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Research Article

HISTOPATHOLOGICAL STUDY OF INFECTED ORNAMENTAL FISHES FROM LATUR DISTRICT (MS) INDIA

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ABSTRACT

The ornamental fishes are called "living jewelers" because of their shape, size, colour and behavior. All these characters decide their market value. The present investigation was aimed at the identification of fish diseases through histopathological observations of some diseased fishes. In the present study, the infected aquarium fishes were collected from various aquariums of Latur, the histological slides are prepared by using the traditional paraffin method and observed under the microscope it was found that the fishes are infected by the fungus which causes the disease saprolegniosis.

Keywords: Ornamental fishes, Bacterial, Fungal, Histopathology.

INTRODUCTION

The ornamental fishes are called "living jewelers" because of their shape, size, colour and behavior. All these characters decide their market value. The ornamental fish industry is tremendously increasing at the global level at the same time microbial disease is the main reason for the big loss in this industry every year. Various fungal and bacterial diseases cause loss of the whole unit if they are not identified and treatment is not given in its earliest stage. The symptoms of the infected fishes are not easily visible with the observation of external features or free-living movement of fish. The examination of infected fishes begins with the observation of the external body of any fish having a disease. These pathogens obtain food by breaking the body tissues of fish or by absorbing the digested food from the intestines. In the gills hyperplasia observe in between the gill lamellae. The parasites feed on the newly formed cells and damage gill tissue.

Fungal diseases of fish have been continuously increasing over the past 20 years. The traditional fungi are composed of members from several different taxonomic kingdoms. Saprolegnia and other typical water molds is the classic secondary host and infect the upper part of the body and for survival it requires poor water quality or general immune suppression. Histopathology is an important disease diagnostic tool, usually in histopathology the test is conducted by making tissue sections. However, the tissue sections are fixed for subsequent study. The present investigation was aimed at the identification of fish diseases through clinical, parasitological, histopathological observations of some diseased fishes.

MATERIAL AND METHODS

Collection of fishes

Common aquarium fishes such as Goldfish (*Carassius auratus*), *Mystus vittatus* showing gross pathological lesions were collected from various aquariums of the Latur district of Maharashtra, India. Diseased fishes were collected from local fish aquariums were brought to the laboratory and sampling was done at the Department of Zoology and Fishery Science and fungal culture was done at the Department of Biotechnology Rajarshi Shahu Mahavidyalaya (Autonomous), Latur. Materials were collected aseptically from different organs like skin, gill, kidney, liver, eye and heart in sterile containers with the liquid medium for primary culture fungus on PDA.

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The histological slides were prepared by using traditional method

After dissecting diseased fish tissues, the samples were quickly washed with physiological saline solution (0.75% NaCl) and immediately fixed in 10% formalin. The following paraffin method was used for the preparation of histological slides. Dehydration: The fixed tissues were gradually dehydrated in upgrading concentration of alcohol with 50%, 70%, 90% and 100% for 15 min, 45 min, 15 min and 15 min respectively. After dehydration, the tissues were washed in xylene for 10 min. Paraffin impregnation*:* The main function of paraffin impregnation is to provide a hard supported block for tissue sections. A paraffin bath with 60^0 C was used. The melting point of the paraffin was $54\text{-}58^0$ C. The tissues were kept in molten paraffin for 30 min to 1 hr. Paraffin embedding was carried out by using metallic moulds and other various types of containers like ice-cube trays and watch glasses were used for this purpose. Blocking out of impregnated tissues: Molten wax was kept in to the mould for solidification. With heated forceps the tissue was transferred so that the face to be cut is firmly embedded in the solidifying layer. After a thin layer is hardened on the outer surface then mould was quickly poured in cold water. After the complete solidification the block was removed from the mould. Trimming and Sectioning*:* The blocks were then trimmed properly to the equal size of the tissue section by cutting the excess paraffin paper around the tissue.

