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Research Article

Investigations on Protozoan Infection in *Cirrhinus mrigala*

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Abstract

Cirrhinus mrigala is one of the most economically important fish in India, they are cultured in ponds and tanks for commercial uses. During culture period many organisms are affected to *C. mrigala*, among them microsporidia is one of the protozoans parasite that infested the fish. In present study, mrigal fish were collected from rearing ponds for parasitological, microsporidian examination by gross and histopathology (muscle, gills, brain and tissue). The present study revealed that presence of the microsporidian in *C. mrigala* fingerlings by wet mount, also histopathological examination strongly confirmed that the presence of spores in gills, primary and secondary lamellae and intracellularly, endothelial cells. Infected fishes revealed that the hemorrhage, intracellular edema and infiltrations of blood cells. The spores were detected in necrotic region and myoseptal boundaries of fish muscles, also in liver and kidney. Histopathological analysis and wet mount observation indicated the presence of microsporidian in collected fishes.

Keywords: Microsporidia; *Cirrhinus mrigala*; Poikilothermic Vertebrates

Introduction

The demand for the animal protein is increased, day by day due to increasing populations of human beings. The production of fish is increased by intensive fish culture practices, requiring high density in ponds and tanks. The culture practice produces the risk and great concern to fish culturists around the world about fish pathogens. Nearly 1200 species of Microsporidia have been identified as parasite to invertebrates and fishes [1-4]. Microsporidia are minute, unicellular organisms living as obligate intracellular parasites in a variety of animals [5]. It's found in the cytoplasm of the host cell and destroy or induce enormous hypertrophy of the cell [6], also it suppress the immunity of the host organism [7]. The most common hosts of microsporidia are fish and shrimp [8], *Danio rerio* [9], *Lepomis macrochirus* [10], shrimp [11]. Microsporidia infection, reduced the productivity of fish farms by inhibition of growth rate. The aquaculture industry is expanded worldwide, even though there is a considerable lack of information regarding the immune response, biology, pathogenesis of microsporidia [12]. In India only few research deal about taxonomy of microsporidians [13], and their parasitic infection to the fish [14,15].

The present observations describes the microsporidian infection in Indian major carp, *Cirrhinus mrigala* (Ham.), on the basis of spore characters and the intracellular developmental stages by wet mount and histological examination.

Materials and Methods

Sample collection

Cirrhinus mrigala fingerlings were collected from rearing ponds and brought to the laboratory in live conditions for further investigations. From the infected fishes, liver, kidney, muscle, gills and brain tissues were dissected out, blotted free of blood and stored at -20°C for further study.

Histological examination of tissues

The collected tissues were ultrathin sectioned (1 micrometer), before fixation, the tissues were cut into 1 mm pieces. Fixation was done by using 25% glutaraldehyde and 32% paraformaldehyde. After fixation, buffer (0.1N sodium cacodylate) wash have been used to remove the excess of fixative. The water from the tissue were remove by dehydration by using upstream alcohol series

from 10 to 100%. For proper infiltration, desiccator has been used. Embedding of the tissues were performed in empty capsules in LR white and kept for polymerization at 70°C upto 48h. In the next step, the tissues with LR white were trimmed and sectioned (1µm) by using ultra-microtome, then the sections were stained with toluene blue for a period of 2 - 3 minutes.

Results and Discussion

There has been a rapid expansion of aquaculture in last few decades. Mainly the Indian major craps *Catla catla, Labeo rohita* and *C. mrigala* are cultured as intensive fish culture practices. Despite the technological advance and achievements, intensive carp farming are very high risk due to disease development. The disease development in carp, are known to be affect the viability of carp culture practice. Disease development in fish farms was due to very high stocking density, poor water quality conditions and imbalances in nutrition. The diseases that encounter in carp by skin, gills, optic, and internal organs. The protozoan parasitic diseases reported to be very common in carp culture in earthen ponds due to poor hygienic conditions [16]. In the present study, parasitic infection has been identified in *C. mrigala* fingerlings. The wet mount preparation of infected gills clearly exhibited the presence of spores (Figure 1a & 1b). The round spores were observed to be very high concentration under light microscopy in fresh mount preparation. The presence of spores in wet mount preparation has been clearly confirmed by microsporidian infection in *C. mrigala* fingerlings. Similar *Rodrigues et al.* [17] stated that Microsporidia infection in fish was confirmed by wet mounts preparation.

