SEASONAL STUDY, HISTOPATHOLOGICAL AND TREATMENT TRIAL ON SAPROLEGNIOSIS IN SOME FISH FARMS.

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ABSTRACT

The aim of this study is to follow up the seasonal occurrence of saprolegniosis which considered an economical important disease due to its regional and international impact on commercial fish culture. The routine examination of 1600 fish including Oreochromis niloticus, catfish (clarias gariepinus), Muggy cephalus and Mugil capito in 22 fish farms including 3 intensive fish farms in Kafr El-Sheikh and El-Bohaira governorates. The seasonal study extended from May 2003 till April 2004and revealed that saprolegniosis occurred in some Oreochromis niloticus as a separate cases following the routine sampling of fish to determine their average weight. In February 2004 an epizootic of saprolegniosis occurred in 9 farms following the passage of a severe cold weather front. The affected species of fish were Oreochromis niloticus and clarias gariepinus but Mugil cephalus and Mugil capito didn't affected although mixed culture by Oreochromis niloticus and Mugil species was practiced. The clinical examination revealed the presence of the characteristic clinical picture of saprolegniosis, in Oreochromis niloticus the cotton wool like masses found mostly on the head specially at the nuchal region and also covering the eyes either unilateral or bilateral while in clarias gariepinus the cotton wool like masses mostly covered the whole body of fish. The wet mount examination of 90 samples of fish from the affected farms revealed the presence of characteristic branched non septated hyphae of saprolegnia with asexual sporangium containing motile zoospores. The histopathological examination of samples from the skin and muscles showing severe damage of skin and muscles of affected fishes with demonstration of the fungal hyphae by PAS reaction where the hyphae appeared bright red. The treatment trials on affected fishes revealed that the treatment of saprolegniosis were very difficult specially in large scale water and when massive skin affection is found. Malachite green, sodium chloride, hydrogen peroxide and potassium permanganate gave good results in small scale water. Potassium permanganate gave good results on large scale water.

Abbreviations: PAS= periodic acid schiff

INTRODUCTION

Fungal diseases of fish have become increasingly important over the past 20 years. The traditional fungi are composed of members from several different taxonomic kingdoms. Saprolegnia and other typical water molds are the classic secondary invaders, infecting more superficial areas of the body and requiring compromise of the exterior of the fish, poor water quality or general immunosuppression (23,24,30).

In the present time mycotic infections with members of the family saprolegniacae are widely reported in freshwater fishes (11,16). Pickering and Willoughby in 1982 (21) described some predisposing factors that increased the susceptibility of fish to saprolegnia infection, these factors included sexual maturation, stress, integumentary damage and evidence of other pathogenic agents, virtually nothing is known about the process of infection and the characteristics that enable this pathogen alone to successfully colonize live fish.

In Egypt saprolegniosis considered one of the most important mycological diseases that infect fish and fish eggs, isolated from fish farms since long time and from this time it induce a great impact on fish culture (1,2, 5, 18).Until recently, little was known about the disease except that it occurred in the cold water months and was frequently manifest in fish exhibiting fungal skin lesions easily recognizable as cottony- white growth on the epidermis of the affected fishes (17,22) and unless fish can be treated the condition is usually lethal. Winter saprolegniosis colloquially termed winter kill syndrome(8,9,13) because it induced very high mortalities causing major financial losses for fish farmers. Saprolegniosis and fungal diseases in general are very difficult to control or treat once they have taken hold, so the aim of this study divided into, epidemiological study to determine the most effective predisposing factors for the occurrence of the disease, the most susceptible species, determination of the clinical picture of saprolegniosis in susceptible species, histopathological study in a trial to detect the exact reasons for high mortalities induced by saprolegnia and finally determine the most effective methods to prevent or control the disease and also determine the most effective drugs in treating the affected fishes in large and small scale water.
MATERIALS AND METHODS

Fish: A total of 1600 fish including of different weight ranges 960 *Oreochromis niloticus* involving 200 fish used for the small scale treatment trial transported to the laboratory (26) and 20 fish used for histopathological study , 400 *Clarias gariepinus*, 240 *Mugil cephalus* and *Mugil capito* in 22 fish farms including 3 intensive fish farms and the others are semi-intensive in Kafr El-Sheikh and El-Bohaira governorates.

-15000 *Oreochromis niloticus*, with weight range 130-150 gram in pond sized 2200 m² used in the large scale treatment experiment.

Seasonal study: Time of occurrence of saprolegniosis, predisposing causes, susceptible species and comparing the disease occurrence in both semi-intensive and intensive farms were recorded.

Clinical study, mortality pattern and post mortum examination: The clinical examination was done for the cultured fishes in the examined farms for the presence of the characteristic clinical picture of saprolegniosis and the mortality rate were recorded in comparing to the total fish cultured according to Stoskopf (26).

