Clinicopathological Studies on Nile Tilapia (*Oreochromus niloticus*) infected with various species of family Streptococcaceae

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ABSTRACT

The present study was planned to investigate the prevalence of family *Streptococcaceae.* among Tilapia nilotica (*Oreochromus niloticus*) and their associated pathological and clinicopathological changes. One hundred and twenty Nile tilapia were used in the experiment. They were subjected to bacteriological examination where 38 (31.66%) were positive for *Streptococcaceae.* Forty-two isolates were obtained from the examined tilapia. The isolated revealed twenty-four *Enterococcus faecalis* (57.15%), five *Aeroccocus viridians* (11.9%), tow *Streptococcus sanguis* (4.76%),eight *Enterococcus durans* (19.05%), and three un identified streptococcus isolate (7.14%).The experimental infection of 300 healthy tilapia nilotica with different isolate of streptococcus was carried out. The leukogram revealed leukopenia in *Aeroccocus viridians*, *Streptococcus sanguis* (after 11 days only) and *Enterococcus durans* infected groups. On the other hand, leukocytosis was noticed in *Enterococcus faecalis* infected group. The biochemical tests revealed an increase in most biochemical parameters (ALT, AST, urea, creatinine and glucose) in most infected groups.The histopathological examination of the experimentally infected tilapia with streptococcus isolates revealed multiple lesions.

INTRODUCTION

Fish diseases, especially the bacterial , are among the major problems in aquaculture *(1).* Bacterial pathogens are the most serious disease problem in tilapia production causing 80% of fish mortalities *(2).* Historically, *Streptococcus* sp. has become more prominent in wild and cultured fish *(3).* Now, *Streptococcus* sp. has recently created a major disease problem in cultured tilapia and is 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 *(4).*

The disease may be subacute but more often it manifests itself as a chronic condition *(1).* Seven species of *Streptococcus* (*S. agalactia, S. dysqalactiae, S. equi, S.equisimilis, S. faecums, S. pyogens* and *S. zoepidemicus*) have been reported as pathogens to fish , but often infections are caused by unspeciated streptococci that differ from other known members of the genus. Also, *Streptpcoccus iniae* has become a very important pathogen of tilapia *(1).*

Among the measured blood chemistry parameters iron, glucose, sodium, and chloride are good indicators for the deteriorating health in tilapia. A study was conducted on blood chemistry and histopathology of Nile tilapia after infection with *Streptococcus iniae.* Moderate histological changes were observed in the gill, tail kidney, liver, spleen, and head kidney of *Strept.iniae*-infected fish.The severity of lesions in the liver, intestine, and trunk kidney was positively correlated with elevated activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), but negatively correlted with sodium, chloride, cholesterol, and total protein. Standardized principal component analysis, discriminated analysis, and canonical correlation analysis were applied to the blood chemical and histopathological data *(5).*

This study was designed to investigate the prevalence of Streptococcaceae among Tilapia nilotica (*Oreochremus niloticus*) through field study and their associated pathological and clinicopathological changes via experimental studies.

MATERIALS AND METHODS

1- Fish

For the field study, 120 Tilapia nilotica (*Oreochremus niloticus*) were collected from Zagazig markets & WorldFish Center, Abbassa, Abou-Hammad, Sharkia. The fish were males and females, each with an average body weight of 100 ± 25 gm. The fish were immediately transported alive in sterile bags to the Fish Health laboratory, WorldFish Center-the fish were subjected to bacterial isolation.

For the experimental study, 300 Tilapia nilotica (*Oreochremus niloticus*) were collected alive from fish health, WorldFish Center. They were of 57.6 ± 25 gm body weight of both sexes and apparently healthy. They were transported in sterile water tanks to the lab. and kept for two weeks in glass aquaria (100 × 40 × 50 cm) under observation for acclimatization. The water was renewed daily and the temperature was adjusted at 24°C ± 2°C. The fish were fed on a balanced diet during the period of the experiment which extendend for 15 days, and they were subjected to bacteriological examination to prove that they were free from bacterial infections.