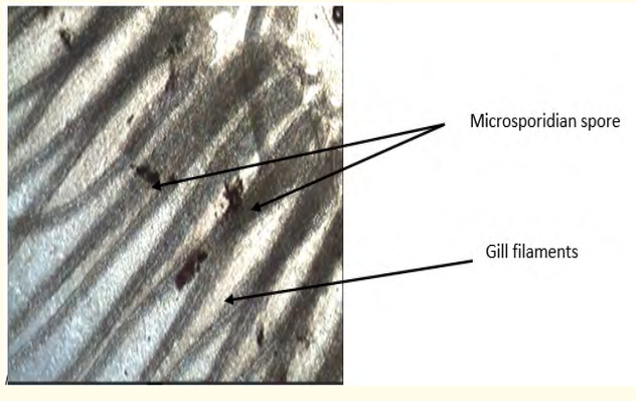

Figure 1a: Wet mount preparation of microsporidian infected gill (45X). Spores can be seen very clearly in the gill filaments as black deposit.

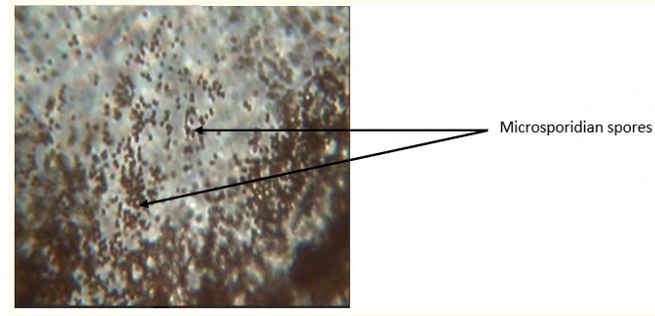

Figure 1b: Microsporidian spores in wet mount preparation in infected gills at 100X magnification. A group of round spores in large concentration can be seen.

Figure 1: Wet mount preparation.

Infection in gills

The microscopic observation of ultra-thin sections of gills has shown primary and secondary gill filaments (Figure 2a and 2b). The microsporidian infection can be seen clearly in gill endothelial cells and connective tissue compartment. The histopathological study indicated the presence of spores in both primary and secondary gill lamella. The microsporidian spores are also seen intracellularly in gill endothelial cells. Hypertrophy and hyperplasia are evident. Other changes like hemorrhage, intracellular edema and infiltrations of blood cells have been clearly observed in ultrathin sections from infected fish. Our result coincide with earlier result of Becker and Speare [18] who stated that microsporidian sp., infected fish, salmonid showed distress with secondary infection in gills. The gill endothelium was preferred site for the development of microsporidian, they were enter either through gill surfaces from water or circulatory system [18]. It exhibit large surface area which is in direct contact with external atmosphere. Mucous cells are also arranged between gill and epithelial cells. A study of microfauna, associated with pathological changes in gills in salmonids has been reported by Bermingham and Mulcahy [19]. This parasitic infection seems to be responsible to necrotic changes, edema and hemorrhagic condition in gill tissues. A parasite also seems to be infiltrated in the connective tissue compartment of gill tissues. Presence of this parasite seems to be responsible for hemorrhagic condition of carp.