After trimming the blocks were kept in the ice chamber for a while before cutting the section. The block was fitted to the microtome machine for sectioning. Sections were cut on a microtome (BRIGHT-No. 5030) fitted with a sharpened microtome knife. The temperature of the water bath was maintained between 55-56℃for stretching the cut out ribbons. Blocks of fish tissues were cut at 6-8µ in thickness. Affixing and deparaffinization*:* The ribbons were attached on the glass slides by means of Mayer's albumen. In 20cc distilled water, 3-5 drops of Mayer's albumen were added, shook and then allowed to dry at 25^0C-34℃. The slides with ribbons containing tissues were kept at 60℃ for ten min for melting, followed by immersing the slides in xylene I and xylene II for ten min each to remove the paraffin. Rehydration of the tissues: The slides with tissues were then transferred to the following grades of alcohol: 100% for 10 min, 90% for 5min, 70% for 5 min and 50% for 5 min. At the end of this process tap water is used to remove alcohol. Staining*:* The slides were then dipped in haematoxyline for 5 min. Then they were held properly with running water. They were then dipped in 0.5% alcohol for 30 sec and were held again with running water. Finally they were dipped in eosin for 1-2 min. Dehydration and cleaning: The slides were then dehydrated gradually keeping in alcohol in the following way: in 70% for 5 min, 90% for 3-5 min and 100% for 15 min. The stained tissues were finally cleaned in xylene for 10 min. Mounting and labeling*:* The permanent mounting of the slides was made by DPX and were labeled according to samples.

RESULTS AND DISCUSSION

Seasonal study of this experiment was that the occurrence of saprolegniosis during the period from 1st January to 28th February 2017 revealed that saprolegniosis occurred among gold fish and *Mystus vittatus*, angel fish, etc as sporadic cases in the examined farms following periodical sampling of fish to determine their average weight. An epizootic of saprolegniosis occurred in February with an incidence rate of 45.9% related to high mortalities following a severe cold weather front. The disease involved gold fish and *Mystus vittatus.* The characteristic clinical picture of saprolegniosis observed sporadic cases in gold fish and *Mystus vittatus*, angel fish, etc., following the routine sampling of fish to determine their average weight in order to calculate the amount of food given. The cotton wool like area found on the head and ulcer areas on the external body surface of fish. During the epizootic of saprolegniosis after these very cold weather front , the characteristic clinical picture of saprolegniosis observed in *Oreochromis niloticus* on the head specially at the nuchal region (Figure 1), some fish the cotton wool like growth covered the eyes either (Figure 2).

During the occurrence of sporadic cases of suspected saprolegniosis and also during the epizootic of the disease, samples were directly taken from the mycelium found on the lesions of skin, head and eyes of affected fishes and examined under the microscope which revealed the presence of the characteristic branched non septated hyphae and the asexual sporangium containing the motile zoospores which allow the genus identification of the fungus (Figure 3). Also the isolates of saprolegnia were identified as *Saprolegnia parasitica* according to its typical morphological characters. Some of the epidermal cell nuclei appeared pyknotic with marked vacuolation (Figure 4). In such cases, the epidermal mucus cells were highly active. The fungal hyphae were demonstrated by PAS reaction where the hyphae appeared bright red (Figure 5). In the muscles, the muscle fibers appeared with marked necrosis where the fibers lost the striation in some segments and the sarcoplasm appeared granular. The muscle fibers showed myolysis and myophagia. The lymphocytes, melanophores and edema were demonstrated in the intermuscular spaces (Figure 6). In advanced cases, Muscle of *Oreochromis niloticus* infected with saprolegnia showing myolysis and macrophage myolysis and macrophage. The results indicated that catching (bad handling) of *Mystus bleekeri*, *Gold fish* represent a great stress on fish through damaging of skin and integumentary system and render fish vulnerable to saprolegniosis. Many published data explained the role of bad handling and damaging of skin as a predisposing factor for the occurrence of saprolegniosis, (Lategan & Gibson, 2003) reported that Saprolegnia infection is a disease brought on by various predisposing factors including wounding of fish. Also some predisposing factors that increase susceptibility to Saprolegnia infection which involve integumentary damage. Similarly (Yanong *et al*., 2010) explained that Saprolegnia and other water

molds are the classic secondary invaders infecting upper areas of the body and requiring poor water for survival. In juvenile ayu (*Plecoglossu saltivelis*) (Wada *et al*., 1993) reported mycotic gastritis caused by Saprolegnia species, the disease occurred after transportation of fish from a hatchery pond to rearing ponds.

