PATHOLOGICAL CHANGES ASSOCIATED WITH STREPTOCOCCAL INFECTION ON *OREOCHROMIS NILOTICUS* AND *CLARIAS GARIEPINUS*

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Abstract

A total number of 1200 fishes (600 *Oreochromis niloticus* and 600 *Clarias gariepinus*) were collected from different commercial fish farms and subjected to clinical, postmortem, bacteriological and histopathological examinations. The prevalence rate of streptococcus infection among the examined fish was 16.2 %. The bacteriological examination revealed the isolation of *Streptococcus faecium* 115 isolates (9.6%), *Streptococcus faecalis* 79 isolates (6.6%). The naturally and experimentally infected fish showed loss of appetite, sluggish movement, swimming close to the surface of the water, lethargic, no escape reflex, darkening of the skin, easily detached and lose of the scales, ulcer formation, uni- or bilateral exophthalmia (pop-eye), in some cases cloudy change and destruction of eye were observed. Haemorrhages on the skin especially in the base of fins and tail, slight abdominal distention, inflammation, congestion of anal opening and vertebral column deformities. Gills were covered with mucus and pale in colour. Internally the liver was pale in some cases and dark brown in others in association with over distended gallbladder and the peritoneal cavity filled with ascetic fluid. In some cases haemorrhage in intestine, enlarged spleen and nearly black, enlarged and congested kidney were noticed. Congested testis was observed. The histopathological changes of artificially infected fish were similar to the naturally infected fish. The histopathological alterations in different organs and tissues were severe congestion, leucocytic infiltrations, degeneration, edema and necrosis. Eye of naturally infected *O. niloticus* with *Streptococcus* `showed severe intraocular haemorrhage.

INTRODUCTION

Bacterial pathogens are the most serious disease problems in fish production causing about 80% of fish mortalities (Plumb, 1999, Woo and Bruno, 1999, Clark *et al.,* 2000 and Shoemaker *et al.,* 2000).

Historically, *Streptococcus sp.* are not serious pathogens of fish, but recently, these bacteria have become more prominent in wild and cultured fish. Streptococcal infection of fish which were rarely reported before 1970 became a major problem worldwide with the intensification of aquaculture (Baya *et al.,* 1990).

Now, *Streptococcus sp.* has recently created a major disease problem in cultured tilapia and considered of high importance in recent years because of the increased reports of infections and the high economic losses caused by gram-positive bacteria in both wild and cultured fish (Eldar *et al.,* 1995).

In Egypt, Nile tilapia (*Oreochromis niloticus*) population facing streptococcosis in several areas, notably in Lake Eltemsah in Ismailia governorate (Badran, 1994) in Kafer-ElShiekh governorate (Khalil, 2000) who recorded massive mortalities from streptococcosis.

Aim of the work: Enlightenment the previous argument the present study was conducted to fulfill the following: Preliminary investigation on *Streptococcus species* pathological effect among infected freshwater fishes and identification of the isolates

MATERIALS AND METHODS

Naturally infected fish:

A total number of 1200 fishes (600 *Oreochromis niloticus* average weight 100-150 gm and 600 *Clarias gariepinus* average weight 200-300) were collected from different commercial fish farms and subjected to clinical, postmortem, bacteriological and histopathological examinations according to Plumb and Browser (1983)

Bacteriological examination

Morphological and biochemical examinations of the isolated bacteria were done according to Elmer et al., (1998).

Histopathological examination was examined according to (Roberts, 2001).

Experimental infection of fish:

Ninety apparently healthy *O. niloticus* were acclimatized under laboratory conditions in glass aquaria supplies with dechlorinated tap-water with continuous aeration. The water temperature was 25 ± 2 C. They were fed twice daily at a rate of 2%of body weight for two weeks. At the end of acclimatization period, fish were used for experimental infections.