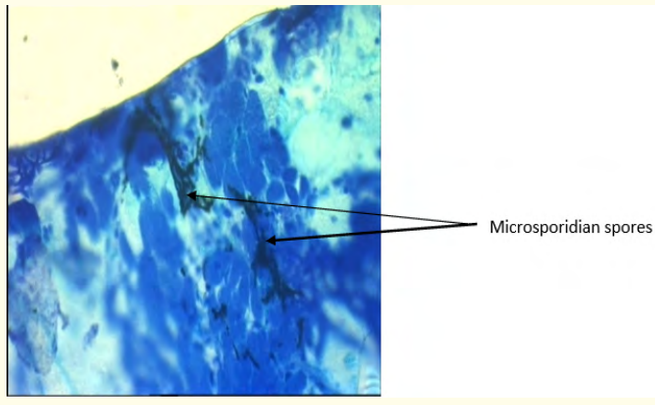

Figure 2a: Microsporidian infection as black patches can be seen (40X).

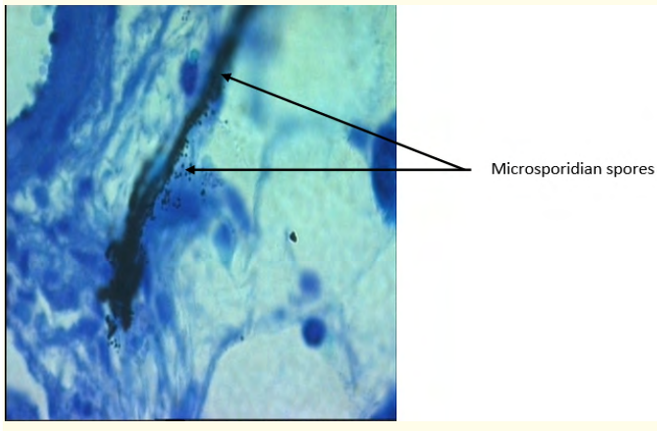

Figure 2b: Microsporidian infection can be seen clearly in primary and secondary gill lamella (100X).

Figure 2: Ultrathin sections of infected gill.

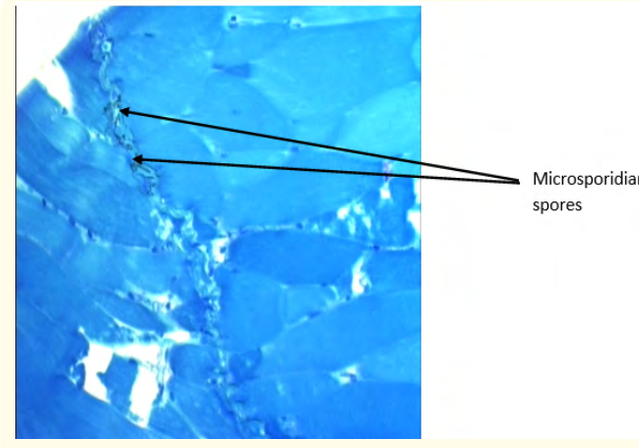

Figure 3a: 40X magnification of microsporidian infected muscle section.

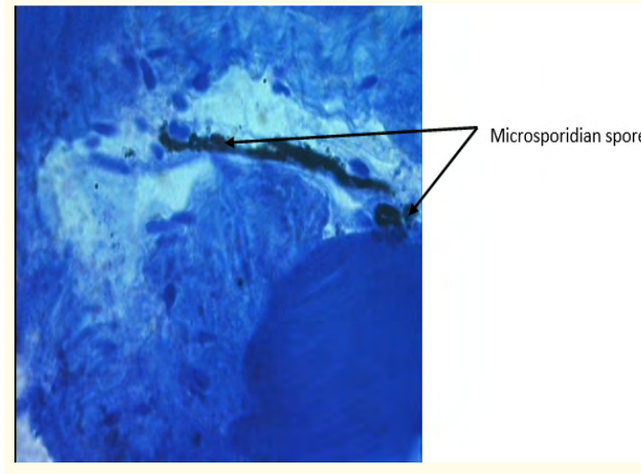

Figure 3b: 100X magnification of microsporidian infected muscle section.

Figure 3: Ultrathin sections of infected muscle.

