, the blood samples were taken from the caudal peduncle and heart with the help of 2 ml disposable syringe containing a drop of 10% EDTA solution as anticoagulant. The hemoglobin content was estimated by hemoglobinometer. The count of erythrocytes and granulocytes etc was carried out by using haemocytometer.

### 3. Results

During present investigation, 175 specimens of *Nandus nandus* were examined out of which, 75 were found infected by metacercariae of *C. complanatum*. The site occupied by the metacercariae is preferentially the gill chamber and behind opercula but in heavily infected fish, the parasites occur throughout the body cavity, liver, intestine and ovaries (Figure 1). The body of the fluke is elongated with rounded ends measuring 5.13 – 8.40 mm in length and 2.20 – 3.24 mm in width. It showed the total prevalence of 42.8% and mean intensity of 2.13 (Figure 2).

#### 3.1 Histopathology of liver

The normal liver is composed of polygonal cells which remain compactly arranged so as to give it the appearance of a dense tissue. Interspersed among the hepatocytes, lie scattered bile passages and blood capillaries (Figure 3).
The histo-pathology of the liver of Nandus nandus infected by Clinostomum complanatum exhibited loosening of hepatic tissue, eccentrically situated nuclei of hepatocytes and necrosis (Fig. 4). Mechanical damage of liver tissue due to larval migration resulted into large space or tunnel around the metacercariae was seen (Fig. 5).

**Figure 1**: Photograph showing a metacerariae of C. complanatum in the body cavity and liver of Nandus nandus

**Figure 2**: Clinostomum complanatum X15

**Figure 3**: Microphotograph of a cross section of normal liver showing hepatocytes (HC), granular cytoplasm (GC) and nuclei (N) X400

**Figure 4**: Microphotograph of a cross section of liver of N. nandus infected by C. complanatum showing necrosis (N) X100
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Figure 5: Microphotograph of a cross section of liver of N. nandus infected by C. complanatum showing loosening of hepatic tissue (LHT), enucleated hepatocytes (EH) and eccentric nuclei of hepatocytes (ENH) X400

3.2 Haematological investigation

During the course of investigation, in non-infected specimens of Nandus nandus, the total haemoglobin content, number of erythrocytes, granulocytes and lymphocytes were observed to be 5.79±0.57g/100ml, 1.266±0.049 x10⁶/mm³, 71.59±4.37x10³/mm³ and 54.93±5.39 x10³/mm³, respectively.

While in the specimens of Nandus nandus infected by Clinostomum complanatum, the total haemoglobin content was 3.51±0.43g/100 ml and the number of erythrocytes 0.878±0.59x10⁶/mm³ and increase in the number of granulocytes and lymphocytes was 95.21±4.01x10³/mm³ and 77.45±3.91x10³/mm³, respectively (Tab. 1).

Table 1: Haematological parameters of non-infected and infected Nandus nandus parasitized by Clinostomum complanatum (values expressed in Mean values ±SD)

| S.No. | Variables         | Non-infected (N=15) | Infected (N=15) |
|-------|-------------------|---------------------|-----------------|
| 1.    | Total Haemoglobin | 5.79±0.57g/100ml    | 3.51±0.43g/100 ml |
| 2.    | Erythrocytes      | 1.266±0.049 x10⁶/mm³ | 0.878±0.59x10⁶/mm³ |
| 3.    | Granulocytes      | 71.59±4.37x10³/mm³  | 95.21±4.01x10³/mm³ |
| 4.    | No. of lymphocytes| 54.93±5.39 x10³/mm³ | 77.45±3.91x10³/mm³ |

4. Discussion

The present study describes the histopathological and haematological alterations induced by digenetic trematode in Nandus nandus. Leidy (1856) created the genus Clinostomum for the forms which develop into adult in the mouth cavity of the avian hosts and have intertesticular space, genital pore behind the anterior testis and uterus with an ascending limb only. C. marginatum (Rudolphi, 1819) was described as the type species and synonymised it as C. complanatum (Rudolphi, 1819). Tabangui and Masilungan (1944) described C. ophiocephali from the gall bladder of Ophiocephalus striatus. Pandey (1973) collected C. piscidium from the buccal cavity and oesophagus of piscivorous birds, Bubulcus ibis (Linn.) and Ardeola grayii (Sykes). The metacercaria is naturally found infecting freshwater fishes, Nandus nandus and Trichogaster fasciatus.
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Histo-pathological investigations made on the histology of liver infected by *C. complanatum* revealed several histological alterations. Beverly-Burton (1963) reported that the presence of metacercariae of this parasite, in some sensitive organs in *Clarias* species do not necessarily imply a debilitating impact on the fish, even at relatively high infection loads. Under present study severe pathological changes have been observed in the liver resulting in the formation of spaces occupying cavity created by metacercariae which might be due to toxic substances secreted/excreted by the parasite. Sommerville (1982) also reported that pronounced inflammatory response and focal haemorrhages accompanies penetration and early migration of metacercariae. The inflammatory reaction, predominated by infiltrating macrophages, is particularly intense around unencysted migrating metacercariae and preceded the eventual enclosure in a fibrous capsule of the encapsulating metacercaria (Yekutiel, 1985).

Harris *et al.* (2005) observed atrophy of the liver tissue of *C. punctatus* due to *E. heterostomum* infection, which may be a result of inflow of nutrients from the host tissue to the metacercariae. They also observed compressed and distorted hepatocytes due to the metacercarial growth in the liver.

Observation made in infected *Nandus nandus* in relation to erythrocytes, lymphocytes and granulocytes counts get confirmations from the works done by various parasitologists like Engelherdt *et al.* (1989) studied haematological changes in Rainbow trout infected with *Proteocephalus neglectus*. Saxena and Chauhan (1993) found increase in lymphocyte count in *Heteropneustes fossilis* infected with *Lucknowia indica*. A higher degree of eosinophilia was observed in *Clarias batrachus* carrying helminth infections (Sinha, 2000). The reduction in RBCs count, Hb value and packed cell volume in the infected catfish occurred as a result of the parasitic infestation that often leads to anemia (Martins *et al.*, 2004). According to Lebelo *et al.*, 2001 and Hassen, 2002 the increase in WBCs count occurred as a pathological response since these WBCs play a great role during infestation by stimulating the haemopoietic tissue and immune system by producing antibodies and chemical substances working as defense against infection.

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