The previously acclimated 90 *O. niloticus* were divided into 3 equal groups (30 fish / group). The first and second groups were injected intramuscularly (I/M) with virulent *Streptococcus faecalis* and *Streptococcus faecium* previously isolated from naturally diseased fish at a dose of 0.5 ml containing $5X10^7$ respectively. The third group was injected by 0.5 ml sterile saline solution and act as control. Daily observation for any abnormal clinical signs and mortalities for 15 days. The recent dead and clinically diseased fish were subjected for bacterial re-isolation.

RESULTS and DISCUSSION

The prevalence rate of streptococcus infection among the examined fish was 194 isolates (16.2%). The bacteriological examination revealed the isolation of *Streptococcus faecium* 115 isolates (9.6%), *Streptococcus faecalis* 79 isolates (6.6%).

Clinical and postmortem examinations:

Regarding to the clinical signs and postmortem lesions, it was revealed that fish infected with *Streptococcus species* showed loss of appetite, sluggish movement, swimming close to the surface of the water, lethargic, no escape reflex, darkening of the skin, easily detached and lose of the scales, ulcer formation, uni- or bilateral exophthalmia (pop-eye), haemorrhage of the eye, in some cases destruction of eye, haemorrhages on the skin especially at the base of fins and tail and abdominal distention. Exophthalmia and cataract were common feature of the disease among the infected fish, exhibited numerous haemorrhagic areas on the body surface particularly at the base of fins and operculum. Gills were covered with mucus and pale in colour. Internally, the liver was pale in some cases and dark brown in others in association with over distended gallbladder and the peritoneal cavity filled with ascetic fluid. In some cases, haemorrhage in intestine, enlarged spleen and nearly black, enlarged and congested kidneys were noticed, congested testis was also observed (Figs 1, 2, 3 & 4). The sluggish movement was probably due to haemorrhagic, edematous and ulcerated fins in addition to anorexia which affected the vital activities of the diseased fish. Swimming close to the surface of the water suggests their extraordinary need for oxygen which could be attributed to coating of gills with perfuse mucus and debris beside decrease the number of erythrocytes caused by the haemorrhage.

Darkening of the skin may be attributed to melanin carrying cells (melanophores bound electron granules of melanin pigments can be moved within the cytoplasm of the cell to give the desired effect (Roberts 2001). Such pigmentation is supposed to share in the defense mechanism as it is a response to a disease condition. The distended abdomen with ascetic fluid could be explained on the basis of renal portal hypertension induced by hepatic renal lesions or decreased osmotic pressure of the blood. Numerous haemorrhagic areas on the body surface particularly at the base of the fins and operculum could be attributed to the bacteria which usually entered the body through small abrasions on the skin through which they gained access to the epidermis and dermis where they multiplied rapidly. Thus, the dermal capillaries become congested and finally ruptured filling interstitial areas with blood (Amlacher, 1970). These signs may be attributed to streptococcal toxins which lead to electrolyte and protein loss together with disturbed circulation. These results are nearly agreed with those recorded by Morita (1975), Roberts (2001), El-Bouhy (2002) and Zeid

(2004) who mentioned that some signs that may distinct for streptococcal disease, include erratic swimming, darkening of the fish, haemorrhage at the base of the fines and of the operculum, pop-eyed appearance, and sometimes with cloudy eyes and swollen abdomen. The corneal opacity (cataract) may be attributed to corneal oedema under the effect of bacterial toxins which leads to osmotic dilution of the aqueous humor, with lens epithelium unable to maintain lens deturgescence against an unusually steep osmotic gradient (Ferguson, 1989) or may be attributed to mould growth on the affected eye. Exophthalmia may be induced by increased permeability of the capillary endothelium leading to escape blood proteins with tissue fluids. The osmotic pressure of the latter is raised at retains more fluid. The permeability of the capillaries was increased due to impaired of the build up of the collagen and chondrotin sulphate under the effect of bacterial exotoxins.

