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Research Article

Parasitic Protozoans in Some Edible Freshwater Fishes of River Asan, District Morena

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Abstract: In the present thesis, an effort has been made to study the various species of *Myxobolus* in freshwater fishes. Total 695 fishes belonging to different genera and 8 species are investigated for this purpose. Nine species of *Myxobolus* have been described. Their incidence, Morphology, and taxonomy are studied and compared. An attempt has been made to study the pathological effects of parasites on different organs viz. skin, muscles and gills. Effect of parasites on the growth of fishes is also noticed. Infection in muscles is caused by *Myxobolus cultus, Myxobolus dujardini*, *Myxobolus cycloid*, *Myxobolus oviforme*, *Myxobolus cognati*, *Myxobolus ellipsoideus, Myxobolus cerebralis.* The infection in Muscles is reported in *Channa striatus, Clarias batrachus, Heteropneustes fossilis, Labeo rohita, Catla catla, Cirrhinus mrigala* and *Wallago attu*. Gills are infected by *Myxobolus oviforme* in *Clarias batrachus*. Myxosporidian infection is noticed in gill lamellae only. Skin and muscles are infected by *Myxobolus oviforme* in *Clarias batrachus* and the infection is reported in melanophores. Calcification is reported in gills only while necrosis is only noticed in the skin.

Keywords: *Myxobolus,* Pathological effect, Sporozoans, *Channa striatus, Clarias batrachus,* Parasites.

1. Introduction

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 agents of various diseases in freshwater fishes.

Among the sporozoan parasites, *Myxobolus* has have been 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 populations 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 becomes a 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 sometimes more than two rods shaped shells which have two polar coiled filaments.

It is reported that spores in the host fish are ingested through the mouth, the spores then shoot off the polar capsule in the digestive tract and fasten firmly to the intestinal wall. The amoeboid young which presumably hatch from spores in the intestine, penetrate lymph vessel and the 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 freshwater fishes of river Asan of Morena district. The work also provides an attempt to describe the pathological effects of *Myxobolus* on different organs of infected fishes viz. skin, muscles, and gills. Effect of *Myxobolus* parasites on the growth of fishes was also studied from all localities.

2. Materials and Methods

The present experimental work was started in the month of January 2009 and observations were made around the year.

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Local fishermen of Asan River were contacted and fishes in a tin container brought in the 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
Kotwal gaon	-	Site E
Silata gaon	-	Site F

A total of 500 fishes of different species, i.e. *Labeo rohita*, *Catla catla*, *Heteropneustes fossilis*, *Clarias batrachus*, *Wallago attu*, *Mystus seenghala*, *Cirrhinus mrigala*, *Cyprinus carpio*, *Puntius ticto* and *Channa striatus* were collected and bring in the laboratory. After quarantine for one day, they selected of equal size 15-25cm length for examining the presence of Protozoan parasite.

In each aquarium 25 fishes of different species maintained. The artificial fish food of floating type pellets which is available in the market was provided to the fishes till the investigation lasted. In this food crude protein 35%, crude fat 3%, crude fiber 4% and moisture was 10%. The fishes were used for study after 7 days of collection.

The age of the fishes before parasitic study was determined in order to study the effect of infection of the parasite in age groups. For determining the age of fishes following method has been used.

2.1 Qayyum and Qasim method

It is length frequency process in which frequency analysis of the species in individuals of anyone age collected on the same data will represent variations in the mean length as the normal distribution. In the experiment, length frequency determined the age of the fishes.

Length in cms	Age in years
1-9	0
10-13	1
14-18	2
19-20	3
21-23	4
24-32	5
33-35	6

2.2 Scale method

It is the simplest and most accurate method for fish age determination. According to this method growth of the fish is at intervals. During summer, the fish feeding intensively grows in size and ridges or curculi are produced on the first formed stratum of the outer layer of the scale, which are composed of a transparent homogenous substance named hyalodentine. But in winter, when the feeding is less, the growth ceases and the cessation in ridge formation results in annuli.

During the first year, ridges are formed with no indication of any annuli. Such fishes belong to 0 age group. Number of annuli shows the age of fish as one annuli for one year.

3. Histopathological Study of Specific Body Tissue

Experimental fishes anaesthesized after is taken out from the aquarium. Then they in Ringer's solution dissected, which prepared freshly in the 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% formalin for 4-6 hours which used as a 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 solutions 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 into 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 400ml of distilled water was added to both stock solutions (David *et al*., 1972).

3.1 Washing and preserving

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

3.2 Dehydration of tissue and 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 minutes.

3.3 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.

3.4 Microtoming

The tissue blocks after trimming for microtomy section put on 820 Spencer Rotary microtome to cut 5µm 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

3.5 Mounting

Now, finally the stained sectioned were mounted with mounting media, Canada balsam.