2-Bacteriological examination

Sampling and isolation were obtained under complete aseptic conditions from the kidney, liver, spleen, and intestine ***(2 , 6)***. The bacterial identification was done by smearing of bacterial colony with drop of distilled water and then stained by gram stain and examined microscopically ***(7)***. The biochemical examinations of the isolates were performed *(7,8)* .The biochemical characters for all suspicious isolates were determined using Commertial API 20 strep system according to the manufacture procedure.

3-Experimental infection

Three handred apparently healthy Nile tilapia were divided into 5 equal groups. Each group was intraprotinial (I/P) injected with 0.5 ml 24hr. bacterial broth-culture (conc. 1.5 x 10^8 CFU /fish), group was inoculated with *Enterococcus faecalis*, group with *Aerococus viridians*, group with *Streptococcus sanguis* and group with *Enterococcus durans* . group inculated intraprotinial (I/P) with 0.5ml sterile saline solution to act as a control. All experimentally infected fish were daily observed for signs of infection and mortalities for 15 days postinfection. The clinically diseased fish were subjected to bacterial reisolation and histopathological examination.

4-Histopathological examinations

Specimens were collected from the organs of experimentally infected fish, and fixed in 10% neutral buffered formalin. Five micron thick paraffin sections were prepared, and stained with hematoxylin and eosin (H&E) and examined microscopically ***(9)***.

5-Clinicopathological examination

a- Blood sample

Blood samples were collected from the caudal vein of unanesthetized fish of all groups. A small amount of blood was placed in a tube containing pot. salt of EDTA and was used as whole blood for leukogram. The remaining blood was placed in plain clean centrifuge tube, centrifuged at 3000 rpm for 5 minutes and serum was separated to estimate the biochemical parameters.

b- Hematological examination

The total leucocytic count using Natt-Herrick's solution as a diluting fluid ***(10)***, blood films were prepared for differential leucocytic count with Giemsa stain ***(11)***.

c. Serum Biochemical parameters

The following biochemical parameters were estimated; serum level of total protein ***(12)***, albumin and globulin ***(13)***, glucose ***(14)***, urea ***(15)***, creatinine ***(16)***, uric acid ***(17)*** and transferases activities (ALT,AST) ***(18)***.

7-Statistical analysis

The obtained results were analyzed statistically. Mean ± values standard errors were calculated ***(19)***. The significance was made ***(20)***. ANOVA single factor (F test) was used.

RESULTS

Thirty-eight (31.66%) out of 120 field examined tilapia nilotica, were positive for streptococcaceae infection. Forty-two isolates were obtained from the internal organs. [liver 17 (40.48%), spleen 6 (14.28%), intestine 12 (28.58%) and kidneys 7 (16.66%)]. The 42 isolates included *Enterococcus faecalis* (24, 57.15%), *Aerococcus viridians* (5, 11.92%), *Streptococcus sanguinis* (2, 4.76%), *Enterococcus durans* (8, 19.05%) and non identified bacterial isolate (3, 7.14%).

The intraprotinial (IP) experimental infection of the healthy Tilapia nilotica with the different isolates of streptococcacea at a dose of 1.5 x 10^8 CFU/fish revealed total mortality of 2 (3.3%), 12 (20%), 2 (3.3%) and 28 (46.7%) for *Enterococcus faecalis*, *Aerococus viridians*, *Streptococcus sanguinis* and *Enterococcus durans;* respectively after 14 day of infection .

Leukogram results

After 24hr. (Table 1): Compared with the control group, shows a significant increase in the total leukocytic count in the *Enterococcus faecalis* infected tilapias gp.(2) and significant decrease in TLC in *Enterococcus durans* infection gp.(5) There was a significant decrease in neutrophil count in the *Streptococcus sanguinis* and *Enterococcus durans* infected gps. (4 and 5) . A significant increase in lymphocyte was noticed in gp.(4) was seen and a significant decrease in gp.(5). Non significant changes in these parameters in the remaning groups.

After four days (Table 2): Shows a significant decrease in TLC in gp. (5) was seen. There was a significant increase in neutrophil count in gps. (2 and 4). A significant decrease in lymphocyte was encountered in gps. (4 and 5).

After eleven day (Table 3): Presents a significant decrease in TLC in gps. (3,4 and 5) was observed. There was a significant decrease in neutrophil in gps. (4 and 5). A significant increase in lymphocyte in gp.(4) was seen but gp.(3) showed a significant decrease.

