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ORIGINAL ARTICLE

Studies on Myxobolus Infection in Edible Fishes with Special Reference to *Myxobolus cycloid* and *Myxobolus mulleri*

Manisha Deshpande
Department of Zoology, Govt. S.L.P. (PG) College, Gwalior (MP)
Email: ananddeshpande158@gmail.com

ABSTRACT

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. Keeping these facts in mind present study highlights the infection from specific species of Myxobolus in fishes with their details.

***Key words**: Myxobolus Infection, Myxobolus cycloid, Myxobolus mulleri*

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INTRODUCTION

Surveys of epidemic diseases of fishes are frequently found to be due to myxosporidian infections. According to Davis (1924), the Wormy halibut of the pacific coast of north America is due to *Unicapsula muscularis*, which invades the muscular tissue of the host fish. The boil disease of the harbel (*Barbus barbus*) and others of European waters, is caused by *Myxobolus peifferi* (Keysselitz, 1908). The intensification of fish culture also produces a number of problems, including the occurrence of fish diseases. Besides this poor sanity conditions at many hatcheries diets also cause some diseases. The diseases have been one of the major checks in the development of aquaculture. Among these diseases, certain Protozoan diseases are responsible for heavy mortality of fishes.

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 divides 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.

In the present study, an attempt has been made to study infection of Myxobolus parasitic Protozoans in some edible fresh water fishes of River Asan of Murena district. The work also provides an attempt to describe the Myxobolous on different organs of infected fishes viz. skin, muscles and gills.

MATERIALS AND METHODS

The present experimental work was started in the month of January 2009 and observations were made round the year. Local fishermen 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 tissues 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 (Lillie, 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.

RESULTS AND DISCUSSION

***Myxobolus cycloid*, Gurley 1893:-**

Channa striatus, *Heteropneustes fossilis* and *Clarias batrachus* fresh water fishes are the host of *Myxobolus cycloid*. A heavy infection of *Myxobolus* is observed, 6-12 parasites are obtained in a slide.

Table 1: Specific site of infection of *Myxobolus cycloid* parasite in each host

Host	Site of infection	Sites
Channa striatus	Muscles	Silata gaon (Site-F)
Heteropneustes fossilis	Muscles	Silata gaon (Site-F)
Clarias batrachus	Muscles	Silata gaon (Site-F)

INCIDENCE

a heavy infection of *Myxobolus cycloid* is observed, 5 fishes out of 20 fishes examined are found to be infected.

MORPHOLOGY

Spores are ellipsoidal in shape, spore valves are relatively thin, symmetrical and smooth. Spores are 2.6µm-2.9µm in length and 1.6-2.2µm in width. A round iodinophilous vacuole is present in sporoplasm. Polar capsules are two in number, pyriform in shape, equal in size. Size of polar capsules are 1.3µm-1.7µm in length and 0.7µm-1.0µm in width. Polar capsules are located at the anterior part of the spore cavity and valves of polar capsules are symmetrical. A sporoplasm occupies the posterior half of the spore cavity.

TAXONOMIC STUDY

Host	-	*Channa striatus*, *Heteropneustes fossilis* & *Clarias batrachus*
Site of infection	-	muscles
Locality	-	Silata gaon (Site F)

DISCUSSION

Of the various species of *Myxobolus* reported so far, the present species conform to *Myxobolus cycloid* from Roach (*Rutilus rutilus*), Gurley (1893).

Gurley (1893) has mentioned the length 4.0µm-4.5µm and width 3.0µm of the spores but he had not given the measurement of the polar capsule. While in the present study, the size of spores is 2.6µm-2.9µm in length and 1.6µm-2.2µm in width. The size of polar capsule is 1.3µm-1.7µm in length and 0.7µm-1.0µm in width. Gurley reported the species from pseudobranchia of Roach (Marine fish), while in present study the species has been reported from muscles of fresh water fish. There are no marked differences between morphology of the two species.

The polar capsules are equal in size. Iodinophilous vacuole is present in both investigations. Polar capsules are located at the anterior part of the spore cavity in both cases. However, the size of polar capsules shows some variations, the present specimen of *Myxobolus* are identified as *Myxobolus cycloid* (Gurley, 1893) on the basis of morphology.