Infection in muscle

Microsporidia, caused lesions in the muscle, especially in trunk muscle of carps. The ultrathin section of muscle from infected mrigal reveals presence of microsporidian spores (Figure 3a and 3b) also necrosis have been clearly seen in connective tissue. The spores have been detected in necrotic region and along myoseptal boundaries. The destruction of myofibrils and necrosis of muscles clearly indicates that the presence of microsporidia in muscular components. Several investigators have reported microsporidian infection in muscle [20]. These protozoa may have entered muscle through necrotic spaces and damaged body surfaces, which is observed in some of infected fishes. Microsporidian found in the fat body and body wall of the fish [21], also it affect the intracellular and caused necrosis in the muscle [22].

Infection in kidney

The head kidney portion of the infected fishes indicated the presence of nephric duct and hematopoietic regions. The histopathological changes in head kidney portion indicated that the spores in the kidney, it may have entered through circulation or renal portal system and crossed the filtration area. The presence of spores in head kidney portion indicates the intensity of this infection in mrigal fingerling. Similarly, the microsporidia found in *L. rohita*, kidney (destroyed the epithelial cells and intercellularly) [23].

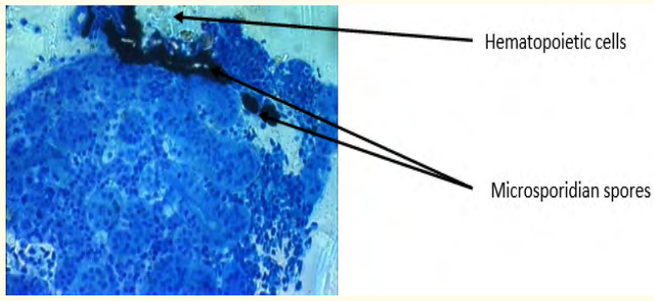

Figure 4a: 40X magnification of microsporidia infected kidney section.

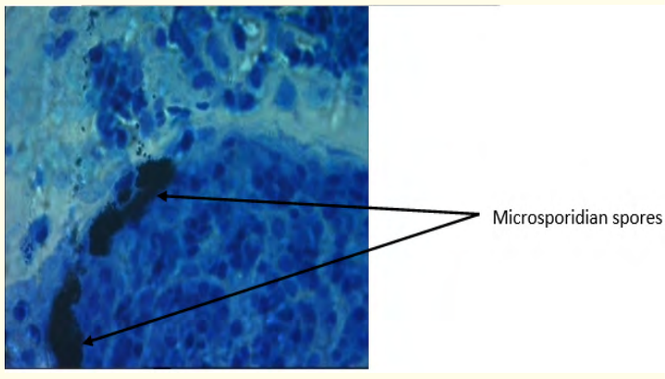

Figure 4b: 100X magnification of microsporidia infected kidney section.

Figure 4: Ultrathin sections of infected kidney.

Infection in brain

The ultrathin sections of brain from infected fingerling have ruled out penetration of spore in brain. Spores are not detected in brain sections. The neuronal cell body, glial cells and neurites can be seen in the sections. Large number of multipolar neurons can be seen. There was no pathological changes detected in brain. This indicates that the parasites have not crossed the blood brain barrier. Similarly, microsporidian infection has never been reported in brain tissue [24]. Sanders., *et al.* [25] stated that microsporidia affected the central nervous system and their skeletal deformities of fish.

Infection of liver

In microsporidia infected liver, showed clear oedema of hepatocyte due to intracellular accumulation of microsporidia. However, intracellularly located microsporidian stages cannot be observed under light microscope. Histopathological observations have also shown necrosis, hypertrophy, infiltration of blood cells and damage to the sinusoids. Results have confirmed the microsporidian

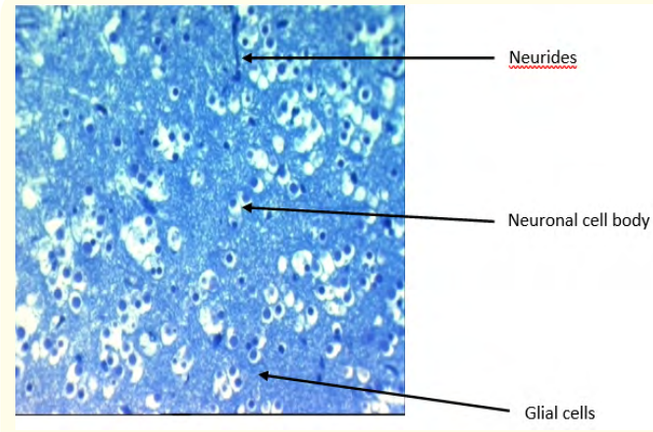

Figure 5a: 40X magnification of brain sections showing no infections.