Other leukocytic cells (monocytes, eosinophils and basophils) showed non significant changes during the periods of experiment.

Biochemical analysis

After 24hr. (Table 4): There was a significant increase in the glucose in *gp.*(5). The other biochemical parameters (total protein, albumin, globulin, uric acid, urea, creatinine, ALT and AST) were not significantly changed.

After four day (Table 5): A significant increase in ALT in all groups was noticed while, AST showed significant increase in gp. (2 and 3). There was a significant decrease in the albumin in gps.(2,4 and 5). The globulin showed a significant increase in *gp. (4).* A significant increase in glucose in gp.(5) was seen, there was a significant increase in urea in gp. (3). A significant increase in creatinine in gps. (3,4 and 5) was seen.

After eleven day (Table 6): A significant increase in glucose in gps. (4 and 5) was seen. There was a significant increase in urea in gp. (5) and a significant increase in creatinine, ALT and AST in gps. (2,3 and 5) was observed.A significant decrease in total protein in gp.(5), albumin in gps.(2,4 and 5) and A/G ratio in gp.(4) was noticed.

Histopathological results

Degenerative changes were found in the liver and exocrinepancreas of infected tilapia (Fig. 1), Mucinous degeneration and leukocytic infiltrations were seen in the lamina propria and submucosa of the intestine (Fig.2). The Nile tilapia infected with *Enterococcus durans and Enterococcus faecalis* showed focal epithelial desquamation of the epithelial covering of the secondary lamellae (Figs 3). The Nile tilapia infected with *Streptococcus sanguinis* and *Aerococcus viridians* revealed necrosis of melanomacrophage centers and depleted splenic lymphocytes. (Fig. 4).

Table 1. Leucogram (mean values ± S.E) in Tilapia nilotica of gp.(1) and experimentally infected groups after 24 hr. post infection.

Group	TLC (10^3 / µL)	Neutrophil (10^3 / µL)	Lymphocyte (10^3 / µL)	Monocyte (10^3 / µL)	Eosinophil (10^3 / µL)	Basophil (10^3 / µL)
(1)	36.67±0.88 ^{BC}	15.45±2.12 ^{A}	19.00±2.13 ^{B}	1.84±0.25 ^{A}	0.24±0.25 ^{A}	0.12±0.13 ^{A}
(2)	39.60±0.11 ^{A}	19.01±0.43 ^{A}	19.01 ±0.49 ^{B}	0.79±0.39 ^{A}	0.53±0.26 ^{A}	0.26±0.13 ^{A}
(3)	35.63±0.66 ^{C}	17.97±0.47^{A}	16.48 ±0.35 ^{B}	1.01±0.23 ^{A}	0.11± 0.11^{A}	0.06± 0.06^{A}
(4)	38.70± 0.11^{AB}	5.73 ±1.13^{C}	30.65 ± 1.07 ^{A}	1.16±0.67 ^{A}	0.77± 0.44^{A}	0.39± 0.22^{A}
(5)	24.00±1.15 ^{D}	10.14±1.08 ^{B}	11.06±0.54 ^{C}	2.08±0.13 ^{A}	0.48±0.02 ^{A}	0.24±0.11 ^{A}

The same Column not followed by the same letter differ significantly (P<0.05) and the highest values were represented with the letter A.

Table 2. Leucogram (mean values ± S.E) in Tilapia nilotica of gp.(1) and experimentally infected groups after 4 days post infection

Group	TLC (10^3 / µL)	Neutrophil (10^3 / µL)	Lymphocyte (10^3 / µL)	Monocyte (10^3 / µL)	Eosinophil (10^3 / µL)	Basophil (10^3 / µL)
(1)	36.67±0.88 ^{AB}	15.45±2.12^{BC}	19.00 ±2.13 ^{A}	1.84±0.25 ^{A}	0.24±0.25 ^{A}	0.13±0.12 ^{A}
(2)	40.20±0.11 ^{A}	19.26 ±0.47 ^{A}	19.29 ±0.49 ^{A}	1.07±0.27 ^{A}	0.44±0.22 ^{A}	0.13±0.07 ^{A}
(3)	33.93±0.40 ^{B}	15.60 ±0.32^{BC}	16.63 ± 0.35 ^{AB}	1.08±0.19 ^{A}	0.48± 0.01^{A}	0.14± 0.004^{A}
(4)	39.20± 0.11^{A}	21.80 ±0.37 ^{AB}	15.35±0.21 ^{B}	1.02±0.44 ^{A}	0.71± 0.07^{A}	0.32± 0.13^{A}
(5)	26.00±2.31 ^{C}	12.45 ±1.72 ^{C}	11.84±0.93 ^{C}	1.26±0.14 ^{A}	0.41±0.21 ^{A}	0.04±0.03 ^{A}

The same Column not followed by the same letter differ significantly (P<0.05) and the highest values were represented with the letter A.