4. Observation

For the study of Parasitic Protozoan as endoparasite 500 specimens of 7 species of freshwater fishes were collected from Asan River of Morena region from selected sites- Chonda gaon, Jaroni, Karua, Girgoni, Kotwal and Silata gaon.

Some of the fishes such as *Channa striatus*, *Heteropneustes fossilis* and *Clarias batrachus* are collected from all sites. *Labeo rohita* and *Wallago attu* are collected from Chonda gaon, Jaroni gaon and Karua gaon. *Catla catla* and *Cirrhinus mrigala* are collected from Chonda gaon. *Mystus seenghala*, *Cyprinus carpio* and *Puntius ticto* have not collected at the time of fishing.

Table 1. Fishes and sites of the collection.

S. No.	Name of fish	Sites					
		A	B	C	D	E	F
1.	*Channa striatus*	+	+	+	+	+	+
2.	*Heteropneustes fossilis*	+	+	+	+	+	+
3.	*Clarias batrachus*	+	+	+	+	+	+
4.	*Labeo rohita*	+	+	+	-	-	-
5.	*Wallago attu*	+	+	+	-	-	-
6.	*Catla catla*	+	-	-	-	-	-
7.	*Cirrhinus mrigala*	+	-	-	-	-	-
8.	*Mystus seenghala*	-	-	-	-	-	-
9.	*Cyprinus carpio*	-	-	-	-	-	-
10.	*Puntius ticto*	-	-	-	-	-	-

Fishes collected from different sites were 90, 75, 80, 100 and 170 so it is clear that abundantly they are present at a Silata gaon site. The infection reported maximum 24.70% at site of Silata Gaon while very less % (1.33) reported at site D (Girgoni Gaon).

Table 2. Infection of *Myxobolus* at different sites.

Site	No. of collected fishes	No. of infected fishes	% of infection
A	90	18	20.0
B	75	11	14.66
C	80	05	6.25
D	75	01	1.33
E	110	18	16.36
F	170	42	24.70

The incidences of infection of *Myxobolus* in all site fishes have shown variation.

Table 3. *Myxobolus* parasitic infection of different fishes from collection site-A (Chonda gaon).

S. No.	Name of fish	No. of examined fishes	Infected fishes	% of infection
1.	*Channa striatus*	17	08	47.05
2.	*Heteropneustes fossilis*	16	03	18.75
3.	*Clarias batrachus*	14	06	42.85
4.	*Labeo rohita*	13	01	7.69
5.	*Wallago attu*	10	00	0.0
6.	*Catla catla*	11	00	0.0
7.	*Cirrhinus mrigala*	06	00	0.0

It shows the percentage of *Myxobolus* parasite infection in 7 species of fishes which collected from site A (Chonda gaon).

The table shows the highest infection in *Channa striatus* (47.05%) which decreases, respectively in *Clarias batrachus* (42.85%), *Heteropneustes fossilis* (18.75%), *Labeo rohita* (7.69%) while other species such as *Wallago attu*, *Catla catla* and *Cirrhinus mrigala* are not found to be infected.

Table 4 shows the percentage of *Myxobolus* parasite infection in 5 species of fishes collected from Jaroni gaon. The highest infection present in *Channa striatus* (100%) which decreases, respectively in *Clarias batrachus* (31.25%), *Wallago attu* (25%), *Heteropneustes fossilis* (15.78%) while *Labeo rohita* not found to be infected.

Table 4. *Myxobolus* parasitic infection of different fishes from collection site-B (Jaroni gaon).

S. No.	Name of fish	No. of examined fishes	Infected fishes	% of infection
1.	*Channa striatus*	2	2	100
2.	*Heteropneustes fossilis*	19	3	15.78
3.	*Clarias batrachus*	16	5	31.25
4.	*Labeo rohita*	34	0	0.0
5.	*Wallago attu*	4	1	25

Table 5. *Myxobolus* parasitic infection of different fishes from collection site-C (Karua gaon).

S. No.	Name of fish	No. of examined fishes	Infected fishes	% of infection
1.	*Channa striatus*	19	2	10.52
2.	*Heteropneustes fossilis*	27	01	3.70
3.	*Clarias batrachus*	24	01	4.17
4.	*Labeo rohita*	10	01	10

It shows the percentage of *Myxobolus* infections in 4 species of fishes which collected from site C (Karua gaon). The highest infection shows in *Channa striatus* (10.52%) which decreases, respectively in *Labeo rohita* (10%), *Clarias batrachus* (4.17%) and *Heteropneustes fossilis* (3.7%).

Table 6. *Myxobolus* parasitic infection of different fishes from collection site-D (Girgoni gaon).

