قسم : الباثولوجيا .
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البرنكوميكورنس

"مرض فطرى يصيب أسماك المزارع المصرية سمك البورى والطوبار العائلة البورية "

محي السعيد عيسى

استخدم في هذا البحث مائة وخمسون سمكة من نوع العائلة البوريه التي تربى في مزرعة اسماك الزاويه (محافظة كفر الشيخ) . هذا العدد من الاسماك كان ضمن ستة الاف سمكة نفقت خلال ٣ - ٥ يوم من ظهور الاعراض.

هذه الاسماك ظهرت عليها أعراض سوء التنفس مع وجود ارتشاحات مائية في منطقة الرأس.

من دراسة التغيرات المرضية الظاهرية ومن خلال استخلاص الغزل الفطرى من الخياشيم ومن الفحص الباثولوجي وكذا التركيب المرفولوجي للغزل الفطرى وكذا الجراثيم امكن تشخيص الفطر أنه من جنس البرنكوميكوز (الرنكوميكوز المهاجر)

وقد أثبتت الدراسة الباثولوجيه للخياشيم وغطاء الخياشيم والكبد وكذا الكليتين أن مسبب نسبة النفوق العالية بين الاسماك في هذا الوقت القصير يمكن أن يرجع الى انسداد الأوعية الدموية الخيشومية بغزل الفطر. واختلال تبادل الغازات في الجهاز الدورى للخياشيم التي ظهرت متآكلة .

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BRANCHIOMYCOSIS "MYCOTIC DISEASE" OF EGYPTIAN FISH FARMS (MUGIL CEPHALUS AND MUGIL CAPITO. "GRY MULLET")

By
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SUMMARY

This study is the description of Branchiomycosis in Gray mullet in Egypt. This disease occured two times in july within 4 years interval. From the morphology of the fungus, size of the spores, thickness of the wall of the hyphae, beside its location in gill tissue, the fungus was identified as Branchiomyces demigrans.

The histopathological alterations in gills, gill covering, liver, kidneys are described, as well as the conterol of the disease.

INTRODUCTION

Branchiomycosis or gill rot is a typical hot weather disease caused by fungus of genus Branchiomyces. The disease usually appear in summer (June, July, August) when water temprature exceeds 20 C, this explains the distribution of the disease in southern Poland, Hungary, Yugoslavia, in specific localities of Germany, Italy, Czechoslovakia, and in southern zone of USSR especially in Okraine.

The effect of the invironmental factors on the appearance of the disease was studied by WOLNY (1954), SCHPERCLAUS (1941, 1954), WOLF (1956) and DYK (1956).

Branchiomycosis caused major economic losses, HUCULAK (1958) reported the occurrence of Branchiomycosis in Coregonus albula reared in ponds with infected carp, with 100% mortality while GYLA et al. (1967) studied Branchiomycosis in Silurus glnis and reported losses up to 50% withen 4-8 days.

The disease was described in Germany for the first time by PLEHN (1912) who had observed mycotic hyphae within branchial vessels which resulted in thrombosis and necrosis of gill tissue ending in death of the affected carp. She named the agent Branchiomyces sanguinis. WUNDSCH (1930) found that the fungal filaments of genus Branchiomces grew extracascularly in the surrounding epithelium in Pike and Tench. He attributed such infection of the fungus Branchiomyces demigrans. In USSR, BESPALYI (1950) briefly described Branchiomycosis in carp. He isolated B. sanguinis and B. demigrans on a singel culture, and therefore, he doubted whether B. demigrans is a different species. Later on IVASIK and DEMCHENKO (1959) demonstrated B. sanguinis in the branchial blood vessels as well as in the liver and spleen of the carp in USSR farms. The fungus did not cause marked pathological changes in the liver and spleen. In Czechoslovakia, TOMANEK (1962) found that Branchiomycosis was evoked by B. sanguinis in the Salmo gardneri. On other hand, in Silerus glanis the disease is caused by B. demigrans (LUCKY, 1970). MEYER and ROBINSON (1973) mentioned that Branchiomycosis occurs in fingerlling Striped bass (Morone saxatilis) and adult Largmouth bass (Microptrus samoides) in fish hatchery of North America. On the basis of location of the filaments in gill tissue and the

demonstration of the hyphae and spores the auther considered the causative fungus to be B. sanguinis.