Table 3. Leucogram (mean values ± S.E) in Tilapia nilotica of gp.(1) and experimentally infected groups after 11 days post infection.

Group	TLC (10^3 / µL)	Neutrophil (10^3 / µL)	Lymphocyte (10^3 / µL)	Monocyte (10^3 / µL)	Eosinophil (10^3 / µL)	Basophil (10^3 / µL)
(1)	36.67±0.88 ^{A}	15.45±2.12 ^{A}	19.00 ±2.13 ^{B}	1.84±0.25 ^{A}	0.24±0.25 ^{A}	0.13±0.12 ^{A}
(2)	39.00±0.57 ^{A}	18.15 ± 2.12^{A}	18.13 ±1.69 ^{B}	2.13±0.53 ^{A}	0.39±0.23 ^{A}	0.19±0.11^{A}
(3)	31.00±0.57 ^{B}	14.69 ± 0.26 ^{A}	13.73 ± 0.31 ^{C}	1.01±0.27^{A}	1.04± 0.22^{A}	0.52± 0.11^{A}
(4)	32.00± 1.15 ^{B}	3.96 ± 1.28 ^{B}	26.12 ± 1.41 ^{A}	1.00±0.59 ^{A}	0.61± 0.35^{A}	0.31± 0.17^{A}
(5)	29.67 ±0.44 ^{B}	6.23 ± 0.22 ^{B}	21.16 ±0.40 ^{B}	1.82±0.25 ^{A}	0.29±0.17 ^{A}	0.15±0.08 ^{A}

The same Column not followed by the same letter differ significantly (P<0.05) and the highest values were represented with the letter A.

Table 4. Some biochemical parameters (mean values ± S.E) in Tilapia nilotica of control and experimentally infected groups after 24 hr. post infection.

Group	ALT U/I	AST U/I	TP. g/dl	Alb. g/dl	Glob. g/dl	A/G ratio	Glu. mg/dl	UA. mg/dl	Ur. mg/dl	Cr. mg/dl
1)	36.67 ±8.82 ^{A}	47.00 ±0.58 ^{A}	2.28 ±0.01 ^{A}	0.84 ±0.09 ^{A}	1.44 ±0.10 ^{A}	0.59 ±0.11 ^{A}	68.33 ±4.81 ^{B}	7.30 ±0.87 ^{A}	25.78 ±2.41 ^{A}	0.22 ±0.02 ^{A}
(2)	34.00 ±2.87 ^{A}	50.33 ±4.33 ^{A}	2.17 ±0.06 ^{A}	0.87 ±0.01 ^{A}	1.29 ±0.07 ^{A}	0.68 ±0.05 ^{A}	68.33 ±0.96 ^{B}	7.73 ±0.12 ^{A}	25.50 ±0.64 ^{A}	0.24 ±0.01 ^{A}
(3)	33.00 ±2.87 ^{A}	49.00 ±0.57 ^{A}	2.19 ±0.06 ^{A}	0.86 ±0.01 ^{A}	1.34 ±0.06 ^{A}	0.65 ±0.03 ^{A}	71.67 ±2.89 ^{B}	8.50 ±0.21 ^{A}	30.00 ±2.89 ^{A}	0.26 ±0.03 ^{A}
(4)	35.00 ±0.58 ^{A}	39.67 ±4.91 ^{A}	2.21 ±0.05 ^{A}	0.87 ±0.04 ^{A}	1.33 ±0.05 ^{A}	0.66 ±0.04 ^{A}	66.67 ±1.92 ^{B}	7.03 ±0.29 ^{A}	29.55 ±3.13 ^{A}	0.22 ±0.01 ^{A}
(5)	40.00 ±0.58 ^{A}	48.67 ±4.33 ^{A}	2.28 ±0.01 ^{A}	0.88 ±0.03 ^{A}	1.40 ±0.04 ^{A}	0.63 ±0.04 ^{A}	95.00 ±0.96 ^{A}	7.40 ±0.15 ^{A}	25.22 ±1.83 ^{A}	0.27 ±0.01 ^{A}

The same Column not followed by the same letter differ significantly (P<0.05) and the highest values were represented with the letter A.