Mycological examination: Direct wet mount examination and isolation: Samples directly taken from the mycelium found on the lesions of skin, head and eyes of affected fishes were taken and examined for the presence of the characteristic appearance of saprolegnia hyphae also parts of the mycelium were inoculated into Sabaroud’s dextrose medium and incubated at 20°C for 48 hrs according to Neish and Hughes 1980 (20).

Histopathological study: Samples from the skin and muscles were taken from the clinically affected *Oreochromis niloticus* and also from apparently healthy fish according to Wolke (29) to determine the histopathological changes induced by saprolegnia and also the saprolegnia fungi was demonstrated in fish tissue using the PAS technique according to Neish (19).

Treatment trial experiment:

Drugs and chemicals:

- Malachite green. Sigma company.
- Sodium chloride. Commercial salt.
- Hydrogen peroxide. 70% concentration. El-Nasr company.
- Potassium permanganate. El-Gamhoria company for chemicals and drugs.

Malachite green, sodium chloride and hydrogen peroxide were used in the treatment trial only on the small scale water at doses 0.3mg / L bath for 1 hrs, 3% dipping for 5 min., 0.01% dipping for 2 min respectively while potassium permanganate used at doses 4 mg / L as flush treatment on large scale water treatment and as a bath for 30 min on small scale water treatment, the characteristic precautions for each drug followed during treatment according to Stickney *et al* (25), Alvarez *et al* (3), Stoskopf
Large scale water experiment: Field experiment on pond sized 2200m² and contain about 3500 m³, the pond have been stocked with 15000 Oreochromis niloticus, their weight range 130-150 gram. The fish suffered from the epizootic of saprolegniosis and the mortality rate averaged 80 kg /day which constitute 3-4% of the total fish cultured. potassium permanganate added at the recommended concentration (total 14 kg potassium permanganate) as flush treatment, stock solution was made freshly and added gradually at the water inlet. The mortality rate was recorded for 1 week post treatment.

Small scale water experiment: 200 Oreochromis niloticus clinically affected with saprolegniosis with weight ranged from 130-150 gm classified into 5 equal groups each group held in an aquarium sized measured 40 x 50 x 120.cm 4 aquarium received Malachite green, sodium chloride, hydrogen peroxide and potassium permanganate respectively at the treatment dose and the last aquarium left as a control group. The fish observed for 1 week post treatment

RESULTS

Results of the seasonal study of saprolegniosis: Determination of the times of occurrence of saprolegniosis during the period from May 2003 till April 2004 revealed that saprolegniosis occurred among Oreochromis niloticus as sporadic cases in the examined farms following periodical sampling of fish to determine their average weight. An epizootic of saprolegniosis occurred in February in 9 farms of the examined farms with incidence rate 40.9%, associated with high mortalities following a severe cold weather front. The disease involved Oreochromis niloticus and clarias gariepinus but Mugil cephalus and Mugil capito not affected even in ponds contain mixed culture of Oreochromis niloticus and Mugil species. Both large and small sized fish were affected. The epizootics of the disease were occurred in semi-intensive farms but in the intensive farms not affected.

Clinical study and mortality pattern: The characteristic clinical picture of saprolegniosis observed as a sporadic cases in Oreochromis niloticus following the routine sampling of fish to determine their average weight in order to calculate the amount of food given. The characteristic cotton wool like masses found on the head and ulcerd areas on the external body surface of fish. During the epizootic of saprolegniosis after the severe cold weather front, the characteristic clinical picture of saprolegniosis observed in Oreochromis niloticus on the head specially at the nuchal region (Fig.1), some fish the cotton wool like growth covered the eyes either
unilateral or bilateral (Fig. 2), at the same time some fish the cotton wool like masses covered the whole body of fish specially caudal peduncle and caudal fin (3). The clinical examination of catfish revealed that the cotton wool like masses covered mostly the whole body of fish. Very high mortality was recorded during the epizootic ranged from 25% to 70% in the affected farms.

Fig.1. *Oreochromis niloticus* naturally infected with *Saprolegnia parasitica* in the early stage showing redness in the dorsum and nuckal region.

Fig.2. Cotton wool like mass of *Saprolegnia parasitica* covered the eye of naturally infected *Oreochromis niloticus*. 
Fig.3. Cotton wool like mass covered the head and body of *Oreochromis niloticus* infected with saprolegniosis

**Mycological examination:**

*Results of direct wet mount examination and isolation:* 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. Also the isolates of saprolegnia were identified as *Saprolegnia parasitica* according to its typical morphological characters.