Figure 1. *Mystus vittatus* infected with *Saprolegnia parasitica* in the early stages showing redness in the dorsum and nuckal region.

Figure 2. Cotton wool like mass of *Saprolegnia parasitica* covered the eyes of naturally infected gold fish**.**

Figure 3. Gill of *Mystus vittatus* infected with Saprolegnia showing marked spongiosis of the epidermis and epidermal hyperplasia.

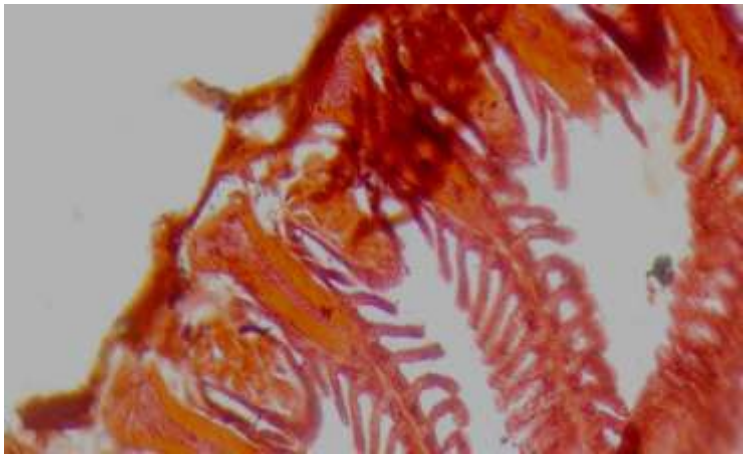

Figure 4. Gill of *Mystus vittatus* infected with Saprolegnia showing marked spongiosis of the epidermis and epidermal hyperplasia.

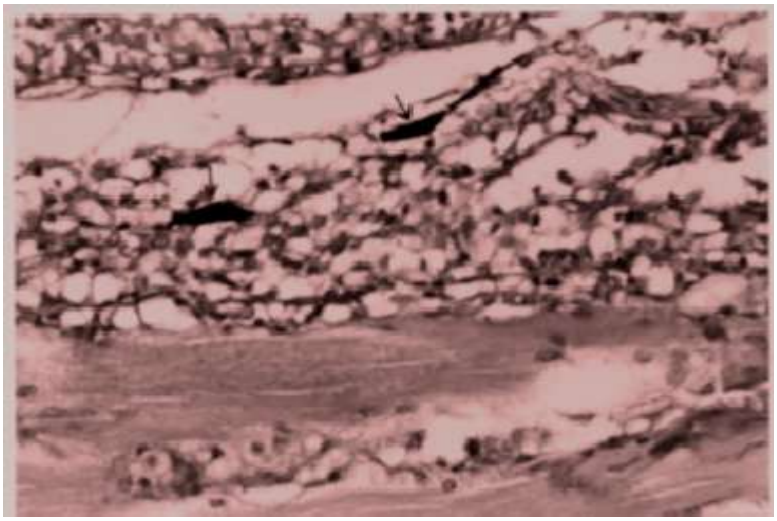

Figure 5. Liver of *Mystus vittatus* naturally infected with *Saprolegnia parasitica* showing severe edema between the muscle fibers, lymphocyticin filtration and melanophores aggregation (arrows).