This literature support the view that the diagnosis and identifecation of Branchiomycosis is based mainly on the microscopical dingings of the fungal elements in gill tissue. In the meantime it has not been finally decided whether one or more species of the fungi of the genus Branchiomyces parasitise the gill of fish as well as there is a lack of information dealing with the histopathological changes in gills and the internal organs of fish infected with Branchiomyces species.

This contribution is to report the occurrence of the Branchiomycosis in egyption fish farms as well as to identify its species based upon the histopathological examination of gills and demonstration of the fungal elements in tissues.

MATERIAL and METHODS

An enzootic occurred during July, 1977 in an Egyption fish farm (Elzawia) in Kafer El-SheikhGovernorate, where the mortality rate among Mugil cephalus and Mugel cpito "Gray mullet" reached 70% within 4-8 days after the appearance of clinical symptoms.

Symptoms manifested itself as fish gathering in groups at the surface of the water and swim close to the shore of the ponds. The fish showed respiratory distress, cease to eat, gather at the inlet, move upstream or remain with the head down near the inlet and eventually die.

Additional outbreak reoccurred in the same farm during July 1981 where fish were stocked in the same ponds and the preceeding signs of impaired respiration was exhibted. water temprature was 25-29C, pH 7-7.5 and oxygen 4ppM. About 6000 fish died within 3-5 days of the appearans of clinical symptoms. Fish varied from 150-200 gm in weight and from 30-35 Cm in length and were 8 months old.

On examination of these fish, the gill filaments appeared swollen, oedematus and surrounded with a great amount of mucous. Gill leaflets showed dark red strips. Sometimes reddish discolouration appear in the form of patches which differ in size from few mm to cm in addition the free portion of the gill filaments appear pale pink in colouration, denuded and muddy gray (Fig. 1). This picture in gill leaflets gave it a marbling appearance. In most cases parts of gill leaflets were necrotic, it looked as if pieces of gills had been cut off, or fish had bitten itself.

Periorfital oedema, in most cases, may extend to involve the region to the snout, and cheek (Fig. 2). The scales fall off in focal patches (Fig. 3). The remaining scales were easy to slough.

There was no pathological changes in internal organs except, in most cases liver, kidneys, and spleen are congested, and the intestine is devoid of food.

Complete necropsy was performed on 150 cases, 100 of them were dead and 50 showed clinical signs. Parts of the gills from the dead and dying fish are washed in water 3 times, and homogenized in morter with the aid of glass beeds. Few drop of water are added to the homogenate and the material is centrifuged, the supematent fluid is descarded, and then an equal amount of water is added, the material is centrifuged for three minutes. This process is repeated three times. The supernatent fluid was discarded. A sample of the sediment is pot on a glass watch under the micrscope and thus the fungal hyphae can be eliminated from the homogenized tissue, BESPALYL, (1950).

Specimens from gills, liver, kidneys and spleen are fixed in phosphate buffer formalin and gill covering is decalcified in acid solution (WAHTOLA, JUN and OWEN, 1970). Paraffin sections of 6-10 microns are prepared, stained with haematoxylin and eosin, periodic acid schiff reaction (PAS) and prussian blue, and examined microscopically.

RESULTS

The finding of histopathological examination of the gills and internal organs explain the clinical picture which was seen in dead and dying fish.

In most of the examined cases extensive dilatation of the branchial blood vessels of gill arches, hemorrhages and oedema especially in sublamellar zone, leucocytic infelteration of gill arch mainely lymphocytes, plasma cells, and macrophages are present. Sometimes focal aggregation oflymphocytes are seen together with eosinophil granular cells (EGC), ovoid shape with eosinophilic coarse granules, its nucleus was flat and excentrically located. Sometimes these cells are aggregated at the base of the primary lamellae forming a discrete and distinctive submucosal layer.