**Histopathological study:** The histopathological examination of skin of *Oreochromis niloticus* naturally infected with *Saprolegnia parasitica* revealed that, the epidermis showed spongiosis (intercellular edema) specially in the superficial layer. Some of the epidermal cell nuclei appeared pyknotic with marked vacuolation (Fig.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 (Fig.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 (Fig.6). In advanced cases, Muscle of *Oreochromis niloticus* infected with saprolegnia showing myolysis and macrophage
cells engulfing the necrotic muscle fiber (Fig.7) then complete necrosis of the muscle fibers was a common picture (Fig.8). The histopathological examination of skin and muscles of control non infected fish revealed normal.

Fig.4 . Skin of *Oreochromis niloticus* infected with saprolegnia showing marked spongiosis of the epidermis and epidermal hyperplasia (arrow). Notice: activated mucus cells (2 arrows). (H&E stain x200).

Fig.5. Skin of *Oreochromis niloticus* naturally infected with *Saprolegnia parasitica* showing unsegmented fungal hyphae of saprolegnia stained bright red. (arrow). (PAS stain x 400)
Fig. 6. Muscle of *Oreochromis niloticus* naturally infected with *Saprolegnia parasitica* showing severe edema between the muscle fibers, lymphocytic infiltration and melanophores aggregation (arrows). (H&E x 400).

Fig. 7. Muscle of *Oreochromis niloticus* infected with saprolegnia showing myolysis (small arrow) and macrophage cells engulfing the necrotic muscle fiber (myophagia) (large arrow). (H&E stain x 1000).
Treatment trial experiment: The mortality records in the large scale water treatment was decreased from 80 kg daily which constitute 3-4 % of the total fish stocked to 15 kg in the first day after treatment which represent less than 1% of the stocked fish, then the mortality was 4 kg daily for 2 days then the mortality stopped as very few number of died fish observed daily not exceed the number of fingers in one hand. The observation of fish in the small scale water treatment revealed that 31, 28, 22 and 26 fish completely curd in each group treated by malachite green, sodium chloride, hydrogen peroxide and potassium permanganate respectively which represent 77.5%, 70%, 55% and 65% of the treated fish. In treatment by potassium permanganate, the hyphae of saprolegnia on the affected lesions stained with brown coloration after treatment (Figs. 9&10) while in treatment by hydrogen peroxide air bubbles and white coloration observed in the whole body of fish.
(Figs 9&10) *Oreochromis niloticus* treated by potassium permanganate showing the brown discoloration of the cotton wool like masses on the affected lesions inside and outside water.
DISCUSSION

The results of periodical examination of fish including Oreochromis niloticus, clarias gariepinus, Mugil cephalus and Mugil capito in 22 fish farms in Kafr El-Sheikh and El-Bohaira governorates in the period from May 2003 till April 2004 revealed that saprolegniosis occurred among Oreochromis niloticus as sporadic cases following periodical sampling of fish to determine their average weight inspite of the temperature of water.

The results indicated that catching (bad handling) of Oreochromis niloticus 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. Many authors (14,20,24) reported that saprolegnia infection is a disease brought on by various predisposing factors including wounding of fish. Also some predisposing factors that increases susceptibility to saprolegnia infection which involve integumentary damage (21). Similarly Yanong (30) stated that saprolegnia and other typical water molds are the classic secondary invaders infecting more superficial areas of the body and requiring compromise of the exterior of the fish. In juvenile ayu (Plecoglossus altivelis) Wada et.al. (27) reported mycotic gastritis caused by saprolegnia species, the disease occurred after transportation of fish from a hatchery pond to rearing ponds, the stress of catching and transportation at the same time may render this fish vulnerable to the disease.

The results also revealed 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. (6) reported that saprolegnia was obtained from 5 separate farms which became affected by winter kill syndrome during 1991 and 1996. Also, Khulbe (15) reported fish mycosis due to saprolegina in a large reservoir in India and recorded that the severity of mycosis was primarily correlated to moderate water temperature and high temperature retarded the disease process. 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 (7,8,9,17,30) firstly that the rapid decrease in water temperature (6 C during the day and at night reach under zero) induced
immunosuppression to such fishes, secondly that the low water temperature favored high levels of saprolegnia species zoospores, the immuno-compromised fish associated with 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.

The results also revealed that *Oreochromis niloticus* and *clarias gariepinus* highly susceptible to saprolegnia but *Mugil cephalus* and *Mugil capito* resistant to the disease, this may be due to that the extreme low temperature constitute a great stress on such fishes but Mugil species have a wide range of thermal tolerance (25).

On comparing the occurrence of the disease in semi-intensive and intensive farms, the results revealed that the semi-intensive farms suffered greatly from the epizootic of saprolegniosis following the severe cold weather front, while no affections were occurred in the examined intensive farms. This may be attributed to that the intensive farms depends on underground water, their temperature greatly higher than that of surface water that semi-intensive depends upon, secondly the circular concrete ponds in the intensive farm constructed as a funnel shape, the column of water in the center reach 3 meter which may give the chance for fish to stay in deep water during the cold weather front.