Table 5. Some biochemical parameters (mean values ± S.E) in Tilapia nilotica of control and experimentally infected groups after 4 days post infection.

Group	ALT U/I	AST U/I	TP. g/dl	Alb. g/dl	Glob. g/dl	A/G ratio	Glu. mg/dl	UA. mg/dl	Ur. mg/dl	Cr. mg/dl
(1)	36.67 ±8.82 ^{B}	47.00 ±0.58 ^{CD}	2.28 ±0.01 ^{A}	0.84 ±0.09 ^{A}	1.44 ±0.10 ^{B}	0.59 ±0.11 ^{A}	68.33 ±4.81 ^{B}	7.30 ±0.87 ^{A}	25.78 ±2.41 ^{B}	0.22 ±0.02 ^{C}
(2)	81.67 ±7.26 ^{A}	60.33 ±4.33 ^{A}	2.17 ±0.05 ^{A}	0.50 ±0.01 ^{CD}	1.67 ±0.06 ^{AB}	0.29 ±0.01 ^{B}	68.33 ±4.81 ^{B}	7.40 ±0.11 ^{A}	26.72 ±1.69 ^{B}	0.25 ±0.02 ^{BC}
(3)	70.00 ±2.89 ^{A}	59.00 ±0.58 ^{AB}	2.07 ±0.13 ^{A}	0.70 ±0.02 ^{AB}	1.36 ±0.14 ^{B}	0.53 ±0.07 ^{A}	75.55 ±2.22 ^{B}	8.57 ±0.20 ^{A}	41.67 ±0.96 ^{A}	0.36 ±0.01 ^{A}
(4)	70.00 ±2.89 ^{A}	39.00 ±4.62 ^{D}	2.19 ±0.13 ^{A}	0.35 ±0.01 ^{D}	1.84 ±0.12 ^{A}	0.19 ±0.01 ^{B}	66.66 ±1.92 ^{B}	8.00 ±1.27 ^{A}	28.99 ±0.96 ^{B}	0.30 ±0.01 ^{B}
(5)	90.00 ±11.54 ^{A}	50.00 ±1.73 ^{BC}	2.10 ±0.06 ^{A}	0.60 ±0.07 ^{BC}	1.50 ±0.12 ^{AB}	0.41 ±0.08 ^{AB}	100.00 ±1.92 ^{A}	8.02 ±0.33 ^{A}	28.00 ±2.89 ^{B}	0.29 ±0.01 ^{B}

The same Column not followed by the same letter differ significantly (P<0.05) and the highest values were represented with the letter A.

Table 6. Some biochemical parameters (mean values ± S.E) in Tilapia nilotica of control and experimentally infected groups after 11 days post infection.