Epithelial lining of the secondary lamellae show hypertrophy with extensive hyperplasia especially at the base of the lamellae (Fig. 4). As a result of rapidly multiplying epithelial cells, in some cases it causes fusion of secondary lamellae, thus the filaments appear as a singel leaflet. In this condition there is a collection of mononuclear cells, lymphocytes and primetive reticular cells among the hyperplastic epithelial cells.

In some cases oedema and separation of the epithelium from the capillary bed of secondary lamellae are demonstrated (Fig. 5). In all examined cases necrosis and sloughing of great numbers of secondary lamellae are seen (Fig. 6). In most cases branchial blood vessels of primary lamellae are dilated but in secondary lamellae showed telangectasia (Fig. 7). In other cases these vessels are destroyed and haemorrhages are seen in lamellae interstitial tissue especially at the point of pentration of the fungus.

Fungal elements are seen concentrated especially at the base of primary lamellae. However some of them are seen at the apex of the secondary lamellae. The hyphae carry spores which are spherical or subspherical, smooth, 6-8 micron in diameter. Number of spores in section of the hyphae are 4-6 spores. The width of the hyphae is 26-40 microns although the wall of the hyphae measured 0.4-0.7 microns. Fungal elements cluster among the necrotic lamellar tissue (Fig. 8), and commonly occur at its base. Hyphae and spores are seen in and around the branchial vessels of gill arches. The wall of the hyphae appear strongly eosinophilic and the spores appear slightly basophilic. Hyphae and spores are PAS positive. Hyphae and spores are seen in the branchial vessels but most of them are demonstrated in interstitial tissue and the epithelial lining the gill filaments.

Histopathological examination of the operculum show spongisis of epidermal cells and extinsive oedema in the subepidermal layer and among its muscle bundels. Oedema extend to the supporting cartilagenous pieces of operculum.

The majority of blood capillries of the liver are dilated and filled with erythrocytes. Sometimes haemorrhages are seen. Siderocytes are aggregated around the centeral veins, where there are great numbers of EGC, and vacular degeneration of hepatic cells is commen, particularly centrilobularly (Fig. 9).

Sever congestion of the renal capillaries together with intertubular haemorrhages are also seen. Renal glomeruli show proligeration of the endothelial lining the capillary loops, with

swelling of the epithelial lining of the Bawman's capsule. Degenerative changes of the renal tubules are characterized by swelling and granulation of its epithelial lining. In some cases the renal tubules especially the first proximal segments are filled with desquamated epithelial cells. In other cases they were filled with homogenous darkly eosinophilic renal casts.

DISCUSSION

The hypertrophy and hyperplasia which appear in branchiomycotic gill lamellae indecates that the fungus exited in the host for a while which causes mild irretation. This proliferative changes in the lamellar epithelium was noticed by BESPALYI (1950), APAZEDE (1963) and LUCKY (1970). The rapidly multiplying cells cause fusion of the distal margins of the adjacent lamellae. In the more advanced stages the inter lamellar spaces of the filaments are partially of completely filled with solid blocks of epithelial tissue and this blanket a great part of the respiratory surface which could lead to death of fish.

In case of high infestation of the gills with great numbers of fungal elements, cause thrombosis and hemorrhages of the branchial vessels, the gill lamellae become necrotic and lead to death of great numbers of fish from suffocation.

The high mortality rate that occurred withen few days may be due to the lodgement of the fungus in the branchial vessels and lamellar tissue. The lamellar-blood circulation becomes stagnant. The epithelium covering the secondary lamellae is degenerated and shed away in a continuous sheat from the capillaries, thus the increasing the diffusion distance from the water to blood. Thus the centeral lamellar spaces are decreased in size, terminating in respiratory collapse and death of great numbers of fish in few days. This can be explained by the detchment fo the epithelium lining the secondary lamellae and extensive oedema of gill filaments which simulates what mentioned by SKIDMORE and TOVELL (1972) in case of zinc sulfate poisoning of Rainbow trout.

Sever congestion of hepatic blood capillaries and haemorrhages were seen in some cases, degenerative changes of hepatocytes could be the result of the direct action of a mycotic toxin (APAZEDE, 1963) or a sequelae to general venous congestion due to mycotic lesions in the respiratory organs of fish.

