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ORIGINAL ARTICLE

Studies on Histological Changes in Fresh Water Fishes after Protozoan Parasite *Myxobolus mulleri* Infection in River Asan at Murena District

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ABSTRACT

Infection to fishes is a major problem for fish farming as they destroy a large production and cause economic loss to fish producers. The infection may be due to fungus, bacteria or protozoa. Infection from different parasites in edible fishes cause economical as well as health related loss. Sporozoan parasites may be found in all organs of fishes but in the skin, they are relatively rare in comparison with other organs. The little knots are formed when the parasite are situated in the skin, while bumps and pimples originate from a process in the muscles. The present paper deals with infection caused in fishes due to Myxobolus mulleri in river asan at district Muerena.

***Key words**: Myxobolus mulleri, River Asan, Murena District*

INTRODUCTION

Many fresh water fishes are infected with sporozoan parasites, these parasites often cause serious problems. Myxosporidians cause black coloured nodular growths on body surface and hence the name black spot disease. Blackish nodular growths were noticed at the base of the caudal peduncle and paired fins of fry and fingerlings of rohu which were identified as Myxobolus cysts. Myxosporidian disease affecting the muscles of fresh water fish can be of considerable significance. *Myxobolus pfeifferi* the causative agent of boil or bulbonic disease of barbel in USSR appeared in an epizootic form in the wild when rivers became polluted (Petrshevskii, 1961). The combination of pathogens and pollution has been balanced for these episodes which can destroy the myofibrils and progressively form cysts giving the fish a bulbonic appearance. Eventually, the cysts rupture resulting into the death of the host in which muscles are damaged and all cellular functions are blocked. These samples were collected from a sewage pond which might be a depot of these protozoan parasites and thus very obviously affected the stocks in the pond (Das and Mukherjee, 1998). Cystic condition in the intercellular space of muscles also indicated the spreading nature of these parasites which could damage the hemopoietic system of fish and described the hyperplastic nature of gill epithelium, degeneration in cases of myxosporidiasis. If these fishes are cultivated in polluted or sewage fed water bodies may carry infections and can succumb to disease, Mc Craren *et al*. (1975), Dykovan and Lom (1978). The present study is aimed to record parasitization of organs such as skin, muscles and gills by various species of Myxobolus organism.

The great majority of the infectious diseases of fishes are mostly caused either by bacteria or by Protozoans. Among the Protozoans the sporozoans are the largest in number. These Protozoans are endoparasites occur in the skin, muscle and gills and are causative agent of various diseases in fresh water fishes. Among the sporozoan parasites Myxobolus is an important parasitic Protozoan, its many species are pathogenic in nature, often causing fatal diseases or even death to host fish. Myxobolus parasitic Protozoans affect fish population by causing mortality, reduction in growth, weight loss, and suppression of reproductive activity. The significance of recognizing these parasites increases with the development of aquaculture.

The life cycle of Myxobolus parasite is not uniform. Infection of the host occurred by spores, two haploid nuclei after fusion become diploid zygote with mononucleus. This zygote grows up in the infected organ of the host and divide by multiple nuclear fission. Vegetative stages produced as trophozoite and they also multiply themselves further by fission. The growth and reproductive phases of trophozoite follow the formation of spores. The spores of Myxozoans are characterized

by two or some time more than two rod shaped shells which have two polar coiled filaments. It is reported that spores in the host fish are ingested through mouth, the spores then shoots off the polar capsule in digestive tract and fasten firmly to the intestinal wall. The amoeboid young which presumably hatch from spores in the intestine, penetrate lymph vessel and blood and reach to different body parts.

MATERIALS AND METHODS

The present experimental work was started in the month of January 2009 and observations were made round the year. Local fisherman of Asan River were contacted and fishes in tin container brought in laboratory. The fishes were collected alive from different study sites.