The results of clinical examination revealed the presence of characteristic picture of saprolegniosis, presence of cotton wool like masses covered the head and ulcerd areas on the external body surface after routine sampling 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 condition appeared 3-4 days following catching and involve few number of fish. During the epizootic, the same picture appeared but various degrees of infection found and the dead fish in some ponds may cover the surface of the water. The easily recognized cottony mycelium on the surface of affected fishes have been probably recognized since antiquity (1,10,12,28) published reports describing these infections found since long time. Similar picture of mortality cited by Fregeneda (13) that saprolegniosis can follow an epizootic course and cause a high mortality rate as has occurred amongst wild brown trout (*Salmo- trutto*) in various rivers between 1980 and 1986.
The identification of the genus of the fungus by of wet mount procedures and isolation increase the incrimination and responsibilities of *Saprolegnia parasitica* to the winter kill syndrome.

The histopathological examination of skin and muscles of *Oreochromis niloticus* 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 disease as a result of impairment of osmoregulation (4). Also the absence of leukocytic infiltration around the invading fungal hyphae may indicate the state of immunosuppression of infected fish. The damaging of integument of fish through the penetration of the hyphae may depriving the affected fish from the protection of the mucus. The damage done 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 (1,5,12) has been tested for effectiveness against the fungi indoors. The treatment trial of saprolegniosi in 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 its effectiveness, Malachite green has been withdrawn from use with food fish (1,12) due to its mutagenic, teratogenic and carcinogenic properties but can be used in ornamental fishes.

From this study it could be concluded that culturing of *Oreochromis niloticus* and *clarias gariepinus* during the winter season requiring great care to avoid the epizootic of saprolegniosis which is an economical 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.

REFERENCES (BIBLIOGRAPHY)


دراسة موسمية وهستوباثولوجية وكذلك إجراء محاولات علاجية على مرض الصابرولجيا في بعض المزارع السمكية

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الغرض من هذا البحث هو المتابعة الموسمية لحدوث مرض الصابرولجيا ودراسة العوامل التي تؤدي إلى حدوث المرض، حيث أن مرض الصابرولجيا من أهم الأمراض التي تؤدي إلى خسارة اقتصادية كبيرة في المزارع والمفرحات السمكية في مصر وفي العديد من دول العالم. وقد أوضح الفحص الدوري لعدد 1600 سمكة شملت 960 من أسماك البلطي النيلى و400 من أسماك القراميط و240 من البور الحر والطوبارة في عدد 22 مزرعة في محافظات كفر الشيخ والبحيرة في الفترة من مايو 2003 وحتى أبريل 2004 حدوث مرض الصابرولجيا في أسماك البلطي النيلى مصاحباً لعمليات الصيد الدوري لمعرفة متوسط أوزان الأسماك وتحديد نسب الغشائية. وقد تم تسجيل حدوث وفاء لممرض الصابرولجيا في عدد 9 مزرع في فبراير 2004 ما يشير إلى انخفاض شديد في درجة الحرارة. وقد وجد أن الأسماك المصابة هي أسماك البلطي النيلي والقراميط ولم تحدث أي وفيات أو إصابات مرتبطة في أسماك البور الحر والطوبارة على الرغم من وجود استزراع زدوج للبلطي البور في نفس الأحواض.

وقد أوضح الفحص الهستوباثولوجي للعينات من الجلد والعضلات للأسماك المصابة وجود أحماض تحلل بالعضلات، تجمعت بالخلايا الكلوية وتتكزر واضح بخلايا الجلد والعضلات. كما أظهر الفحص وجود ظاهرة مزدوجة للنضرومبولجيا الطفيلي باللون الأحمر اللماع، وعدد عمل محاولة علاجية على الأسماك المصابة وجد أن علاج مرض الصابرولجيا ولا يijkeب英语، استخدام الأخفض مالكية كوريد الصوديوم، فوقع أكسيد الهيدروجين، كوريد الصوديوم، وصوديوم الهيدروجين كوريد في اليوم التالي للعلاج مماثل في اليوم التالي 15 كجم يومياً (1% تقريباً من كثافة الحوض) إلى 80 كجم يومياً (3-4% تقريباً من كثافة الحوض) وقد لوحظ أن الاستجابة العلاجية تعتمد على مدى الإصابة، وعدد عمل محاولة علاجية على عدد 200 سمكة مرضة بغطر الصابرولجيا، بالأخضر ملاكيت، كوريد الصوديوم، فوقع أكسيد الهيدروجين وبرمنجات البوتاسيوم أعطت نتائج إيجابية بنسب 77.5%, 70% و55% بالتبغ.