Bacteriological examination:

It revealed 194 different pathogenic isolates from gills, kidneys, liver, spleen and ascetic fluid. Two species were identified as Streptococcus bacteria according to morphological and biochemical characters. The result of distribution of streptococcus infection and their incidence in different tissues and organs of naturally infected fishes showed that the highest percentage of bacterial fish pathogens were isolated from kidneys (31.72%), liver (29.85%), ascetic fluid (23.13%) and spleen (15.98%) table (2). The isolates from kidney and liver may be attributed that, this organs more or less considered being tropism for this bacteria or due to the nature of septicemia occurred by the microorganism and proved the pathogenicity of this bacteria to fish. The pathogenicity may be attributed high capacity for tissue invasion and toxic products. Presence of high percentage of the isolated bacteria in fish tissue revealed high concentration of these pathogens in the pond water. This observation was in coincidence with the finding of El-Bouhy (2002) and Zeid (2004) who recorded pure, dense bacterial growth isolated from kidney, liver, spleen and the eye of diseased fish.

Histopathological examination:

The histopathological changes of artificially infected fish were similar to the naturally infected fish. The histopathological examinations of *O. niloticus* of the eye infected by *Streptococcus sp1* showed severe intraocular haemorrhage (Fig. 9). Choroid tissues were inflamed, haemorrhage often occurs in the iris, and the orbital adipose tissue with leukocytic infiltration and melanomacrophages (Fig. 10), with leukocytic infiltration in cornea. Thickening of sclera was observed.

Congestion of the internal organs with pale liver and bloody ascitic fluid. These lesions were in accordance with the findings met by Romalde and Toranzo (1999), Roberts (2001), El-Bouhy (2002), Zeid (2004) who reported that abdominal cavity

contained yellow or reddish yellow fluids, the stomach and intestine may empty, the liver was pale but sometimes mottled red, spleen was enlarged and dark red in appearance were common postmortem lesions observed in streptococcal infection.

The hepatopancreas of *O. niloticus* infected by *Streptococcus faecalis* showed severe congestion of hepatoportal vein beside dilated engorged sinusoids with blood (Fig. 11 & 20). The hepatocytes suffered from vacuolar degeneration (Fig. 12). Minute focal areas of necrosis were been seen beside dissociation and atrophy of pancreatic acini, which appear inactive with loss of zymogenic granules and had leucocytic infiltrations. A slight fibroblastic proliferation infiltrated with leucocytes was seen in the hepatoportal area especially around the bile duct.

The liver of catfish *C. gariepinus* showed dark brown pigment scattered among the hepatocytes. Sometimes the epithelial lining of the bile duct showed vaccular degeneration.

The liver showed vaccular degeneration which induced by the non lethal damage to cells by toxic metabolism of the infected bacteria. The failure of sodium-potassium pump leading to entrance of sodium into the cell followed by water leading to cellular swilling.

Congestion of hepatoportal vein at sinusoid is indicator of the septicaemia as the liver was damaged by the blood borne pathogenic bacteria and its toxins.

The over distended gall bladder could be attributed to enteritis or to the encountered constriction of the common bile duct by the periductal fibrosis due to persistence of the toxic effect. The observed hepatic lesion is chronic as pointed out by the fibrous tissue proliferation.

The spleen showed swelling of the wall of blood sinuses, excessive proliferation of multiple melano-macrophages centers which are large in size and dark in color. Severe congestion of spleen sinusoids beside prominent stroma of both ellipsoids and MMC which suggested the picture of honey combed appearance of spleen. Focal areas of depletion of hemopoietic elements and white pulp were encountered (Fig. 13), (Fig. 21), in the spleen substantiates the cytolytic and fibrolytic capacities of the streptococcus bacteria for destroying the host defense system, increase number of melanomacrophage centers may be due to increase of the body catabolism or may be due to anorexia.