The following sites were selected for collection of fishes-

Chonda gaon	-	Site A
Jaroni gaon	-	Site B
Karua gaon	-	Site C
Girgoni gaon	-	Site D
Kutwal gaon	-	Site E
Silata gaon	-	Site F

HISTOPATHOLOGICAL STUDY OF SPECIFIC BODY TISSUE

Experimental fishes anaesthesized after taken out from aquarium. Then they in Ringer's solution dissected which prepared freshly in laboratory before dissection. Ringer solution prepared by adding- 20ml 0.154 KCl solution; 20ml 0.11M $CaCl_2$ solution; 960ml 0.154 M NaCl solution. The body organs viz. skin, muscles and gills were quickly removed and fixed in 30% formaline for 4-6 hours which used as buffer. These dissected organs cut into small size of 3-6mm thickness in order to penetration of fixative and fixed for 72 hours in two stock solution for proper penetration and observation of Myxobolus sporozoans.

STOCK SOLUTION- A:

Prepared- 0.2 M Na_2HPO_4 solution

Add 400ml 0.2 M Na_2HPO_4 solution in to 1000ml of 4% formaline buffer solution

STOCK SOLUTION- B:

Prepared- 0.2 KH_2PO_4 solution

Add 400ml 0.2 M KH_2PO_4 solution into 1000ml 12.98 M HCHO.

Then 400 ml of distilled water was added to both stock solution (David *et al*., 1972).

WASHING AND PRESERVING:

When organs were properly fixed, the excess fixative removed by washing of organs in tap water, then transfer them into 70% alcohol (C_2H_5OH) for 3-4 hours.

DEHYDRATION OF TISSUE ORGAN:

After washing the tissue properly dehydration process started through a series of C_2H_5OH viz. 30%, 50%, 70% and 90% with one change in each concentration and with 45 minute duration in each case for the dehydration, then dehydration confirm with xylol. Finally, the tissue were passed through absolute alcohol for 60 minute.

EMBEDDING:

The dissected organs then transferred to a bath of molten paraffin wax in an embedding oven for infiltration and impregnation and kept at 45-60^{0}C for one hour.

MICROTOMING:

The tissue blocks after trimming for microtomy section put on 820 Spencer Rotatory microtome to cut 5 µm sized serial sections. The ribbon of tissue section, so obtained fixed on a slide with the help of Meyer's albumin, flattened on hot plate, passes through one change of xylene then treated with descending series of graded C_2H_5OH, stained with Ehrlich's Hematoxylin-eosin, washed with water, dehydrated in ascending series of graded C_2H_5OH and xylene.

Ehrlich's haematoxylin (Lillia, 1965):

Distilled water	-	100.00ml
Alcohol (C_2H_5OH)	-	100.00ml
Glycerine	-	100.00ml
Haematoxylin	-	1.5gm
Ammonia alum	-	3.0gm

Eosin (Lillia, 1965):

Distilled water	-	50.0ml
Absolute alcohol	-	5.0ml
Acetic acid	-	1 drop
Aqueous picric acid	-	5.0ml
Potassium dichromate	-	0.25gm
Eosin	-	0.5gm

Mounting: Now, finally the stained sectioned were mounted with mounting media, Canada Balsam.

RESULT AND DISCUSSION

The percentage of Myxobolus infection in different tissues are presented table-28. Muscles showed the highest percentage of Myxobolus infection (78.94%), gills (15.30%) and skin (4.76%).

Table 1: Showing infection percentage in fish organs

Total number of parasites in all fishes	Organs of fishes	Percentage
219	Skin	4.76%
	Gills	15.30%
	Muscles	78.94%