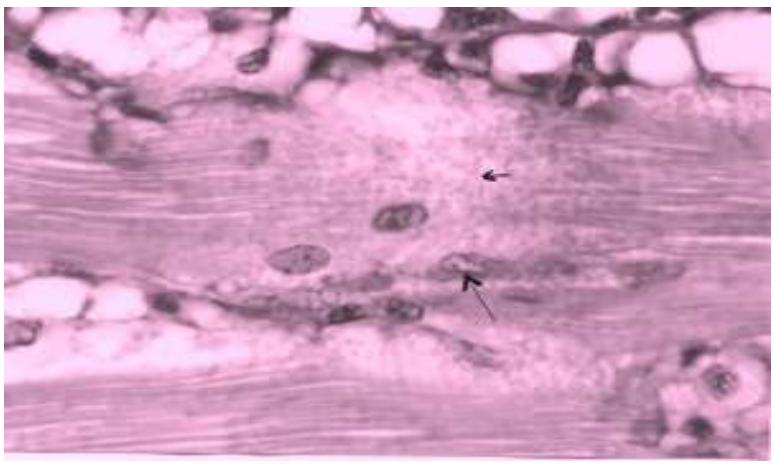

Figure 6. Liver of *Mystus vittatus* infected with Saprolegnia showing myolysis (small arrow) and macrophage cells engulfing then ecrotic muscle fiber (myophagia) (large arrow).

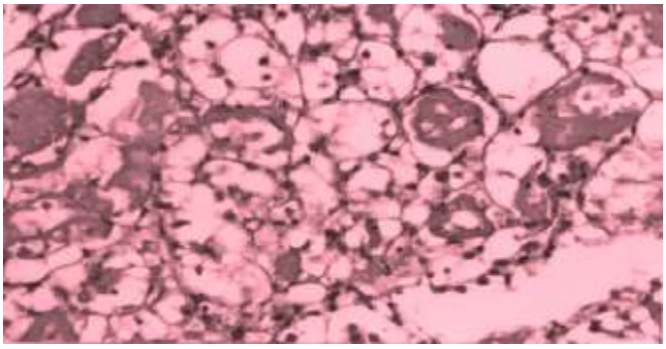

Figure 7. Liver of *Mystus vittatus* infected with Saprolegnia showing zunkerś necrosis, edema and lymphocyticin filtration.

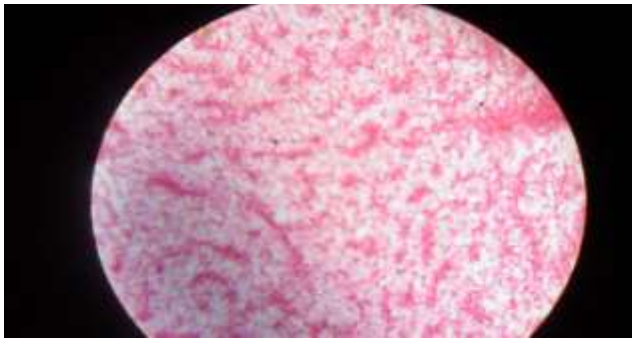

Figure 8. Liver of *Mystus vittatus* infected with Saprolegnia showing necrosis, edema and lymphocyticin filtration.

