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BACTERIOLOGICAL AND HISTOPATHOLOGICAL STUDIES ON AEROMONAS HYDROPHILA INFECTION OF NILE TILAPIA (*OREOCHROMIS NILOTICAS*) FROM FISH FARMS IN SAUDI ARABIA

(With 1 Table and 9 Figures)

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دراسات بكتيريولوجية وهستوباثولوجية عن الإصابة ببكتريا الأيرمونس لأسماك البلطي النيلي المستزرعة بالمملكة العربية السعودية

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تم إجراء دراسات بكتريولوجية وهستوباثولوجية لأسماك البلطي النيلي المستزرعة تحت نظام التربية مكثف في مياه آبار ذات درجة ملوحة مابين ٤-٧ جم/لتر . أشارت الطرق المورفولوجية والبيوكيميائية عن وجود بكتيريا الأيرمونس هيدروفيلا بالأسماك. تم أخذ العين والكبد – البنكرياس والطحال والأمعاء وتثبيتها لقرنية العين. وكذلك إلتهاب بالعضلات المدعمة للجدار المتصلب للعين. كان هناك إستحالات دهنية وتنكرز لخلايا الكبد وكذلك إلتهاب للنسيج البنكرياسي . علاوة على ذلك كان هناك نشاط ملحوظ لمراكز الخلايا الأكولة المحملة بالميلانين في الطحال. لوحظ أيضا تجمع المخاط في الأمعاء. تم مخاطبة أصحاب المزارع لتحسين عوامل جودة المياه وتخزين العلائق بطريقة سليمة وذلك لتقليل أو تلافي الوفيات وخاصة عندما ترتفع درجة حرارة المياه إلى ٤٠ – ٥- درجة مئوية.

SUMMARY

Bacteriological and histopathological studies on *Aeromonas hydrophila* infection of the Nile tilapia (*Oreochromis niloticus*) raised under semi-intensive culture system in Saudi Arabia using well water of 4-7 g/L salinity were conducted. Morphological and biochemical methods showed isolates of *Aeromonas hydrophila*. The eyes, hepatopancreas and spleen were processed for light microscopy. Corneal spongiosis, ulcer

formation and inflammation of the muscles supporting the sclera were observed. Fatty change, necrosis and pansteatitis were common findings in the hepatopancreas. Moreover, activation of the melano-macrophage center in the spleens was noticed. Fish farmers/owners should improve water quality and feed storage to avoid or minimize the mortalities occur when the water temperature increases to 40-50°C.

Key words: *Bact. & Histopathology, Aeromonas, Hydrophila, Nile Tilapia.*

INTRODUCTION

Motile Aeromonad Septicaemia (MAS) is a common bacterial cause of fish mortality (Austin and Austin, 1987). This disease can be fatal with no gross clinical signs. It can also result in the formation of deep dermal ulcers and organ haemorrhage (Thune et al., 1993). *Aeromonas hydrophila* can be isolated from coastal and brackish water where salinity is low (Boira, 1996). Recent reports have indicated the occurrence of *A. hydrophila* in marine cultured Atlantic Salmon (Candan et al., 1995) and in cultured sea bss from the Aegean Sea (Doukas et al., 1998) and in Nile tilapia from Upper Egypt (Amin et al., 1985).

The Nile tilapia (*Oreochromis niloticus)* has been introduced to the aquaculture industry in Saudi Arabia. Private fish farms were able to raise the species under semi-intensive culture system using well-water of 4-7 g/L salinity. These farms are facing mortalities when the water temperature raised to 40-50°C. During this period, the morbid fish had signs of haemorrhagic septicaemia. The purpose of the present study was to isolate and identify possible bacteria causing mortalities in these farms, and to describe the lesions histopathologically.

MATERIAL and METHODS

Fish farms:

Four different farms located in the western region of Saudi Arabia were selected and assigned as F1, F2, F3 and F4. The F1, F2 and F4 have concrete tanks, while F3 has both concrete tanks and earthen ponds. These farms use well water of 4-7 g/L salinity. The stocking density in these farms varried from 50-70 fish/m^3.

