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ON *THELOHANELLUS OPHTHALMICUS* (MYXOZOA : MYXOSPOREA) AND HISTOPATHOLOGICAL CHANGES DUE TO THIS PARASITE IN THE GILLS OF *CATLA CATLA*

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INTRODUCTION

Nigrelli and Smith (1938), while reviewing the results of earlier studies, stated that myxozoans did little, if any, harm to the host tissue. This view is still commonly held inspite of reports of pathological changes.

Nigrelli and Smith (1940) were the first to classify the host reaction to myxozoan invasion as an inflammatory reaction. They pointed out that changes caused by *Henneguya exilis* in the gills of *Ictalurus punctatus*, as described by Kudo (1929), were typical of inflammatory response, although this had not been recognised by Kudo himself.

Jakowaska and Nigrelli (1953) observed extensive damage associated with accumulation of lymphocytes and fibroblasts in host infected with *H. electricus*. They also found degenerative changes in the liver, kidney and heart. They did not, however, consider that this was a typical inflammatory response.

Myxobolus lintoni caused marked changes in the epidermis and hypodermis of skin leading to deformation of lesions which eventually developed into large tumour masses in *Cyprinodon variegatus* (Nigrelli and Smith, 1938). The tissue response in such cases manifests itself by the proliferation of fibroblastic material which forms a supportive framework for the developing spore mass. In addition to the local reaction of cyst production, hyperplasia of epithelium with increase in number of dermal gland cells, mucous and squamous cells was observed in *Ameiurus nebulosus* when infected with *H. ameiurensis* (Nigrelli and Smith, 1940). However, some of the myxozoan parasites appear to be innocuous and may not induce any response in the host tissue (Greven, 1956).

M. exiguus infecting the gills of mullet has been reported from the Black Sea (Petrushevskii and Schulman, 1961). The gill filaments were obliterated by cycts which burst causing extensive haemorrhage.

Aisa (1972) did not observe any histological changes in the gills of tench *Tinca tinca*, infected with *M. ellipsoides braemaeformis*. The only effect observed was that of mechanical pressure resulting in the reduction of cell layers surrounding the cyst, deformation or atrophy of the secondary lamellae, but the condition of the fish was not impaired appreciably.

Taylor and Haber (1974) reported granuloma formation as the host response in the trout infected with *Myxosoma cerebralis*. McCraren, Landolt and Hoffman (1975) described different manifestations of *Henneguya* infection in Channel catfish according to the tissues parasitised and site of spore formation ; there were two types of gill infections causing intra-and interlamellar cysts.

Dykova and Lom (1978) studied in detail the histopathological changes caused by *H. psorospermica* and *H. creplini* and observed various types of tissue reactions. They also found changes brought about by a change in the temperature. Kalavati and Narasimhamurti (1985) studied the histopathological changes brought about by *H. waltairensis* in *Channa punctatus*. Early stages induced hypertrophy of the host tissue with associated vacuolization of the cell cytoplasm. In later stages macrophages accumulated and the rupture of the cyst was associated with haemorrhage. Obviously, the histopathological changes produced by myxozoan parasites vary and are of different types.

Catla catla is one of the commonly reared carps in India. Gills, fins, eyes, spleen and intestine are usually found infested by the myxosporean parasite, *Thelohanellus ophthalmicus* Haldar, Das and Sharma, 1983 ; gill is the most frequently infected organ. An attempt has, therefore, been made to study the effect of *T. ophthalmicus* on the gill of *C. catla* and the corresponding host response by empoying various histological stains.

ABBREVIATIONS USED

Ca	Cartilage	NP	Neutrophil
CGN	Capsulogenous nucleus	NPC	Neck of polar capsule
Cy	Cyst	PC	Polar capsule
CyW	Cyst wall	PlC	Plasma cell
DS	Degenerating spores	PF	Polar filament
EP	Eosinophil	RZ	Repairing Zone
Fb	Fibroblast	S	Spores
GF	Gill filaments	Sb	Sporoblasts
IBS	Interbranchial septum	SGL	Secondary gill lamellae
ILC	Interlamellar cells	SP	Sporoplasm
IV	Iodinophilous vacuole	SPNi	Sporoplasmic nuclei
LC	Lymphocyte	SW	Spore wall

MATERIAL AND METHODS

Catla catla were examined for myxosporean parasites. Spores were mounted in glycerine jelly to clear the coils of polar filaments inside the polar capsules. Dry smears of spores were stained with Giemsa after fixation in acetone free methnol. Permanent

preparations were made with Heidenhain's iron haematoxylin after fixation in hot Schaudinn's fixative (50°C).