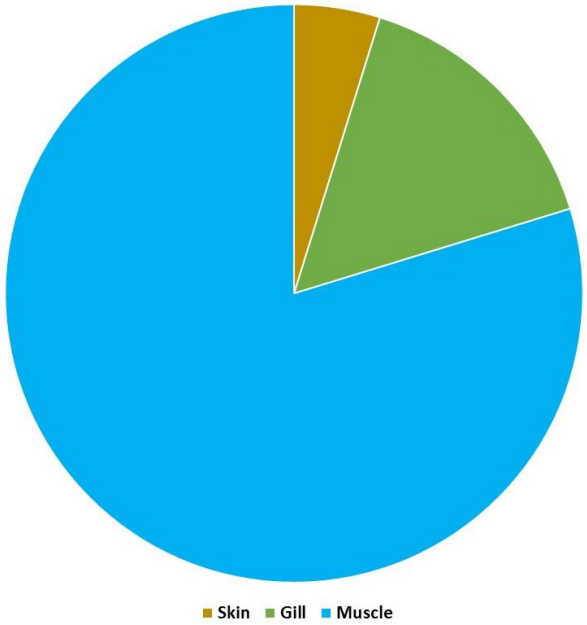

Fig. 1: Pie chart showing infection percentage in different organs of fishes

***Myxobolus mulleri*, (Butschli, 1982) INFECTION:**

Channa striatus, a fresh water fish is the host of this species of *Myxobolus*. Scanty information of *Myxobolus mulleri* is observed and the intensity of infection is rather poor, only 2-4 parasites are observed in slide. A low infection of *Myxobolus mulleri* is observed only 3 fishes out of 70 fishes examined are found to be infected. During observation pimples on the skin, fins and near lower jaw seen infected. The pimples are dark reddish in colour and giving ugly appearance to fish just like as measles and mumps at different locations. Spores are oval in shape in frontal view and lemon shaped in lateral view. Spore valves are relatively thin, symmetrical and smooth. Spores are 3.0µm-

3.5µm in length and 2.0µm-2.5µm in width. They are present in various sizes. Spine like projections occur in posterior side of endoplasm of spore. Polar capsule are two in number which are pyrifrom in shape. Size of polar capsule are 1.2µm-1.5µm in length and 0.6µm-0.8µm in width. Polar capsule are present at the anterior part of the spore cavity. Polar filaments are coiled with 6 to 12 turns in polar capsule, situated perpendicular to the longitudinal axis of the capsule. The sporoplasm is without a vacuole.

Table 2: Measurement of *Myxobolus mulleri* parasites in fresh water fish

Range	Experimental findings
Length of spore	3.0µm-3.5µm
Width of spore	2.0µm-2.5µm
Length of polar capsule	1.2µm-1.5µm
Width of polar capsule	0.6µm-0.8µm

Das *et al*. (2000) reported the percentage of Myxobolus infection in different body tissue in which liver showed the highest percentage of cysts (37.5%) followed by kidney (25%), heart (20%), gills (15%) and skin and muscles (12.5%) in tissue section. In present study the pathology of Myxobolus parasites is a spontaneous case. In present observation muscles showed the highest percentage of cyst (78.94%) followed by gills (15.30%) and skin (4.76%). In present observation locality Silata gaon out of the six localities is highly infected with myxobolus. On this area is sewage treated and the water seems to be much polluted but its research are scanty.

Histopathological changes were more pronounced in the skin. Polymorphonuclear leucocytes were abundant in the subepidermal and epidermal layers which showed marked thickening. According to Das *et al*. (1991) and Das and Mukherjee (1998) melanophores may be engulfed by dermal macrophages which migrate through the epidermis to release them into the surface mucous. Gordon (1959) has stated that extensive melanophore damage malpighian cells or macrophages within the epidermis may contain large number of melanosomes in their cytoplasm. Melanin derived from melanocytes containing large number of melanin granules, while in the present study necrosis is noticed only on the skin at a place where infection is present and melanophores are observed in rupture form by *Myxobolus oviforme* in *Clarias batrachus*. No calcification is reported in this case. In present observations pimples in *Channa striatus* infected within *Myxobolus mulleri*, on the skin, fins and near lower lips. The pimples were reddish in colour giving ugly appearance to fish just like as measles and mumps in human beings (at different locations). In the present investigation, it is found that the highly infected locality is Silata gaon (site F) in Murena district while least infected is Jaroni gaon. Rare cystic nodules are observed on the body and at the base of fins of infected fishes. A few number of Myxobolus cysts are observed from body pigmentation.