The kidney *O. niloticus* infected by *Streptococcus faecalis* showed that hemopiotic element among the renal tubules showed variable degrees of activation and depletion, minute focal areas of coagulative necrosis of some tubules manifested by pyknotic nuclei and esinophilic hyaline cytoplasm (Fig 14). This may be due to sever toxic effect of the bacterial toxins. Some renal tubules suffered from hydropic

degeneration. Sometimes the epithelial lining of the tubules desquamated in the lumina in the tubules in the form of renal casts. Other cases showed deposition of hyaline droplets in the tubular epithelium (Fig. 19). Numerous melanin carrying cells aggregations were seen under the renal capsules beside congestion of intertubular blood vessels. Congestion and oedema seems to play a role in the enlargement of the kidney and spleen. Focal activation of the hemopoietic tissue and hyaline droplet degeneration beside congestion and haemorrhage among the renal tubules may back to that the kidney is the target organ in many diseases. A reason for this may be the affinity of the organ for circulatory particular antigen. The hemopoietic hyperplasia is a general response through out the kidney to the initiation of infection (Roberts 2001). The kidney droplet degeneration suggests the existence of a glomerular disease which can results in protein leakage into the filtrate and decreased osmotic pressure with its consequences.

The testis *O. niloticus* infected by *Streptococcus faecium* showed severe inflammation manifested by sever congestion of blood vessels, and migrated number of R B Cs into the somniferous tubules and mixed with spermatides (Fig. 15, 16 & 17).

The intestine showed hyaline degeneration of muscular coat (Fig. 18) which resulted from sever and persistent of strong stress (bacterial exotoxins and metabolites).

The behavior of infected African catfish and tilapia was similar, although tilapia reacted more dramatically (El-Bouhy 2002).

However, many other bacterial infection in tilapia cause the same or similar clinical signs and postmortem lesions (Plumb, 1999 and Clark *et al,* 2000), it was therefore considered burden to make isolates from various tissues of moribund fish.

Experimental infection of the fish:

The result of experimentally infected fish was shown in table (1). The clinical signs and postmortem lesions of the experimentally infected fish were appeared as loss of escape reflex, restlessness and swam near the water surface. After three days from injection, no marked gross lesions were noticed except hemorrhage at the base of the fins. After 9 days dead fish has slightly exophthalmia (Fig. 7) ascetic fluids in the abdomen with congestion of the internal organs (Fig. 8).

The survived fish were having opacity of the eyes and ulcers on the skin. Re-isolation of the injected bacteria was succeeded from all dead fish. The control group showed neither clinical signs nor post mortem changes, only one mortality was recorded with no streptococcal bacteria were isolated from control group.

Table 1. Experimental infection of *O. niloticus* with *Streptococcus spp.*

Dose / days	No. of injected fish	Number of dead *O. niloticus*		
		Control	*Streptococcus faecalis*	*Streptococcus faecium*
1	30	-	-	-
2	30	-	-	-
3	30	-	6	-
4	30	-	6	3
5	30	-	9	6
6	30	-	3	12
7	30	-	-	3
8	30	-	3	3
9	30	-	3	-
10	30	-	-	3
11	30	-	-	-
12	30	-	-	-
13	30	-	-	-
14	30	-	-	-
15	30	-	-	-
Mortality rate		0%	100%	100%

Table 2. Distribution of Streptococcus isolates in clinically infected Nile tilapia (*O. niloticus*) and African catfish (*C. gariepinus*).

Fish species	Organ			
	Kidney	Liver	Ascitic fluid	Spleen
	%*	%*	%*	%*
O. niloticus	32.04	30.10	26.21	11.65
C. gariepinus	31.51	29.7	21.21	17.58

%* from the infected fish in the same species.