For scanning electron microscopy, spores were fixed in 4% glutaraldehyde in phosphate buffer, washed, dehydrated and critical point dried in carbon dioxide. Specimens were gold coated in sputter coater and finally scanned under JEM 1200 EX JEOL make electron microscope.

For histopathological studies infected gill filaments of small sized *C. catla* were isolated and cleaned free from adhering mucous, using a fine camel hair brush without causing damage either to the lamellae or to the cysts. Gill filaments were fixed either in 10% neutral formalin, Carnoy's fluid or alcoholic Bouin's fluid and sectioned at 5-7μm thickness. Sections were stained with haematoxylin-eosin, periodic acid-Schiff, alcian blue method, and Giemsa's stain to study the various histological changes.

RESULTS

Thelohanellus ophthalmicus Haldar, Das and Sharma, 1983

Syn. : *T seni*, apud Chakravarty and Basu, 1948

[Fig. 1 (a-b), Plate I (a-g)]

Cyst : Milky-white coloured present mostly in the fins and gills ; spherical in the fins [Plate I (c)], 0·152-0·475mm in diameter, oblong in the gill arch [Plate I (b)] 0·442-0·608 × 0·190-0·494mm and elongated in the gill filaments [Plate I (a)] 0.323-1·235 × 0·190-0·437mm in size ; located in the proximal side of gill filaments. Rarely, cysts are also seen in the spleen and intestinal wall.

Spore : Pyriform in valvular view, anterior end narrow and rounded, posterior end broad [Fig. I (a), Plate I (d) (f)]. Saucer-shaped in sutural view [Plate I (g)]. Valves symmetrical and uniformly thick with prominent and straight sutural ridge [Plate I (g)]. Sutural line visible with scanning electron microscope [Plate I (g)]. Polar capsule one, pear-shaped, more than half of spore length, a small tubular neck visible at the anterior end, contains 6-7 coils of polar filaments [Fig. I (a), Plate I (d)]. Polar filament thread-like when extruded [Fig. I (b)] ; rounded capsulogenous nucleus present at the periphery of polar capsule near the posterior end on the lateral side [Fig. I (a), Plate I (e)] Sporoplasm cup-shaped, granular, homogeneous [Fig. I (a), Plate I (d)]. An oval iodinophilous vacuole present in the sporoplasm [Fig. I (a), Plate I (e)]. Two sporoplasmic nuclei present in the sporoplasm lateral to iodinophilous vacuole, placed obliquely in most of the spores but horizontal in some [Fig. I (a), Plate I (e)].