Fish:

Oreochromis niloticus are produced regularly in these farms for marketing. The number of fish sampled per farm was 5 to 10 fish. The total number of examined fish from all farms was 120. Three fish were randomly selected from each farm for histopathology.

Bacteriology:

Bacteriological examination was done on both morbid and healthy fish. Fish were transported to the laboratory on ice. Samples for microbiological examination were taken aseptically from the posterior kidneys and external ulcerations then inoculated in TSA and Rimler-Shotts media supplemented with 0, 2, 5% Na C1 at 25°C for up to 48 h. Twenty-four isolates from all fish farms were identified using commercial API 20E system (Biomerieux Lab., France).

Histopathology:

Fish were carefully examined grossly for the presence of any gross lesions and killed by over exposure to clove oil. The eyes, hepatopancreas and spleens were taken immediately, fixed in 10% formalin, dehydrated in alcohol, embedded in paraffin, sectioned at 4-6 u, stained by haematoxylin and eosin and examined by light microscopy.

RESULTS

I- Bacteriological examination:

All isolates from *O.niloticus* of F1, F2, F3 and F4 were identical morphologically and biochemically identical, and identified as A. hydrophila (Table 1).

II- Pathology:

Gross lesions:

Oreochromis niloticus showed ecchymosis, loss of scales and few ulcers in the trunk area. The eye orbits had redness at the margins. Corneal opacity and slight exophthalmia were observed in the eyes of morbid fish. Bloody peritoneal fluid was also found. The livers were enlarged and had pale patchy areas. The gall bladder was engorged with bile. Spleenomegaly and accumulated whitish exudate in the intestinal lumen were common findings.

Histopathologyical lesions:

1) Eyes:

The stratified squamous epithelium of the cornea showed spongiosis (Fig. 1). In some areas there was necrosis of the epithelium

lining the cornea leaving an ulcer (Fig. 2). The bottom of these ulcers had remnants of degenerated epithelium as well as red blood cells (Fig. 3). The collagen fibers of the substantia properia lost their fibrillar arrangement (Fig. 2,3). The muscular layer supporting the sclera showed lymphocytic and red blood cells infiltration (Fig. 4).

2) Hepatopancreas:

The hepatic tissue showed areas of fatty change with the typical signet-ring appearance. Focal coagulative necrosis with pyknotic nuclei was intermingled with the fatty areas (Fig. 5). The pancreatic tissue was also infiltrated with fat cells (Fig. 6). Pansteatitis was expressed by the presence of neutrophils, mononuclear cells, lymphocytes and red blood cells (Fig. 7, 8).

3) Spleens:

Enlargement and activation of Melano-Macrophage Center (MMC) was observed (Fig. 9).

DISCUSSION

Aeromonas hydrophila has been studied extensively. Several reports on isolation, pathogenesis and typing methods including molecular biology have been reported. Aeromonas spp. from the organs of healthy tilapia such as kidney, spleen and livers were isolated. Moreover, Aeromonad spp. were isolated from low and high salinity environments (Torres et al., 1990; Boira, 1996; Hamilton, 1996 and Doukas et al., 1998).

The present study showed identical isolates of *A. hydrophila* in *O.niloticus* from the farms using morphological and biochemical methods. These results were similar to the reports in tilapias and sea bass (Doukas et al., 1998 and Yambot, 1998). *Oreochromis niloticus* raised under Saudi Arabia environmental conditions had mortalities and exhibited signs of haemorrhagic septicaemia, especially in temperatures between of 40-50°C. Abrupt temperature change is known to be one of the predisposing factors which contribute to the infection of *A.hydrophila* (Leung et al., 1994).

The gross lesions described in this study were the same to the naturally and experimentally induced lesions reported previously (Roberts & Sommerville, 1982; Roberts, 1993; Thune et al., 1993 and Yambot, 1998). The spongiosis of the corneal epithelium, fibiral disorganization of the substantia properia and the ulcers observed in the tilapia eyes are reponsible for the corneal opacity observed grossly.

There are several lesions involved in the aetiology of corneal opacity which include corneal oedema, stromal fiber disorganization and keratitis

(Borucinska et al., 1998). While, the haemorrhagic myositis was contributed for the redness of the eye oribit observed grossly.