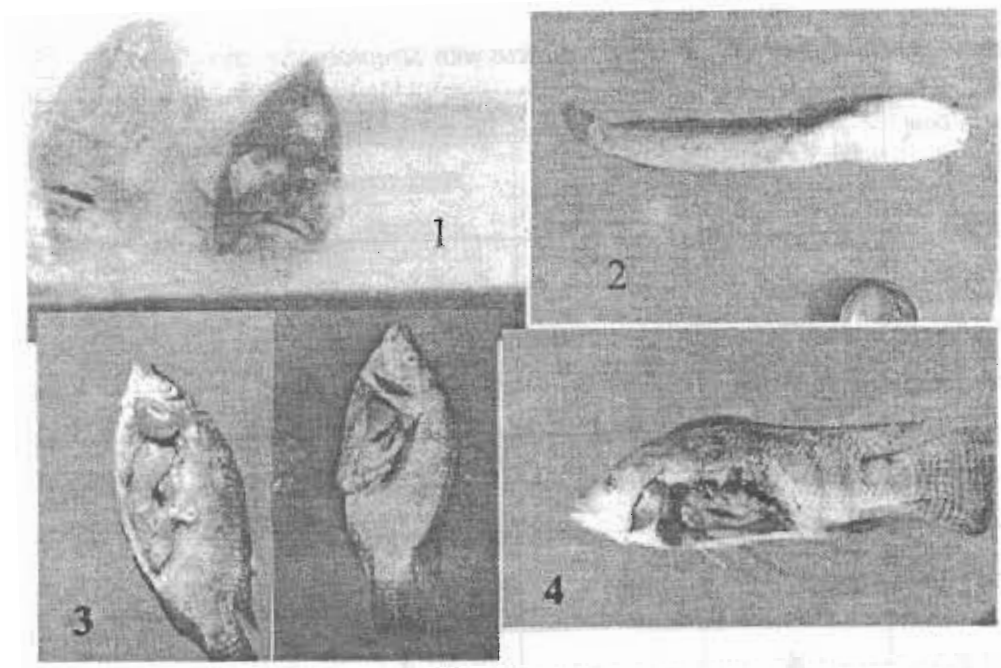

Fig 1. *O. niloticus* naturally infected with *Streptococcus spp.* showing corneal opacity.
Fig 2. *C. gariepinus* naturally infected with *Streptococcus spp.* showing skin ulceration and haemorrhages in the base of the fins.
Fig 3. *O. niloticus* naturally infected with *Streptococcus spp.* showing pale liver and congestion of internal organs.
Fig 4. *O. niloticus* naturally infected with *Streptococcus spp.* showing bloody ascitis and inflammation of intestine.

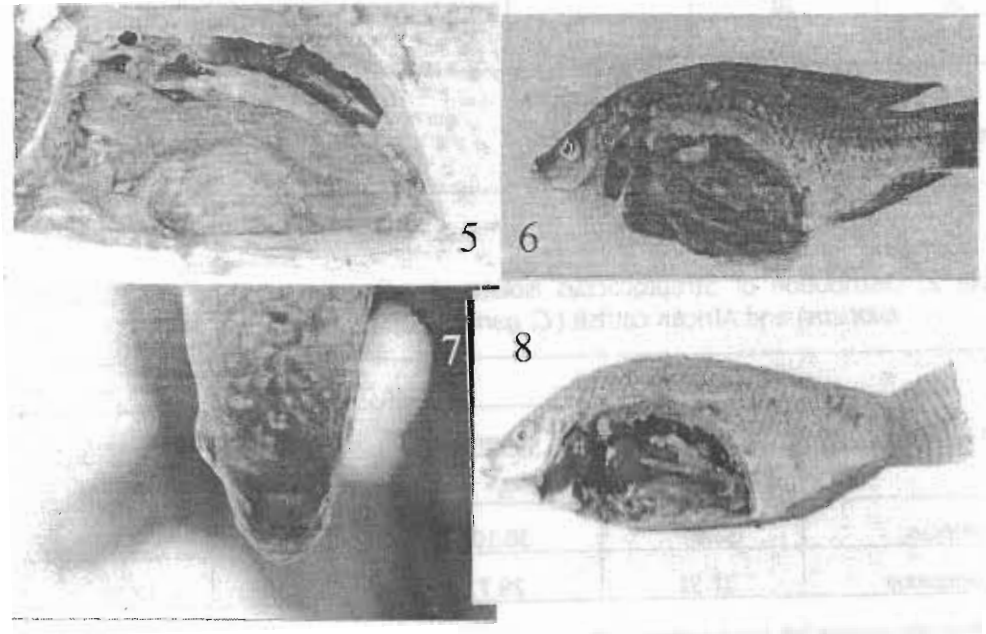

Fig 5. *O. niloticus* naturally infected with *Streptococcus spp.* showing enlarged and congested kidney.
Fig 6 *O. niloticus* naturally infected with *Streptococcus spp.* Showing ascitis.
Fig 7. *O. niloticus* injected with *Streptococcus faecalis* showing slight exophthalmia.
Fig 8. *O. niloticus* injected with *Streptococcus faecalis* showing congestion of the internal organs.