S. No.	Name of fish	No. of examined fishes	Infected fishes	% of infection
1.	*Channa striatus*	23	01	4.34
2.	*Heteropneustes fossilis*	30	0	0
3.	*Clarias batrachus*	22	0	0

It showed the percentage of *Myxobolus* parasite infection in 3 species of fishes which were collected from site D (Girgoni gaon). The highest infection showed in *Channa striatus* while *Heteropneustes fossilis* and *Clarias batrachus* were without *Myxobolus* parasite.

Table 7. *Myxobolus* parasitic infection of different fishes from collection site-E (Kotwal gaon).

S. No.	Name of fish	No. of examined fishes	Infected fishes	% of infection
1.	*Channa striatus*	30	06	20
2.	*Heteropneustes fossilis*	36	04	11.11
3.	*Clarias batrachus*	42	08	19.05

It showed infection of *Myxobolus* parasites in 3 fish species present in site E (Kotwal gaon).

The highest infection showed in *Channa striatus* (20%) which decreased respectively in *Clarias batrachus* (19.05%), *Heteropneustes fossilis* (11.11%) while *Catla catla* were without *Myxobolus* parasite.

Table 8. *Myxobolus* parasitic infection of different fishes from collection site-F (Silata gaon).

S. No.	Name of fish	No. of examined fishes	Infected fishes	% of infection
1.	*Channa striatus*	62	25	40.32
2.	*Heteropneustes fossilis*	65	07	10.76
3.	*Clarias batrachus*	43	10	23.80

It showed infection of *Myxobolus* parasites in 3 fish species present in site F (Silata gaon).

The highest infection showed in *Channa striatus* (40.32%) which decreased respectively in *Clarias batrachus* (23.80%) and *Heteropneustes fossilis* (10.76%).

Table 9. *Myxobolus* parasitic infection of different fishes from all sites.

S. No.	Name of fish	No. of examined fishes	Infected fishes	% of infection
1.	*Channa striatus*	153	43	28.10
2.	*Heteropneustes fossilis*	193	18	9.32
3.	*Clarias batrachus*	161	31	19.25
4.	*Labeo rohita*	57	2	3.50
5.	*Wallago attu*	14	1	7.14
6.	*Catla catla*	17	0	0.0
7.	*Cirrhinus mrigala*	06	0	0.0

It showed the percentage of *Myxobolus* parasite infection in seven species of collecting fishes from all collection sites. The table shows the highest infection in *Channa striatus* (28.1%) while decreasing respectively in *Clarias batrachus* (19.25%), *Heteropneustes fossilis* (9.32%), the lowest percentage in *Labeo rohita* (3.50%) while *Catla catla* and *Cirrhinus mrigala* reported without *Myxobolus* parasite, it indicates their resistivity against *Myxobolus* pathogenic parasites. From Table 2 it is clear that site F (Silata gaon) is highly infected in comparison to site A (Chonda gaon). Site D (Girgoni gaon) is negligible infected fish population area.

4.1 Protozoan Parasites Present in Fishes

Protozoan parasites of class Sporozoa are endoparasite in nature which transmitted to the host as spores. These spores usually protected by multiple fission and have thick wall surrounded spores. Each sporozoan is survived in the specific host and is intracellular or intercellular in nature. Conidiosporidian sporozoan is unique in their shape and each spore consists of (1-6) polar filamentous. The membrane of spores may be single layered or sometimes two layered.

The conidiosporidian have a single host as they complete their life cycle in single host. Conidiosporidium is exclusively parasite of the lower invertebrates like honeybees and vertebrates like fishes (Kudo, 1920 & 1934).

Spores of class conidiosporidia are of different shape and size and chitinous (Kudo, 1921). The spores consist of two to six shells. The shell may possess various processes. The polar capsules of *Myxobolus* spore contain a polar filament with coils in number from one to six which united at one site, except in the family Myxididae, in which one polar capsule is present near each of the poles of the spore. Below or between the polar capsules there is a sporoplasm. Ordinarily, a

young spore has two sporoplasm nuclei which fuse into one by autogamy prior to germination.

In Myxobolidae there is a glycogenous substance in a vacuole which stains mahogany red with iodine and is known as iodinophilous vacuole.

The Myxosporidae are exclusively parasite of lower vertebrates, especially fishes. Both marine and freshwater fishes have been found to harbour in various regions, a few occur in Amphibia and Reptiles but no species have been found to occur in either Aves or Mammals.

When a spore gains entry into the digestive tract of a specific host fish, the sporoplasm leaves the spore as an amoebula which penetrate through the epithelium of the gut and after a migration, enters the tissue of a specific organ, where it grows into a trophozoite at the expense of the host tissue cells, and the nucleus divided repeatedly. Some nuclei surrounded by dense cytoplasm called sporonts.

