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ORIGINAL ARTICLE

Histological Changes in Fresh Water Fishes after Parasitic Protozoan Infection in River Asan, District Murena

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ABSTRACT

Surveys of epidemic diseases of fishes are frequently found to be due to myxosporidian infections. Sporozoan parasites may be found in all organs of fishes but in the skin, they are relatively rare in comparison with other organs. Myxoboluscerebralis, which attacks the supporting tissue of salmonidfish is known to be responsible for the so called twist disease which is often fatal especially to young fishes and occurs in an epidemic form. Henneguyasalminicola (sporozoan) invades in diameter, it is thus responsible for the so called tapioca disease of salmon fish. This is the reason to conduct the aforesaid histological observations.

Key word: Histological Changes, Protozoan Infection, River Asan

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INTRODUCTION

Sporozoan parasite *Kudothyrsites* attacks the body muscles fibres of the barracouta in which the infected muscles become liquefied. This condition is known as milky barracouta or pap snoek and may affected as much as 5% of the commercial catches (Willis, 1949). 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 (*Barbusbarbus*) and others of European waters, is caused by *Myxobolus pfeifferi* (Keysselitz, 1908). The little knots are formed when the parasite are situated in the skin, while bumps and pimples originate from a process in the muscles (Duijn, 2000). *Marbus nodulosus* commonly known as knot or pimple disease is a collective name for several diseases caused by sporozoan. The symptoms are the formation of little knots in the skin, often closely resembling to those caused by Ichthyophthirius or large pimples or bumps, if there are only little knots in the skin as is usually the case in carps and in related groups (Duijn, 2000). 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, McCraren, *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.

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 Asanriver was 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-

Chondagaon	-	Site A
Jaronigaon	-	Site B
Karuagaon	-	Site C
Girgonigaon	-	Site D
Kutwalgaon	-	Site E
Silatagaon	-	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 was 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 is presented table 1. Muscles showed the highest percentage of Myxobolus infection (78.94%), gills (15.30%) and skin (4.76%).

Table 1: The percentage of Myxobolus infection in different tissues of Fish

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

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 Silatagaon 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 is scanty.

RESULT AND DISCUSSION

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 *Clariasbatrachus*. 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 Silatagaon (site F) in Murena district while least infected is Jaronigaon. Rare cystic nodules are observed on the body and at the base of fins of infected fishes. A few numbers of Myxobolus cysts are observed from body pigmentation.