Table-2 shows measurement of spores and polar capsules in all fishes while table-3 shows comparison of size of spore and polar capsules between experimental findings and previous findings

Table 2: Measurement of *Myxobolus cycloid* (*M. cycloid*) parasites in all fresh water fishes

Range	***Channa striatus***	***Heteropneustes fossilis***	***Clarias batrachus***
Length of spore	2.8µm-3.0µm	2.6µm-3.0µm	2.6µm-2.9µm
Width of spore	1.6µm-2.3µm	1.7µm-2.3µm	1.5µm-2.1µm
Length of polar capsule	1.3µm-1.7µm	1.2µm-1.6µm	1.3µm-1.8µm
Width of polar capsule	0.8µm-1.0µm	0.7µm-1.2µm	0.6µm-1.0µm

Table 3: Measurement of *M. cycloid* parasite in fresh water fishes and previous findings

Range	**Experimental findings**	**Previous findings**
Length of spore	2.6µm-2.9µm	4.0µm-4.5µm
Width of spore	1.6µm-2.2µm	3.0µm
Length of polar capsule	1.3µm-1.7µm	No report
Width of polar capsule	0.7µm-1.0µm	No report

***Myxobolus mulleri*, Butschli, 1982:-**

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.

INCIDENCE

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.

MORPHOLOGY

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.

TAXONOMIC STUDY

Host - *Channa striatus*
Site of infection- Gills
Locality - Silata gaon (Site F)

DISCUSSION

Of the various species of *Myxobolus* reported so far, the present species conforms to *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.

Table 4: Measurement of *Myxobolus mulleri* parasites in fresh water fish

Range	Experimental findings	Previous findings
Length of spore	3.0µm-3.5µm	8.0µm-9.0µm
Width of spore	2.0µm-2.5µm	6.0µm
Length of polar capsule	1.2µm-1.5µm	3.0µm-3.6µm
Width of polar capsule	0.6µm-0.8µm	2.0µm-2.5µm

PATHOLOGICAL STUDY OF MUSCLES

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 cycloid* and *Myxobolus mul*leri in *Channa striatus*. Muscle tissues exhibited a variety of changes characterized by focal to multifocal areas of degeneration with loss of cross striations of myofibrils. Areas of marked Zenker's necrosis with mild to moderate infiltration of leucocytes and oedema were evident at many places. Myxosporidian cysts were observed inside the muscle tissue surrounded by fibrous tissue and melanin pigment.

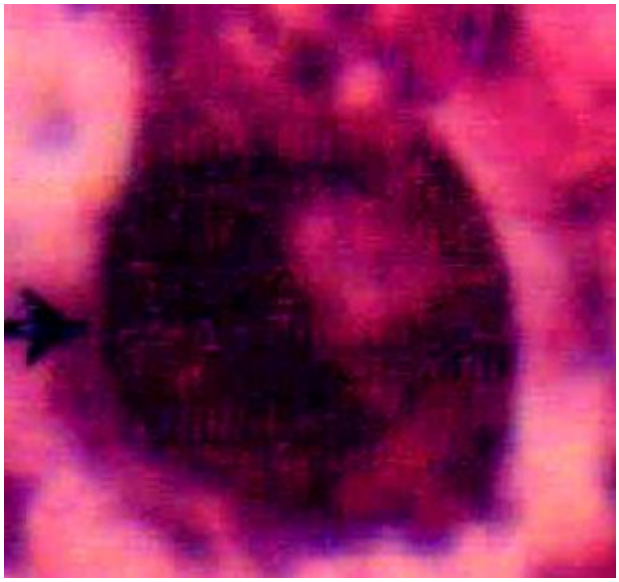

Plate1: *Myxobolus cycloid* in muscles

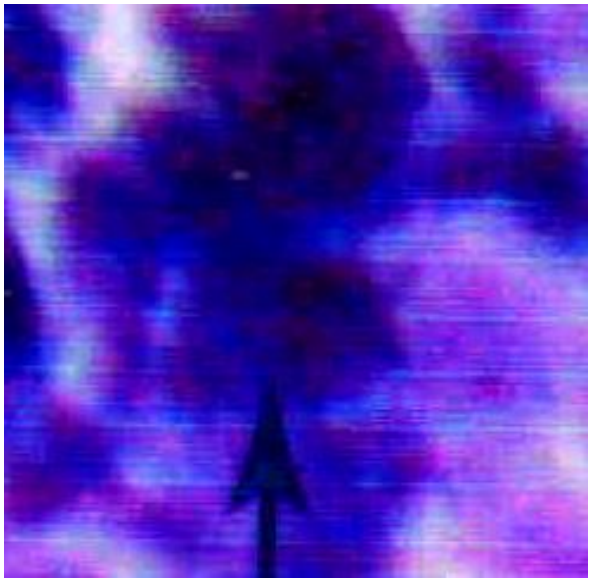

Plate 2: *Myxobolus mulleri* in muscles

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