Groups	ALT U/I	AST U/I	TP. g/dl	Alb. g/dl	Glob. g/dl	A/G ratio	Glu. mg/dl	UA. mg/dl	Ur. mg/dl	Cr. mg/dl
(1)	36.67 ±8.82 ^{C}	47.00 ±0.58 ^{C}	2.28 ±0.01 ^{A}	0.84 ±0.09 ^{A}	1.44 ±0.10 ^{A}	0.59 ±0.11 ^{AB}	68.33 ±4.81 ^{B}	7.30 ±0.87 ^{A}	25.78 ±2.41 ^{B}	0.22 ±0.02 ^{C}
(2)	100.00 ±5.77 ^{AB}	73.00 ±3.46 ^{A}	1.91 ±0.15 ^{AB}	0.43 ±0.02 ^{B}	1.48 ±0.16 ^{A}	0.30 ±0.05 ^{BC}	75.00 ±0.96 ^{B}	7.20 ±0.58 ^{A}	36.67 ±2.89 ^{AB}	0.38 ±0.01 ^{A}
(3)	118.33 ±19.22 ^{A}	78.33 ±0.67 ^{A}	2.03 ±0.05 ^{AB}	0.77 ±0.09 ^{A}	1.26 ±0.13 ^{A}	0.64 ±0.15 ^{A}	76.66 ±1.92 ^{B}	9.10 ±0.17 ^{A}	33.33 ±7.69 ^{AB}	0.33 ±0.01 ^{B}
(4)	70.00 ±2.89 ^{BC}	40.33 ±3.33 ^{C}	1.97 ±0.11 ^{AB}	0.26 ±0.01 ^{B}	1.71 ±0.13 ^{A}	0.16 ±0.02 ^{C}	95.00 ±6.73 ^{A}	8.60 ±1.15 ^{A}	31.67 ±0.96 ^{AB}	0.26 ±0.01 ^{C}
(5)	105.00 ±18.93 ^{AB}	57.00 ±1.15 ^{B}	1.70 ±0.16 ^{B}	0.35 ±0.08 ^{B}	1.35 ±0.21 ^{A}	0.28 ±0.08 ^{BC}	98.33 ±2.89 ^{A}	8.40 ±0.20 ^{A}	45.00 ±6.94 ^{A}	0.30 ±0.01 ^{B}

The same Column not followed by the same letter differ significantly (P<0.05) and the highest values were represented with the letter A.