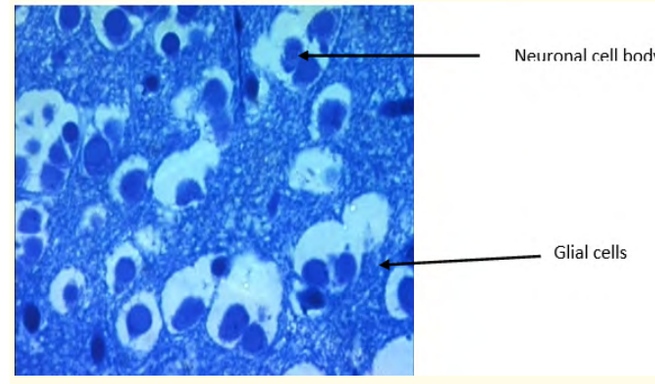

Figure 5b: 100X magnification of brain sections showing no infections.

Figure 5: Ultrathin sections of infected brain.

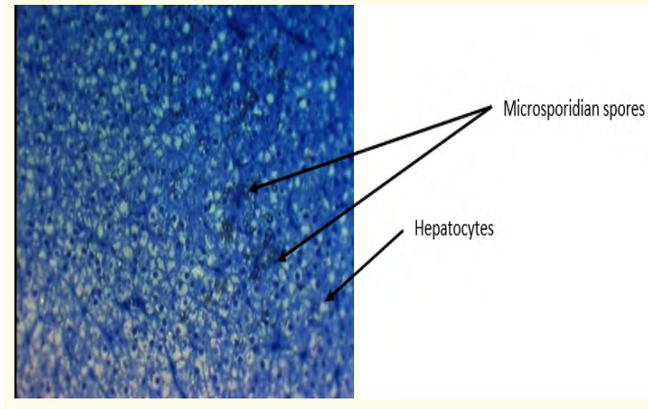

Figure 6a: 40X magnification of liver showing microsporidia.

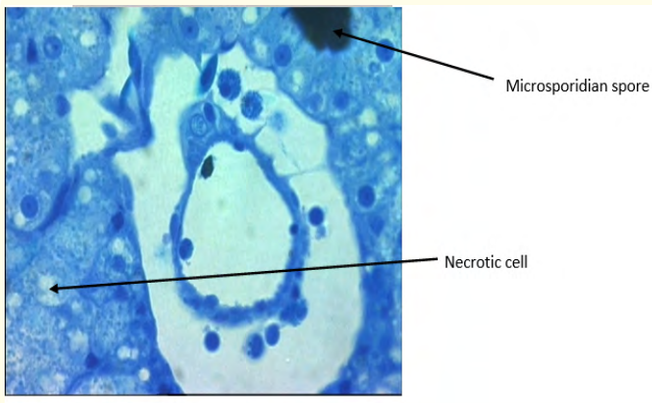

Figure 6b: 100X magnification of liver showing microsporidia infection.

Figure 6: Ultrathin sections of infected liver.

infection in liver. Our result agreement with result of Alarcon *et al.* [26] who stated that microsporidia infection was confirmed liver of *Cyclopterus lumpus* when subjected to histopathology.

Conclusion

Histopathological analysis of tissue from infected fish indicated the presence of microsporidian spores. The spore damaged the tissues mainly liver, muscle, gill, kidney. The microsporidian spore were not observed in brain tissue this could be probably the pathogen failed to cross blood-barrier. The study indicated that the presence of microsporidian infections in the culture ponds. To avoid the economic losses by pathogen, there is need of proper hygiene condition, balancing stocking density of population and water management (renewal of water) and composition.

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