Oedema in the operculum and periorbital tissue as well as the degenerative changes of renal tubules and the epithelial lining the Bawman's capsule support the view of the general venus congestion and toxic effect of the fungus.

Fungel elements could not demonstrated in the hepatic tissue of any one of examined cases which dose not correlate with the result obtained by IVASIK and DEMCHENKI (1959).

According to morphology of the fungus, diameter of the hyphae, thickness of its wall and diameter of the spores as well as location of the fungal elements within the gill of the Gray mullet, the fungus was considered Branchiomyces demigrans which usually migrates from the branchial blood vessels to the lamellar interstitial tissue and its epithelial covering.

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CONTROL OF THE DISEASE

In order to remove the excess of organic matter which helps the gross and multiplication of the fungus as well as the rise in the acidity of water, an immediate colliction of dead and dying fish were done, water level and current of water in ponds were raised to maximum and feeding of fish was discontinued.

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MYCOTIC DISEASE OF EGYPTIAN FESH FARMS

LEGINDS

Fig. (1): Branchiomycotic gills.
Upper : Gill filaments swollen, oedematus, surrounded with great amount of mucus.
Middle : Gill leaflet showing patches of reddish discolouration.
Lower : Gill leaflet denuded and muddy gray in colouration.

Fig. (2): Gray mullet.
Oedema of periorbital, snout and cheek region.

Fig. (3): Gray mullet.
Notice, shedding of the scales in focal patches.

Fig. (4): Gill filament showing:
Extensive lamellar hypertrophy and hyperplasia with highly infestation by Branchiomycis hyphae.

Fig. (5): Gill filament.
Notice, oedema and desquamation of epithelial lining secondary lamellae, mycotic element at its base.

Fig. (6): Gill filament showing:
Extensive necrotic changes of secondary lamellae, fungal elements inbetween.

Fig. (7): Primary lamellar oedema and telangiectasis in secondary lamellae.

Fig. (8): Notice:
Necrosis and sloughing of secondary lamellar epithelium, Branchiomycis spores inbetween.

Fig. (9): Liver of branchiomycotic Gray mullet showing, severe congestion of the hepatic vessels which appear surrounded with EGC (arrow).

Fig. (1)

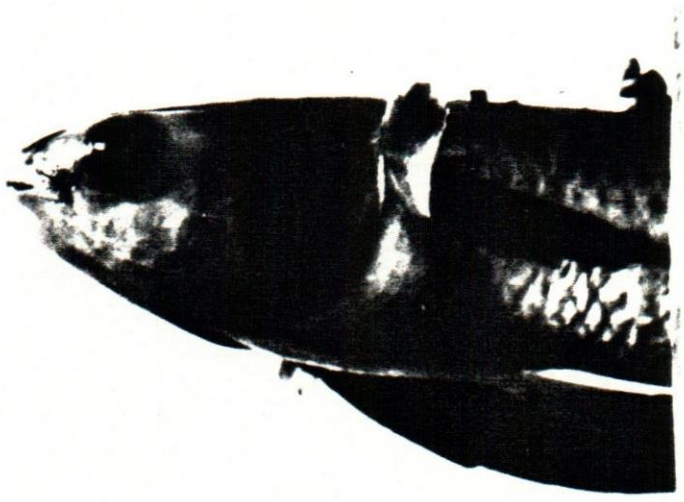

Fig. (2)