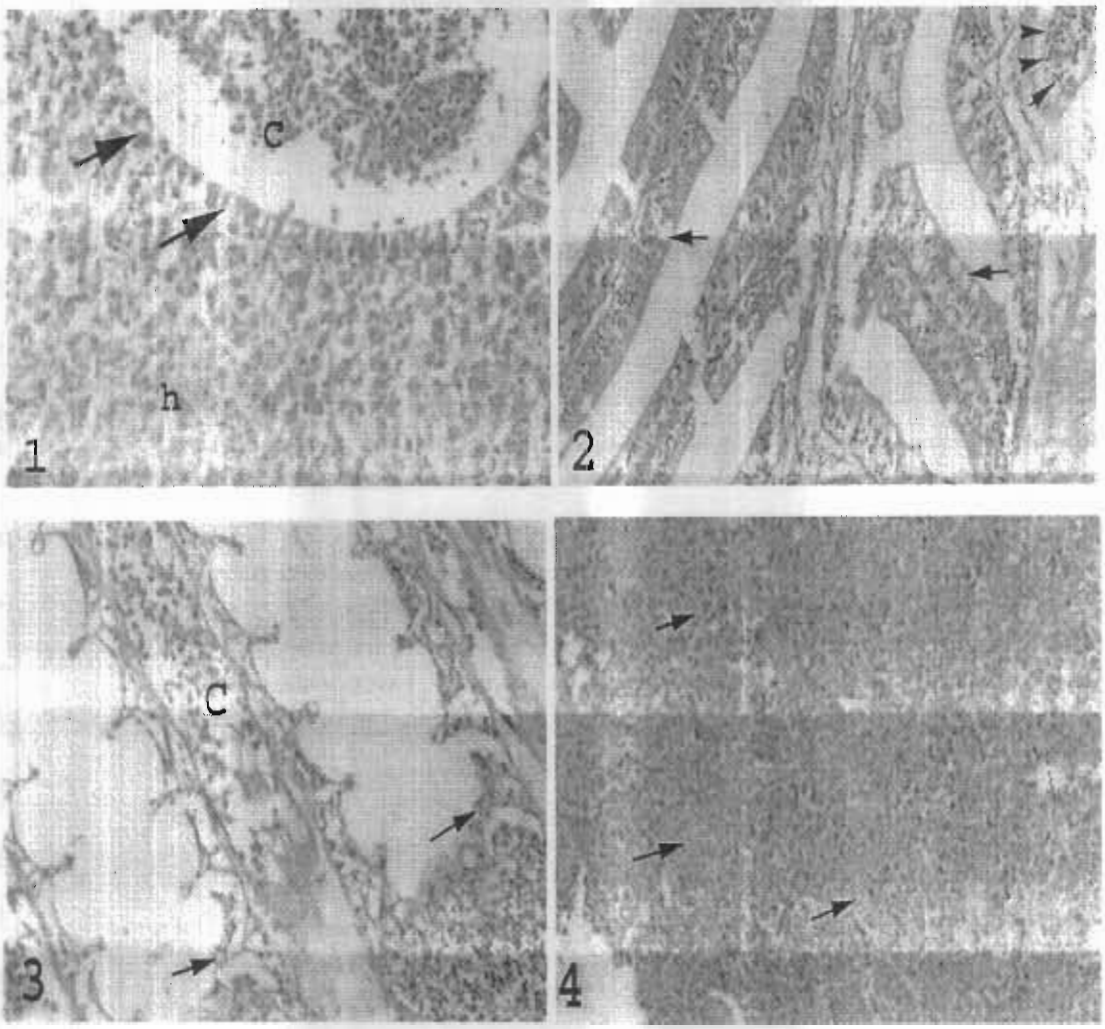

Fig. 1.Phatomicrogroup of hepatopancreas, of Tilapia nilotica infected by *Streptococcus sanguis* showing mild congestion (c), focal degeneration and necrosis in the hepatocytes (h) and acinar pancreatic cells (arrows). H & E stain, x 250.

Fig. 2.Phatomicrogroup of intestine, of Tilapia nilotica infected by *Enterococcus durans* showing mucinous degeneration (arrows) with focal desquamation in the epithelial lining and monomuclear cells infiltration in the lamina propria and submucosa (arrow heads). H & E stain, x 250.

Fig. 3.Phatomicrogroup of gills, of Tilapia nilotica infected by *Enterococcus durans* 7 days postinfection, showing congestion (c) and focal epithelial desquamation in the epithelial lining of the secondary gill lamellae (arrows). H & E stain, x 250.

Fig. 4.Phatomicrogroup of spleen, of Tilapia nilotica infected by *Streptococcus sanguis* showing parenchymal edema with atrophy and necrosis of melanomacrophages center (arrows). H & E stain, x 100.

DISCUSSION

In this study, the prevalence of Family streptococcaciae infection in the field examined Tilapia nilotica was 31.66%. The prevalence of streptococcus infection in Tilapia nilotica was 24.8% in Zagazig City, Egypt *(21)* .

The bacteriological examination of the internal organs (liver, spleen, intestine, and kidney) of collected tilapia revealed 42 isolate of streptococcaciae [*Enterococcus faecalis* (24 , 57.15%) , *Aerococcus viridians* (5 , 11.9%) , *Streptococcus sanguinis* (2 , 4.76%) , *Enterococcus durans* (8 , 19.05%) and unidentified bacterial isolate (3 , 7.14%)]. *(22)* Gram positive-catalase negative coccal isolates (24.23%) as *Aerococcus viridans*, *Enterococcus durans/hirae*, *Enterococcus faecium*, *Lactococcus garvieae*, *Lactococcus lactis lactis*, *Leuconostoc Spp.* and *Streptococcus anginosus* have been isolated from seven rainbow trout farms *(22)*. In this study, we identified the bacterial isolates using the morphology *(7)*, biochemical tests and API system.

The experimental infection of healthy Tilapia nilotica with the different bacterial isolates *Enterococcus faecalis*, *Aerococus viridians*, *Streptococcus sanguis* and *Enterococcus durans* induced mortalities [2 (3.3%) , 12 (20%), 2 (3.3%) and 28 (46.7%)] respectively. Many *Streptococcus* species were isolated and were pathogenic to fish and were found naturally in the environment and may become endemic in aquaculture *(23)*.. The cultured Nile tilapia (*Oreochromus niloticus)* was challanged with *Strept. Iniae* and the fish mortality increased following immersion infection and the organism was isolated from ≥92% dead or moribund fish *(24)*.

Aerococus viridans and *Enterococcus durans* infected groups suffered leucopenia, possibly, due to the stress of infection manifested by high mortality rate, associated with necrotic splenic lymphocytes and melanomacrophage center ,besides renal hematopoietic tissue. *Enterococcus faecalis* group and *Streptococcus sanguinis* infected groups showed leukocytosis due to stimulation of bone marrow. Histopathologically mononuclear cell infiltration in the lamina propria and submucosa of intestine was detected in the *Enterococcus faecalis* infected group presented mild congestion and mononuclear cell infiltration in the primary lamellae and gill arches. Cellular infiltration and numerous cocci in most organs, besides the eyes, meninges, (meningeal granuloma), and kidneys were recorded *(25)*. The bacteria in the hepatic subcapsular capillaries and some liver tissue with granulomatous reactions were seen. Numerous cocci were found in the splenic sinuses, epicardium and myocardium. Leukocytic infiltration in the intestine, spleen, posterior kidney and the brain of ornamental cyprinid was recoded *(26)*.