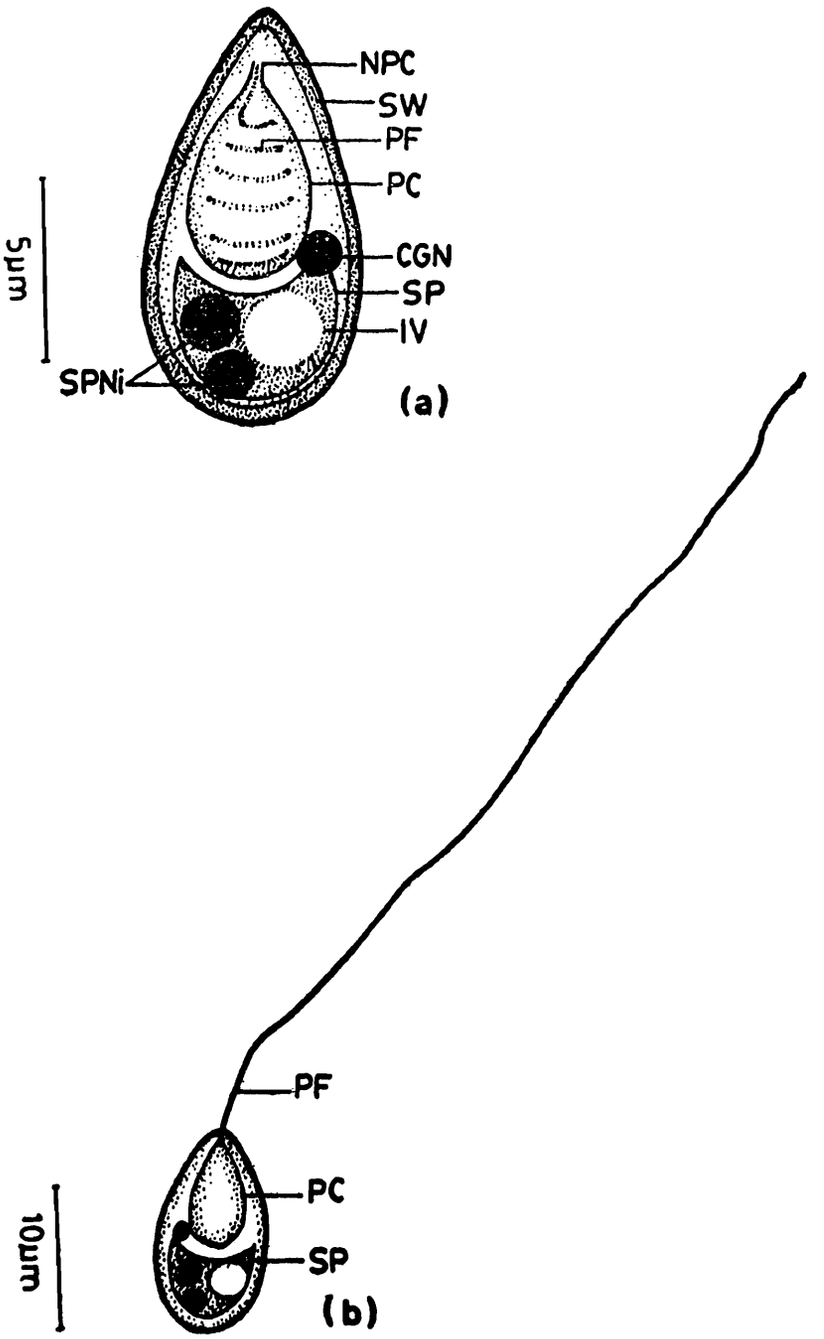

Fig. 1 : *Thelohanellus ophthalmicus* Haldar, Das and Sharma, 1983
(a) A spore in valvular view : Schaudinn/Iron haematoxylin,
(b) A spore with polar filament extruded : Methanol/Giemsa.

*Measurement :

Spore

length	11-14	(12·4, 0·952)	breadth	7-9	(7·967, 0·741)
Polar capsule					
length	6-7	(6·233, 0·359)	breadth	4·5-6	(4·933, 0·359)
Polar filament		62-71			
Iodinophilous vacuole		1·7-2× 1·1-2			

(Figures within parentheses indicate mean and standard deviation of fifteen specimens)

* All the measurements are in microns (µm) unless otherwise stated

Host : *Catla catla* (Hamilton)

Site of infection : Gills, fins, spleen and intestine

Locality : Harike (Punjab) ; India

Dates of collection : 19th and 20th February 1986

Remarks : Polar filaments of present specimens are quite long as compared to those in the original description [27·5-50·5 (39·4)]. Haldar, Das and Sharma (1983) reported this parasite from the eye of *C. catla*.

Chakravarty and Basu (1948) redescribed *T. seni* (Southwell and Prashad, 1918) Kudo, 1933 from the gills of *Catla catla*. But from the figures and measurements given by Chakavarty and Basu (1948) the specimen seems quite different from those described by Southwell and Prashad (1918). The spores, as illustrated by Southwell and Prashad (1918), are pointed at the anterior end and have a smaller polar capsule whereas in the diagram given by Chakravarty and Basu (1948) spores are narrower with bluntly pointed anterior end and a larger capsule. It is evident that Chakravarty and Basu's specimens are quite different from those described by Southwell and Prashad (1918). However, they resemble *T ophthalmicus* Haldar, Das and Sharma, 1983 (which is also described from *Catla catla*) in shape and size.

HISTOPATHOLOGICAL STUDIES

Gills of *Catla catla* were found heavily infested with *Thelohanellus ophthalmicus*. The cysts were present between the gill filaments on the proximal side of the gills near the gill arch [Plate I (a)]. Cysts showed heavy burdens of parasites [Plate II (c), (d)]. Observations on fully formed myxozoan cyst show that cyst wall is formed of a layer of stratified epithelium, a band of connective tissue and a layer of granulated cytoplasm beneath it. It contains a large number of immature spores (sporoblasts) along the periphery [Plate II (e)] and lumen is filled with mature spores embedded in the matrix of mucous material [Plate II (e), (f)]. The connective tissue band is positive to PAS and Alcian blue [Plate II (g), (h)].

Large amount of mucous is secreted in the infected fish. The damage done to the gill tissue is quite extensive. Mechanical pressure, due to increase in size of the cyst, results in destruction of gill lamellae [Plate II (c)], displacement of musculature of the interbranchial septum [Plate III (a)] and necrosis of the tissue surrounding the cysts [Plate II (c), III (b)]. Cartilage [Plate II (d)] and the secondary lamellae are replaced by the developing cyst [Plate II (c)]. Secondary gill lamellae are completely destroyed in the proximal area of the gill filaments and also above the cysts on the distal side of gill filaments [Plate II (c), III (b)]. Damaged epithelial cells and pillar cells exfoliate. This results in atrophy, degeneration and other necrobiotic changes.