The fatty change and the hepatic necrosis observed suggested a hepatotoxic effects resulting from the bacterial toxin produced. A. hydrophila is known to produce toxins, haemolysins, extracellular products (ECPs) and acetylcholinestrase (Ljungh and Wadstrom, 1982 and Nieto et al., 1991).

This study showed pansteatitis of the peri-adipose tissue of the pancreas and suggested that an improper feed storage had occurred in these farms. It is known that the temperature of feed storage, air and light can increase the amount of lipid peroxidation (Guarda et al., 1997). Fat altered liver is very common in farmed fish (Roberts and Bullock, 1989). Fat oxidation is one of the most important factors causing feed deteriorations and plays a role in inducing metabolic changes that could be the basis of different diseases, among these is fat steatitis (Guarda et al., 1997). Roberts et al. (1979) attributed pansteatitis to vitamine E deficiency or insufficient antioxidant protection in rainbow trout.

Melano-macrophage centres (MMCs) in higher teleosts are known to play an important role in phagocytosis (Ellis et al., 1976; Agius, 1979 and Macchi et al., 1992). Activation of the MMC in the spleens observed in this study suggesting phagocytosis.

CONCLUSIONS

Aeromonas hydrophila was isolated and identified morphologically and biochemically from Nile tilapia (*O.niloticus)* raised under semi-intensive culture system in Saudi Arabian fish farms. The infection might be responsible for the mortalities occurring at 40-50°C. Histopathological findings were related to the aetiology. Fish farmers should be more concerned during periods of high temperature as this cause stress and subsequently bacterial infection. Likewise, proper temperature should be maintained during feed storage, as higher temperature causes feed oxidation.

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FIGURE LEGENDS

Fig. 1: The corneal epithelium of Nile tilapia eyes (*O. niloticus)* showing spongiosis (Head arrow). Haematoxylin and Eosin. X 128.

Fig. 2: Necrosis of the corneal epithelium (E) resulted in ulcer formation (Arrow), S (substantia properia), I (iris), R (retina). Haematoxylin and Eosin. X 12.8.

Fig. 3: The bottom of these ulcers had remnants of degenerated epithelium and red blood cells (Arrow). The substantia properia (S) showing loss of the fibril arrangement. Haematoxylin and Eosin. X 32.

Fig. 4: The muscular layer supporting the sclera (M) showing red blood cells (Arrow) and lymphocytes (wide Arrow). Haematoxylin and Eosin. X 32.

Fig. 5: The livers of Nile tilapia (*O. niloticus)* showing areas of fatty change (Arrows) and focal necrosis with pyknotic nuclei (N). Haematoxylin and Eosin. X 32.

Fig. 6: The panreatic tissue (P) showing infiltration with fat cells (F). Haematoxylin and Eosin. X 32.

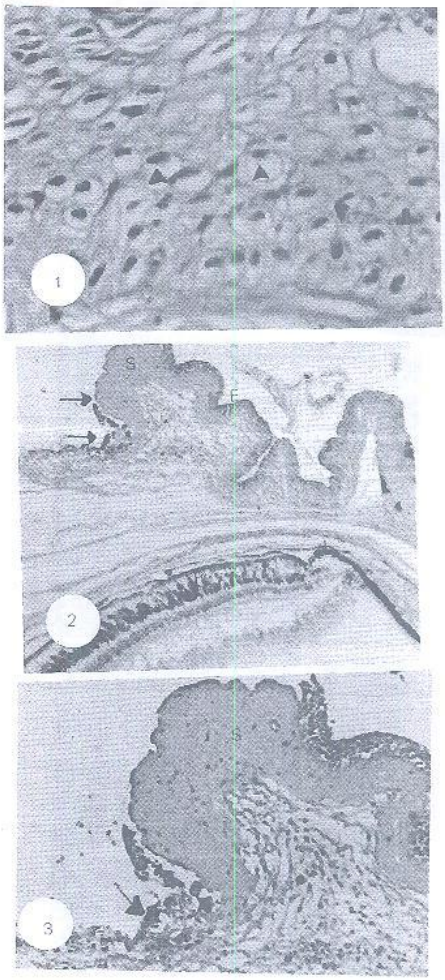
Fig. 7: Pansteatitis (ST) around the adipose tissue (F) of the pancreas. Haematoxylin and Eosin. X 12.8.