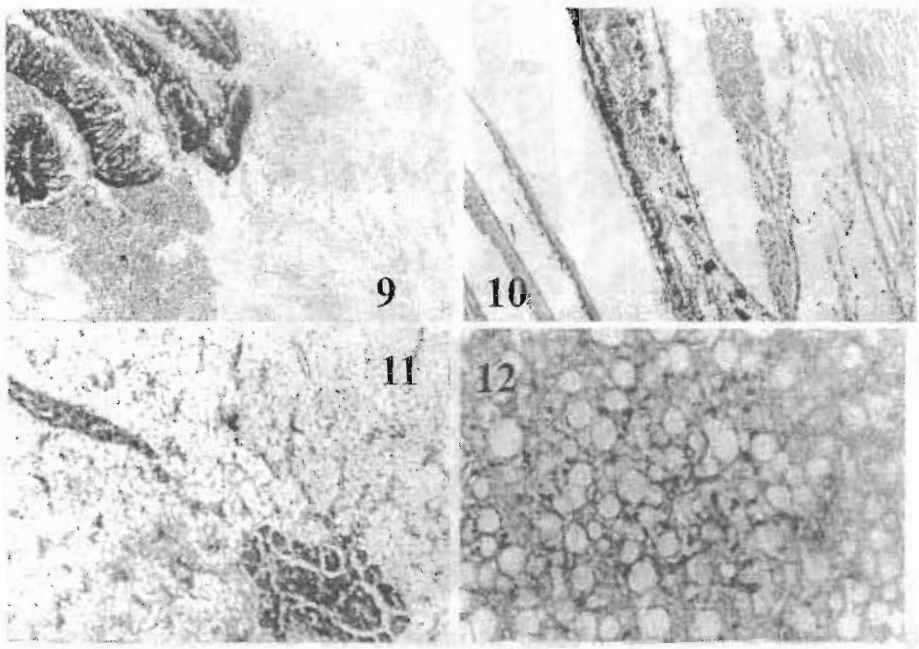

Fig 9. Eye of naturally infected *O. niloticus* with *Streptococcus sp1* showing severe intraocular haemorrhage, H&E., X 300.

Fig 10. Eye of naturally infected *O. niloticus* with *Streptococcus Sp1* showing severe haemorrhage in the orbital adipose tissue with leukocytic infiltration and melanomacrophage cells, H&E., X 300.

Fig 11. Liver of naturally infected *O. niloticus* with *Streptococcus faecalis* showing severe congestion of hepatoportal vein and sinusoids. H&E, X 150.

Fig 12. Hepatopancrease of injected *O. niloticus* with *Streptococcus faecalis* showing severe vacuolar degeneration of hepatocytes., H&E, X 600.