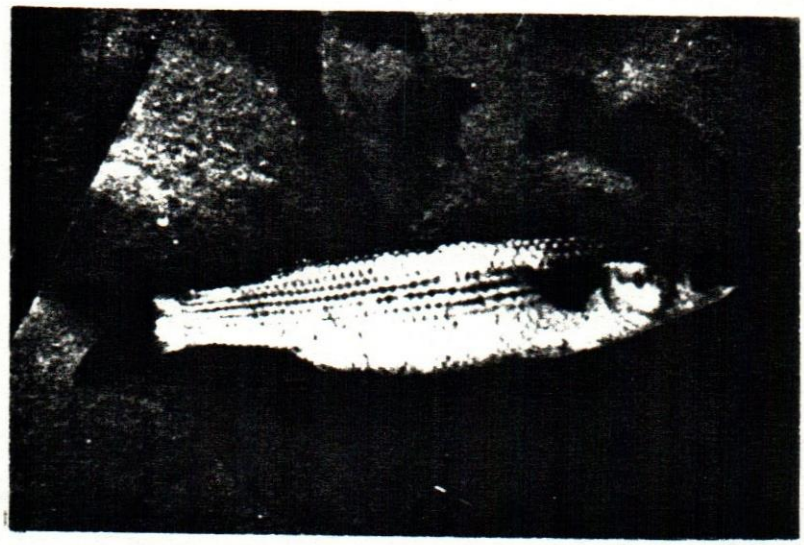

Fig. (3)

Fig. (4)

Fig. (5)

Fig. (6)

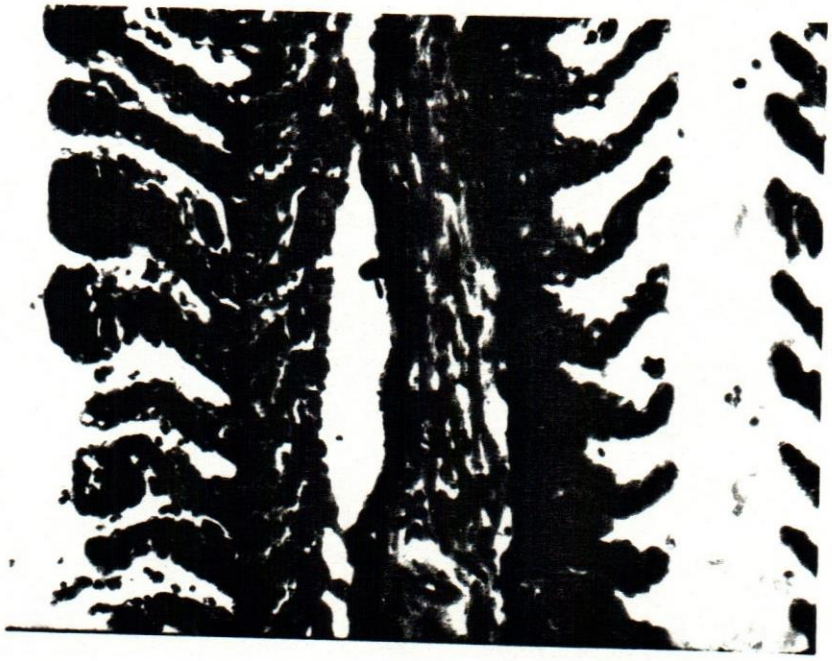

Fig. (7)

Fig. (8)

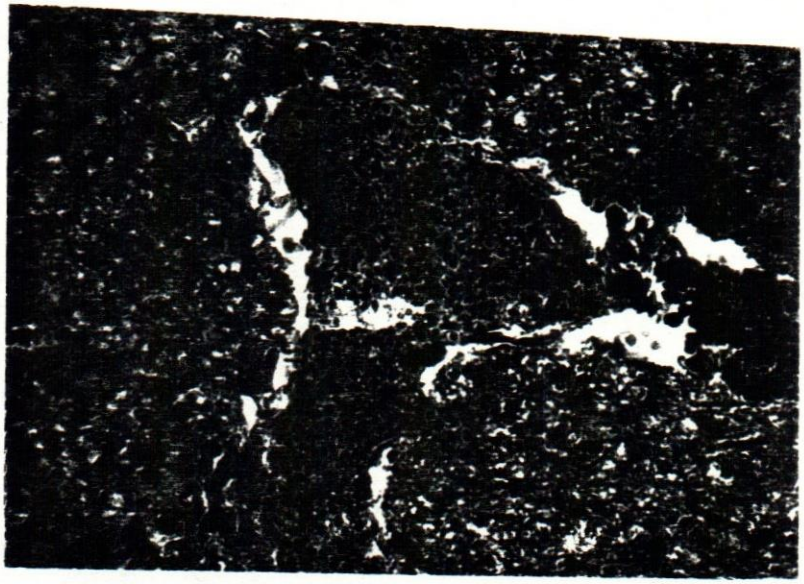

Fig. (9)