Fig. 8: Higher magnification of Fig. (7) showing mononuclear cells (Head arrow), neutrophils (Arrow), lymphocytes (Wide arrow) and red blood cells. Haematoxylin and Eosin. X 320.

Fig. 9: The melano-macrophage centre (MMC) of the spleen showing enlargement and activation. Haematoxylin and Eosin. X 32.

Table 1: Cultural and biochemical characterstics of *Vibrio alginolyticus* isolated from the black tail area of *Penaeus monodon.*

Tests	Reactin
Gram stain	-
Morphology	Rods
Motility	+
Growth with Nacl (0%)	+
Growth with NaCi (2%)	+
Growth with NaCl (5%)	-
Growth on Rimler-Shotts medium	+
Oxidase	+
B-galactosidase	+
Arginine dihydrolase	+
Lysine decarboxylase	-
Ornithine decarboxylase	-
Citrate utilization	-
H_2S production	-
Urease	-
Tryptophan deaminase	-
Indole	+
Voges-Proskauer	+
Gelatin hydrolysis	+
Acid from:	
Glucose	+
Manitol	+
Inositol	-
Sorbitol	+
Rhamnose	-
Sucrose	+
Melibiose	-
Arabinose	+
Amylodose	+
Gas from glucose	+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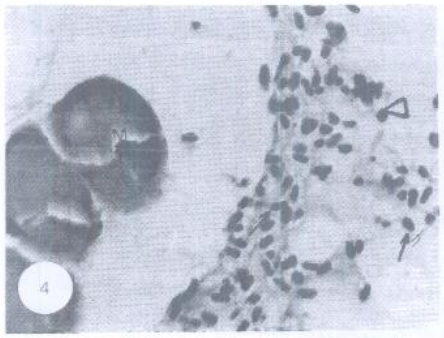
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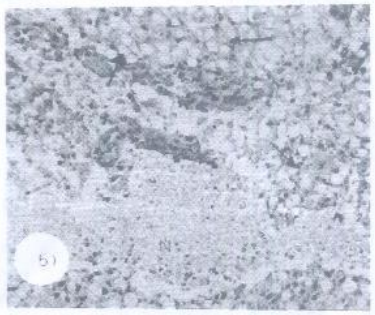
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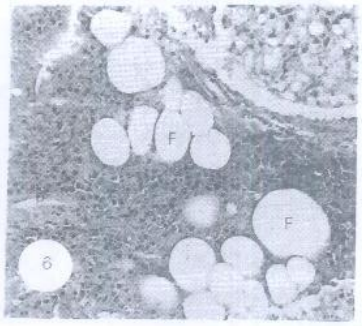
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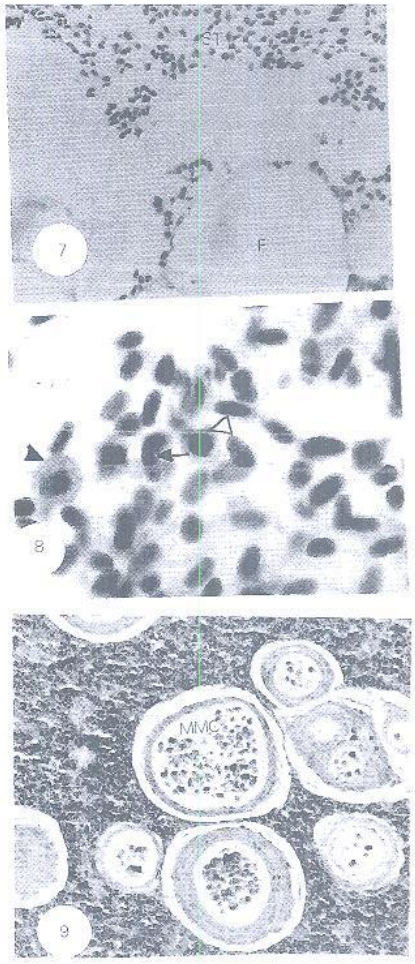
ST
F
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MMC
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