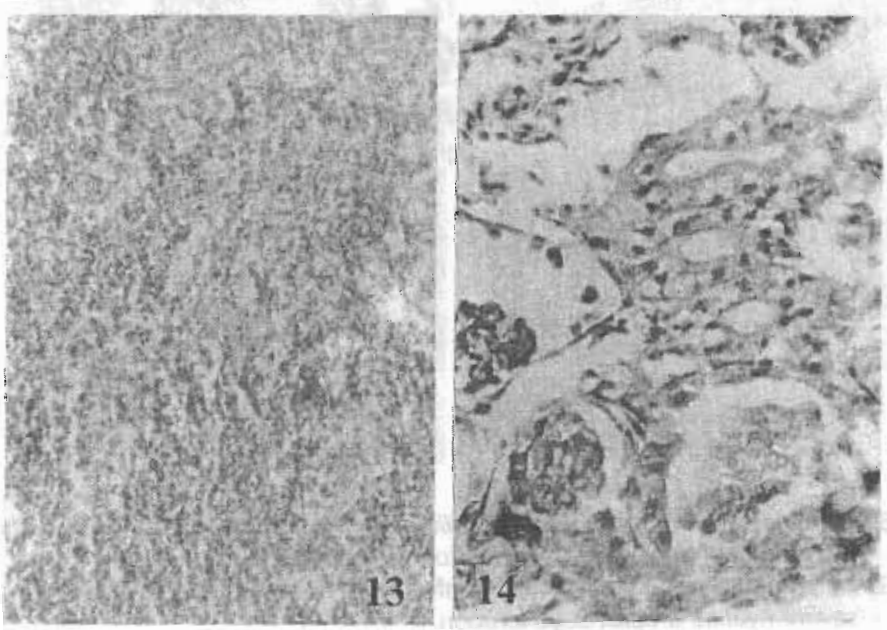

Fig 13. Spleen of injected *O. niloticus* with *Streptococcus faecalis* showing hyaline droplet degeneration of the white pulp. H&E, X 300.

Fig 14. Kidney of injected *O. niloticus* with *Streptococcus faecalis* showing hyaline droplet degeneration. H&E, X 600.

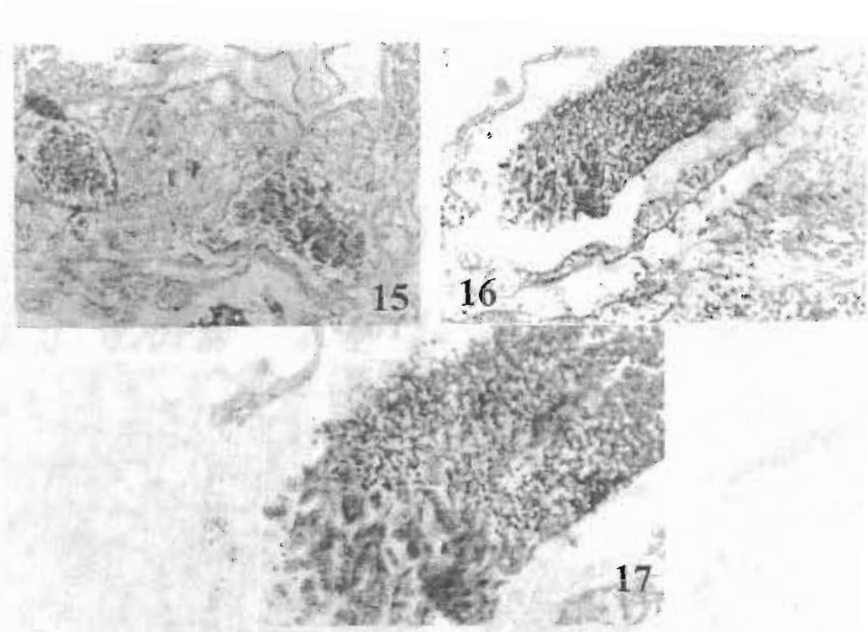

Fig 15. Testis of naturally infected *O. niloticus* with *Streptococcus faecium* showing severe congestion of blood vessels. H&E, X 600.

Fig (16&17). Testis of naturally infected *O. niloticus* with *Streptococcus faecium* showing severe inflammation manifested by mixed R.B.Cs. and spermatides with leukocytic infiltration in seminiferous tubules. H&E, X 300.