Clubbing of the gill filaments takes place due to excessive proliferation of interlamellar cells, filling up spaces between secondary lamellae [Plate III (c)].

Tissue reaction is manifested by cyst formation or fibrosis due to the proliferation of fibroblastic material which forms a supportive framework for the developing spore mass [Plate II (c), (d)]. Cysts are produced by the host around the parasite to minimise the irritation caused due to the presence of parasite.

In addition, focal accumulation of a large number of neutrophils, eosinophils, some lymphocytes and a few plasma cells in the immediate vicinity of the infection is observed, showing a typical inflammatory response. Only a few erythrocytes are observed in this area [Plate III (d-h)]. Repairing of the host tissue at the site of infection is also seen [Plate IV (a), (b)] where fibroblasts, lymphocytes, a few plasma cells and neutrophils are observed among the degenerating spores [Plate IV (c), (d)].

DISCUSSION

An acute infection of *Thelohanellus ophthalmicus* Haldar, Das and Sharma, 1983 is observed in the gills of *Catla catla*. The epithelium of gills forms a barrier between the fish's blood and the surrounding water. Gaseous exchange needed to sustain life takes place through this barrier. Any morphological alteration by the parasites hinders the respiratory, secretory and excretory function of this organ.

Clubbing of the gill filaments due to excessive proliferation of interlamellar cells is an adaptive measure to protect the gill filaments from continual irritation caused by the lack of an adequate external gill covering. Clubbing of the gill lamellae was also observed by Takashima (1982).

Large amount of mucous is secreted which is an indication of the irritant nature of the parasite. It is presumed that abundant mucous mechanically disrupts gill function and causes asphyxiation of the fish.

Host response to this parasite is quite adequate as large number of neutrophils, eosinophils, lymphocytes and plasma cells are observed in the immediate vicinity of the cyst. Repairing of the infected tissue is also clearly observed in the gills. A large number of inflammatory cells are present along with the degenerating spores. A few fibroblasts are also seen in this repairing zone indicating a strong host response for this parasite. This is not typical of granuloma formation, as observed by Dykova and Lom (1978) in the gills of a perch as tissue response to *H. psorospermica*, as the pseudoepithelial cells are not observed surrounding this mass in the present study. Dykova and Lom (*loccit*) found that when the cyst is full of mature spores an inflammatory reaction is mounted resulting in the rapid replacement of the cyst by gtanulomatous tissue. Their observations are in agreement with those of Finn and Nielsen (1971a, b) and Lucky (1970) who found that host tissue response to *Myxobolus ellipsoides* begins only when the parasite has reached a certain minimum size. Dykova and Lom stated that at first relatively small cysts are overlaid by massive hyperplasia of the adjacent epithelium. This is followed by invasion of the parasitic mass by macrophages, which

remove the spores by phagocytosis. Later infiltration by fibroblasts and histiocytes complete the typical granuloma formation. The outer layers of the granuloma are formed by pseudo-epithelial cells derived from mesenchymal cells. In contrast to this, in the present studies cysts are not invaded by inflammatory cells ; instead, infiltration of neutrophils, eosinophils, lymphocytes and plasma cells is observed close to the cyst. The spores are seen in this inflamed mass ; the latter is infiltrated by fibroblasts resulting in repairing tissue adjacent to the cyst. Almost similar findings were given by Hoffman, Putz and Dunbar (1965), while discussing the histopathology of *Myxosoma cartilagini* in centrarchid fish.

Recently Kalavati and Narasimhamurti (1985) studied the histopathological changes in the gills of *Channa punctatus* infected with *Henneguya waltairensis* but the host response was poor in that case. They did not observe hypertrophy of the host tissue and vacuolisation of the associated cytoplasm. They also observed that when the cysts were mature, degenerative changes appeared more conspicuous and were associated with accumulation of macrophages ; the rupture of the cyst was associated with haemorrhage with this parasite. Rupturing of the cyst is not observed in the present studies.

An early healing response to *Henneguya ameiurensis* in the barbels of *Ameiurus nebulosus* was seen by Nigrelli and Smith (1940). However, they could not observe later stages of repair. Besides the mucoid material in the cysts, they observed lymphocytes, some fibroblasts and occasionally melanophores.

In conclusion, degeneration and repairing of the host tissue as host response, confirms the pathogenic nature of this parasite.

SUMMARY

Thelohanellus ophthalmicus Haldar, Das and Sharma, 1983 was found infecting the gill filaments of a freshwater fish, *Catla catla*. Morphological studies on this parasite have been carried out using light microscopy and scanning electron microscopy. Histopathological changes caused by *T ophthalmicus* in the gills of *Catla catla* have been studied in detail using various histological stains.

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