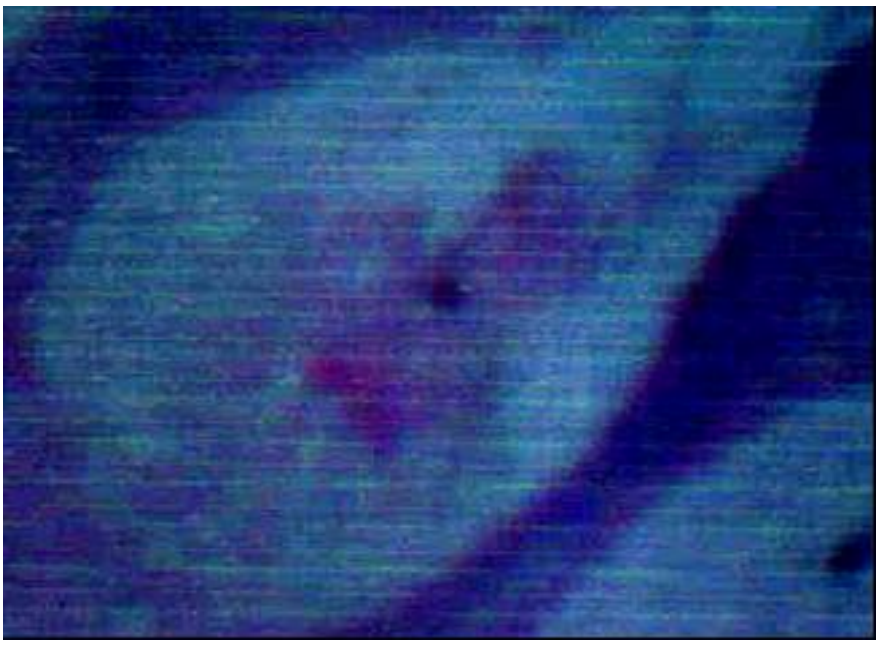

Plate 1: Skin showing rupture of melanophores

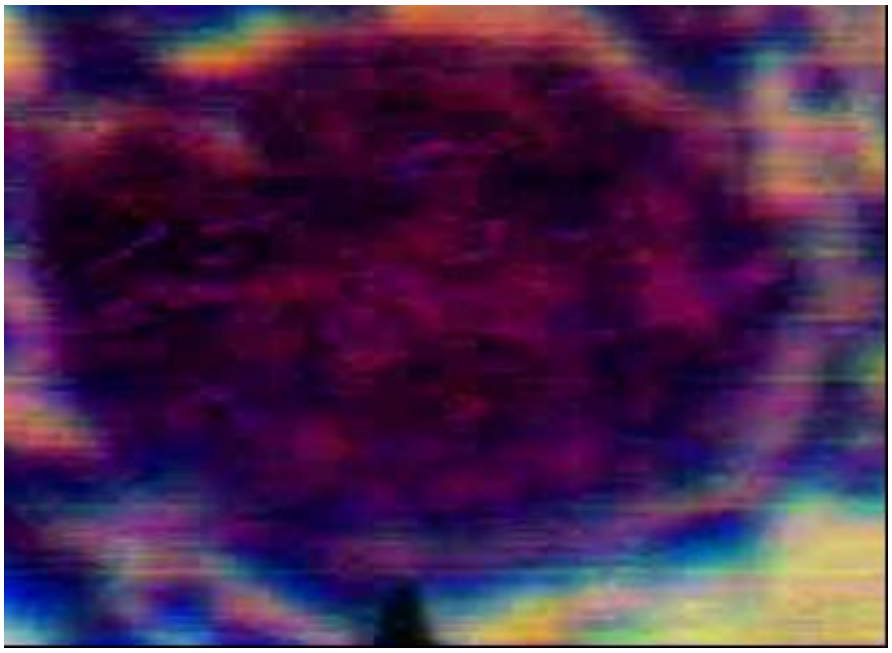

Plate 2: Calcification in muscles

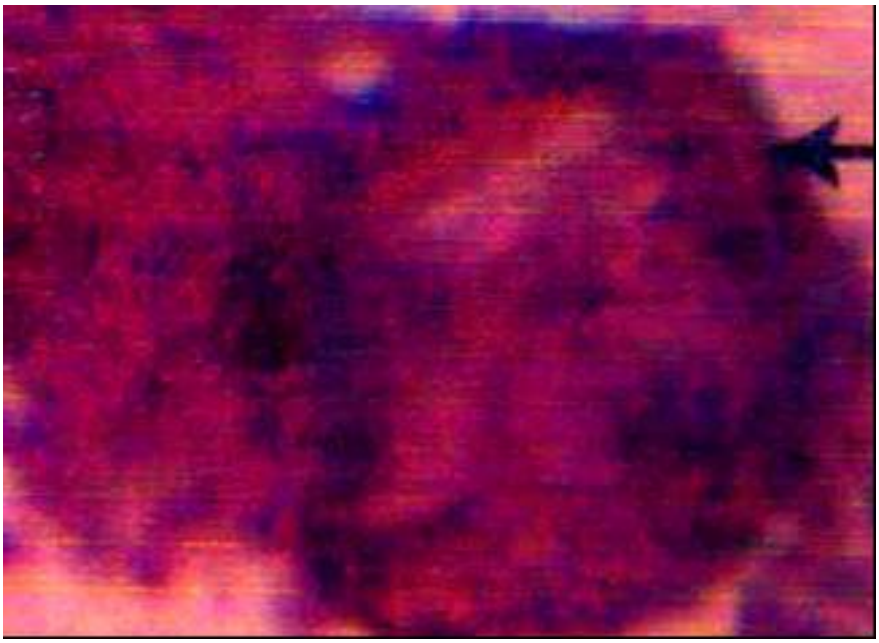

Plate 3: Degeneration in muscles

Plate 4: Swelling in gill lamellae

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 *Channastriatus* while in *Clariasbatrachus* 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 *Channastriatus*, *Heteropneustes fossilis*, *Labeorohita*, *Wallagoattu* and *Clariasbatrachus*. The highest calcification is found in *Channastriatus* due to the infection of *Myxobolus oviforme*, however, calcification by Myxobolus cycloid in *Clariasbatrachus* is

low infection, while in *Labeo rohita* and *Wallagoattu* calcification is the lowest. In *Channastriatus* the blood capillaries of intermuscular region are highly scattered and ruptured due to calcification by *Myxobolus oviforme* and *Myxobolus mulleri*. In *Labeorohita*, *Wallagoattu*, *Heteropneustes fossilis*, the blood capillaries are shrunken by *Myxobolusoviforme* parasite. Mishra, *et al*. (1982) reported enzootic nature of myxosporiadiasis in Indian major carps.

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.A few number of Myxobolus cysts of different size are observed in muscles.

Gills are more infected by *Myxobolus oviforme* in *Clariasbatrachus*. The spore of *Myxobolus oviforme* lies in connective tissues somewhat near the blood vessels. Free end of gill lamellae seems to be swollen. Gill lamellae seems as bulging form of eyeball but cells of gill lamellae are not calcified as seen in muscles. Blood vessels are degenerate and showed necrosis. Myxosporidian infection is noticed in gill lamellae only. Gill lamellae are fused at the tip, necrosis in gill filaments is observed.

Das and Mukherjee (1998) also reported fusion of gill lamellae at the tip due to hyperplastic changes. Their findings were suggestive of these changes due to extraordinary pressure on the respiratory functions of the fishes. Sanaullah and Ahmed (1980) and Dey, *et al*. (1988) have reported myxosporidian infection in carps, *Catlacatla* and describe hyperplastic gill epithelium varying degree of degeneration.

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