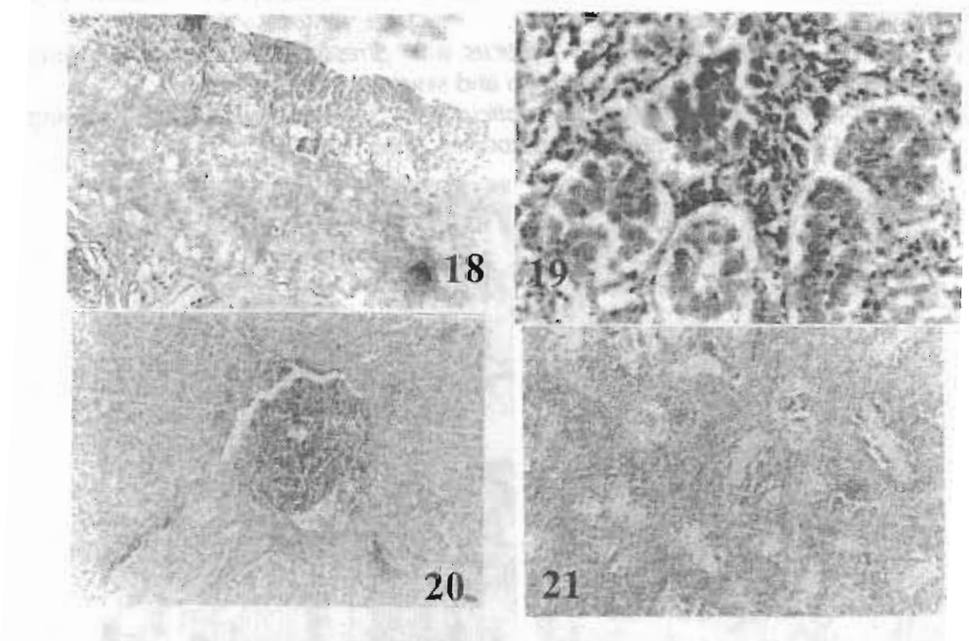

Fig 18. Intestine of naturally infected *O. niloticus* with *Streptococcus faecium* showing hyaline degeneration of muscular coat. H&E, X 600.

Fig 19. kidney of *C. gariepinus* naturally infected with *Streptococcus faecalis* showing severe hyaline droplet degeneration.

Fig 20. Liver of *C. gariepinus* naturally infected with *Streptococcus faecalis* showing congestion of blood vessels and sinasoids.

Fig 21. Spleen of *C. gariepinus* naturally infected with *Streptococcus faecalis* showing tight fibrosed ellipsoids

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التغيرات المرضية المصاحبة للإصابة بالميكروب السبحى الممرض فى أسماك البلطى النيلى والقرموط الأفريقى المستزرعة

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٢. *المعمل المركزى لبحوث الثروة السمكية- العباسة ابو حماد - الشرقية.*

تم إجراء البحث على عدد ١٢٠٠ سمكة (٦٠٠ بلطى نيلى - ٦٠٠ قرموط أفريقى) والتى تم تجميعها من المزارع السمكية وفحصها لتحديد نسبة الإصابة والتغيرات المصاحبة للميكروب السبحى. وكانت نسبة الإصابة ١٦,٢ % فى الأسماك المصابة وتم عزل ميكروب الستروبتوكوكس فيكيم بنسبة ٩,٦% والستروبتوكوكس فيكاليز بنسبة ٦,٦%. وكانت العلامات المرضية فى الأسماك المصابة، فقدان فى الشهية، كسل فى العوم، العوم قريبا من سطح الماء، عدم الإستجابة للمؤثرات الخارجية، تساقط القشور، قرح وجحوظ فى العين وفقدانها، تعفن الذيل والزعانف، إستسقاء، إلتهاب فتحة الإخراج، نشوهات فى العمود الفقارى والخياشيم كانت باهتة فى اللون ومغطاة بكثافة عالية من المخاط. أما الأعضاء الداخلية فكانت محتقنة مع وجود سائل إستسقائى أصفر.

كانت التغيرات الباثولوجية للأسماك المصابة طبيعيا صناعيا متشابهة. وتمثلت التغيرات الباثولوجية للأسماك المصابة فى إحتقان شديد، زيادة عدد خلايا الدم البيضاء، إرتشاح أوديمى، أنزفة وتنكرز مع إحتفان شديد داخل العين.