Site of infection in muscles is reported in present investigation. The muscles are stretched and hyperchromatic leading increased thickeness of muscles due to infections of *Myxobolus shekhai* sps., *Myxobolus cultus*, *Myxobolus dujardini*, *Myxobolus cycloid* in *Channa striatus* while in *Clarias batrachus* and *Heteropneustes fossilis* infected by Myxobolus cycloid and vegetative growth of the cysts is also noticed. In the investigation of both specimens the muscular cells are found to be ruptured. The calcification of cells is observed by all species of Myxobolus. Many fishes are taken for this study such as *Channa striatus*, *Heteropneustes fossilis*, *Labeo rohita*, *Wallago attu* and *Clarias batrachus*. The highest calcification is found in *Channa striatus* due to the infection of *Myxobolus oviforme*, however, calcification by Myxobolus cycloid in *Clarias batrachus* is low infection, while in *Labeo rohita* and *Wallago attu* calcification is the lowest. In *Channa striatus* the blood capillaries of intermuscular region are highly scattered and ruptured due to calcification by *Myxobolus oviforme* and *Myxobolus mulleri*. In *Labeo rohita*, *Wallago attu*, *Heteropneustes fossilis*, the blood capillaries are shrunken by *Myxobolus oviforme* parasite. Mishra *et al*. (1982) reported enzootic nature of myxosporiadiasis in Indian major carps.

Of the various species of *Myxobolus* reported so far, the present species conforms with *Myxobolus mulleri* reported from various parts like ovaries and kidney of minnos and the gills of Squalius cephalus (Butschli, 1982). Butschli had mentioned the length of spore 8.0µm-9.0µm and the width

6.0µm. the length of polar capsule were 3.0µm and width 2.0µm-2.1µm, where as in the present study the length of spore is 3.0µm-3.5µm and width 2.0µm-2.5µm. the length of polar capsule is 1.2µm-1.5µm and width is 0.6µm-0.8µm. Butschli also reported this species from ovaries, kidney and gills, while present species is reported from gills only. The structure and coiling turns of polar capsule are the same in both investigations. Two polar capsules are equal in size and spine like projections present at the posterior end of the spore as reported by Butschli (1982). Same species is also found in present investigation. Moreover, the present species is identified from *Channa striatus*, fresh water fish of Silata gaon (site F), while Butschli reported the species from marine fish. On the basis of the above similarities, the present specimens of *Myxobolus* are identified as *Myxobolus mulleri* Butschli (1982). He reported this species cause pimple disease in *Squalius cephalus* as observed in present study.

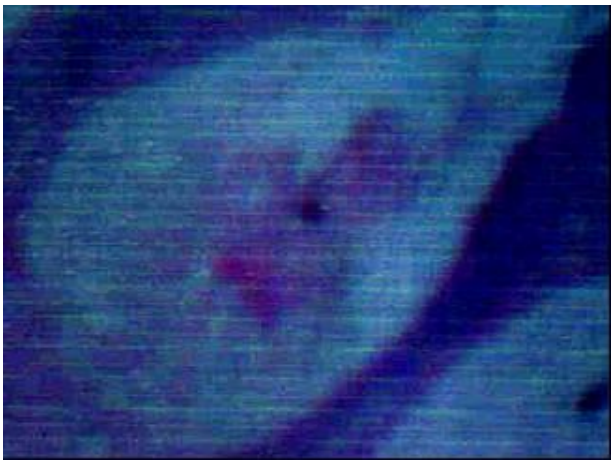

Plate1: Skin showing rupture of melanophores

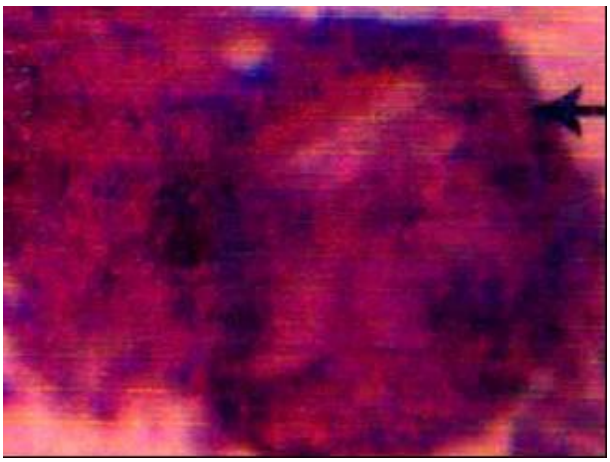

Plate 2: Degeneration in muscles

Plate 3: Swelling in gill lamellae

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