The sporonts grow and their nuclei divide several times, becoming 4-18 nuclei, each nuclei surrounded with a small amount of cytoplasm called sporont. The sporont which develops into a single spore called monosporoblastic sporont. Sporont, which formed two spores known as disporoblastic sporont. The central area of trophozoite mostly takes part in spore formation and peripheral parts after modification become covered of spore.

This is ordinarily referred to as a Myxosporidian cyst.

If the infection site of this parasitic stage is near the body surface of the host, the cyst rupture and the mature spores become free and swim in water. On the other hand, if the infection is internal in the host's organ, spores will not be free and will remain internal to host up to the survival of the host. When the hosts die, body disintegration will start, as the spores become free after disintegration of fish and these spores will transfer to the new host.

The primitive Myxosporidia are coelozoic in the organ of the host, such as skin, muscles, gills and other body parts. The spores liberated amoebulae like into a specific body part of the host; these spores grow into multinucleated amoeboid trophozoites. These amoeboied trophozoite form pseudopodias. This trophozoite takes part in budding which may be exogenous or endogenous so one trophozoite may form one to many spores.

The site of infection by Myxosporidia varies among different species, they have found in almost all types of body organs and their tissue, although each species has its specific site of infection in one to several species of fishes.

In the case of freshwater fishes, the gills and skin are mostly parasites by Myxosporidia when the infection is concentrated in the fins or skin, the resulting changes are quite conspicuous. The infections in the gills usually manifest by whitish pustules, which can be detected with the naked eye, but under primitive stage, the infection, are detected only by microscopical examination. Certain histological changes in the host fish have been noticed.

During observation and identification of *Myxobolus* parasites the review of Myxidium, Jayasri and Hoffmann (1982) used.

The collected fishes of Chonda gaon, Jaroni gaon, Karua gaon, Girgoni gaon, Kotwal gaon and Silata gaon examined. It has been observed that *Channa striatus*, *Heteropneustes fossilis*, *Clarias batrachus*, *Labeo rohita* and *Wallago attu* are infected by *Myxobolus* Protozoan parasite while *Catla catla*, *Cirrhinus mrigala* and *Mystus seenghala* are healthy i.e. free from *Myxobolus* parasites. On the basis of spore following species of *Myxobolus* identified which present indifferently caught fishes of selected sites-

Myxobolus cultus
Myxobolus dujardini
Myxobolus cerebralis
Myxobolus oviforme
Myxobolus cycloid
Myxobolus mulleri
Myxobolus ellipsoideus
Myxobolus pfeifferi
Myxobolus cognati

5. Discussion

During the present investigation, an attempt has been made to study the infection of *Myxobolus* in some edible freshwater fishes of Morena region. This study is based on taxonomic status and pathological effects of *Myxobolus* species. Out of 695 specimens of all 8 species of fishes, 81 specimens are found infected. Ten species of fishes, as taken in the present investigation 5 species of fishes *Channa striatus*, *Clarias batrachus*, *Heteropneustes fossilis*, *Labeo rohita* and *Wallago attu* are found infected, while no infection is noted in three species of fishes such as *Catla catla*, *Cirrhinus mrigala* and *Mystus seenghala*. Other two species of fishes *Cyprinus carpio* and *Puntius ticto* were not captured in net at the time of fishing (Table 1). Silata Gaon (Locality F) is found highly infected in comparison to other localities, while Jaroni Gaon (Locality B) is least infected. No infection is found in two other localities C and D (Table 2). I recognize ten species of *Myxobolus*: *Myxobolus cerebralis*, *Myxobolus dujardini*, *Myxobolus cultus*, *Myxobolus oviforme*, *Myxobolus mulleri* and *Myxobolus cognati*. Identification of *Myxobolus* species is done on the basis of the review of Myxidium given by Jayasri and Hoffman (1982) and Disease of fish (Duijn, 2000). Identification, description, and discussion of all reported *Myxobolus* species are already given in Section III for the purpose of convincing. Maximum numbers of *Myxobolus oviforme* are observed in all infected fishes such as *Channa striatus*, *Clarias batrachus*, *Heteropneustes fossilis*,

Labeo rohita and *Wallago attu*. *Myxobolus mulleri*, cause pimple formation. Pimples are present on the skin, fins and near lower lips of infected fishes. Polar filaments are not visible; the sporoplasm is present as a vacuolated form at posterior end of the spore. The most infected fish is *Channa striatus* but the least infected are *Wallago attu.* Pathological effect of *Myxobolus* species is observed on different organs viz. skin, muscles, and gills. Skin and muscles are rarely infected. Skin infected only in case of *Myxobolus oviforme* in *Clarias batrachus*. Muscles are found to be infected in *Channa striatus*, *Clarias batrachus*, *Heteropneustes fossilis*, *Labeo rohita* and *Wallago attu.* The growth of fishes is also affected by *Myxobolus* infection. The growth of fishes is reduced.

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