The stress so catching and transportation at the same time may render this fish vulnerable to the disease. The results also show–that an epizootic of saprolegniosis occurred in February in 9 farms from the examined farms associated with high mortalities 5 days following a severe cold weather front. In Mississippi, (Bangyeekhun *et al*., 2001) reported that saprolegnia was obtained from 5 separate farms which became affected by winter kill syndrome during 1991 and 1996. Also, (Khulbe *et al*., 1995) reported that fish mycosis is occurred due to Saprolegnia in a large reservoir of India and recorded that this verity of mycosis was primarily due to moderate water temperature and high temperature retarded the disease process (Figure 7 and 8). The occurrence of this epizootic in the examined farms in February associated with very high mortalities among affected farms may be a combination of the 2 related factors (Ahmed, 1992; Bly *et al*., 1992; Carballo & Muñoz, 1991). Firstly that the rapid decrease in water temperature (60C during the day and at night reach under zero) induced immune suppression to such fishes and then low water temperature favors high levels of Saprolegnia species zoospores, in the immuno-compromised fishes there is rapid proliferation of Saprolegnia and production of high levels of zoospores, the free-swimming zoospores attached to skin and muscles of fish, encysted and later germinated to penetrate the skin and muscles and after days the gross fungal lesions led to the observed fish mortalities**.** On comparing the occurrence of the disease in semi-intensive and intensive areas, there results show that the semi-intensive farms suffer easily to the epizootic of saprolegniosis following this very cold weather front, while no infections have occurred in the examined intensive farms. This may be attributed to that the intensive farms depend on underground water, their temperature greatly higher than that of surface water that semi-intensive farms depend upon, secondly the circular concrete ponds in the intensive farm constructed as a funnel shape, the column of water in the center reach 3meter which may give the chance for fish to stay in deepwater during the cold weather front.

The results of clinical examination shows that the presence of characteristic cotton wool like masses which cover the head and ulcer areas on the external body surface of fish, the parts of the body that is subjected to damage from the nets may be the parts covered by lesions of Saprolegnia. The conditions appeared 3-4 days following catching and involve few numbers of fish. During the epizootic, the same condition was seen but degrees of infection found in fishes were variable and the dead fish in some ponds may cover the surface of the water. The easily recognized cotton mycelium on the surface of affected fishes has been probably recognized since antiquity (Bly *et al*., 1993; Khulbe *et al*., 1995; Neish, 1977) published reports describing these infections founds in longtime**.**

The histopathological examination of skin and muscle of *Mystus bleekeri*, *Gold fish* infected with *Saprolegnia parasitica* revealed severe damage of skin and muscles where myolysis, myophagia, marked necrosis of the muscle

fibers and in advanced cases complete necrosis of the muscle fibers was a common picture .Also the epidermis of the skin showing spongiosis, marked vacuolation and the fungal hyphae demonstrated in the tissues. The severe damage of skin and muscles may explain the high mortalities associated with the diseases as a result of impairment of osmoregulation (Alderman, 1985). Also the absence of leukocytic in filtration around the invading fungal hyphae may indicate the state of immune suppression of infected fish. The damaging of integument of fish through the penetration of the hyphae may be depriving the affected fish from the protection of the mucus. The damaged one by these fungi can be directly related to tissue necrosis in the immediate area of the hyphae.

Prevention and treatment of *Saprolegnia parasitica* infection of fish have attracted a lot of attention for a long time and vast array of chemicals (Khulbe *et al*., 1995; Neish, 1977; Saheen, 1986) has been tested for effectiveness against the fungi indoors. The treatment trial of saprolegniosis affected pond applied only by potassium permanganate while the calculated dose of Malachite green, sodium chloride and hydrogen peroxide was not economic. The result of potassium permanganate treatment revealed that the drug was effective as the mortality rapidly decreased, the first day after treatment and stopped completely after 4 days of treatment but still the treatment or control of saprolegniosis is very difficult due to the economic cost of the drug or previous uncontrolled mortality. The results of small scale treatment revealed that Malachite green was highly effective followed by sodium chloride and then potassium permanganate followed by hydrogen peroxide. Although it is effective, Malachite green has been withdrawn from use with food fish (Khulbe *et al*., 1995; Neish, 1977) due to its mutagenic, teratogenic and carcinogenic properties but can be used in ornamental fishes.

CONCLUSION

From this study it could be concluded that culturing of *Mystus vittatus* and *catfishes* during the winter season requiring great care to avoid the epizootic of saprolegniosis which is uneconomical, disease induced high mortalities through its damaging effects on skin and muscles. Continuous exchange of water and entrance of new one as well as raising the column of water in ponds to more than 2 meter during the cold weather front, also the use of potassium permanganate as a prophylaxis or a treatment in the early beginning of the disease could be a useful interference.

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