The biochemical tests revealed increased ALT and AST with decreased in TP and Alb. in most infected groups. These biochemical changes agree with the degeneration and necrosis in hepatic tissues of the infected fish. Similar histopathological changes in the liver, intestine, and trunk kidney, associated with increased activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and lowred total protein were recorded *(5)*. Experimentally infected fish with Streptococcous sp., showed hepatic congestion 3days PI and hepatocellular degeneration with hyperplastic bile ducts , with periductal fibrosis infiltrated with mononuclears 2weeks PI *(21)*.

Our results revealed an increased urea in all the infected groups. The gill lamellae of the infected tilapia where congestion, hyperplasia and degenerated epithelium. Isolated *Streptococcus iniae* from red-tail black shark (RTB shark), revealed necrosis and tissue degeneration *(26)* .

The increased glucose level, in this study, could be due to the stress induced by the systemic infection and septicemia which accelerate gluconeogenesis, resulting in elevated mortality rate. *Strept. faecalis* and *Strept. iniae* are associated with mortalities among the freshwater *Oreochromis sp.* and *Lates calcarifera (27).*

The present study revealed elevated creatinine in all the infected groups, which could be due to degenerated renal tubules. Similar changes were reported *(28)* glumerulonephritis and thickened basement membrane of Bowman's capsule were recorded in streptococcus-infected fish. Isolated *Streptococcus iniae* from red-tail black shark (RTB shark) revealed necrosis and degeneration of the renal tubules *(26)*. The kidneys showed mild tubular cloudy swelling and vacuolar degeneration, besides pyknotic nuclei. Furthermore, the kidneys showed degeneration and necrosis of most renal tubules with piknotic nuclei and karyolysis, besides markedly thickened walls and thrombosis of the intertubular blood vessels, 2 weeks PI.

It could be concluded the Family Streptococcaceae still reprsesnt a problem for fish-production.

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الملخص العربى

دراسات باثولوجية اكلينيكية على اسماك البلطى النيلى المصابة بانواع مختلفة من عائلة الميكروبات السبحية

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تمت الدراسة بعد تجميع ١٢٠ سمكة من الاسواق بمدينة الزقازيق والمركز الدولى للابحاث بالعباسة وبعد إجراء الفحوصات البكتريولوجية على الأعضاء الداخلية للسمك (الكبد، الطحال، الأمعاء، الكلية) أظهرت النتائج ٣٨ حالة إيجابية وذلك بنسبة ٣١,٦٦% بعد إجراء إختبارات العزل والتصنيف .تم تصنيفهم الى أربعة أنواع *Enterococcus faecalis* (٢٤-٥٧,١٥%)، *Aeroccocus viridians* (٥-١١,٩%)، *Streptococcus Sanguinis* (٢- ٤,٧٦%)، *Enterococcus durans* (٨- ١٩,٠٥%) ، وبعض الأنواع غير المعرفة (٣- ٧,١٤%).

وجد أن معدل النفوق أثناء إجراء التجربة على النحو التالى *Enterococcus faecalis* ٢ (٣,٣%) ، *Aerococcus viridians* ١٢ (٢٠%) ، *Streptococcus Sanguinis* ٢ (٣,٣%) ، *Enterococcus durans* ٢٨ (٤٧,٦%) . وقد اجريت العدوى التجريبية على ٣٠٠ سمكة فى حالة جيدة وتم تسجيل الوفيات وقد لوحظ نقص في مكونات الدم من كرات دم بيضاء في الأسماك المصابة فى بعض المجموعات بالمقارنة مع المجموعة السليمة وتدهور في وظائف الكبد حيث أرتفع في مستوى الانزيمات ونقص في البروتين وكذلك وظائف الكلى ارتفع الكرياتنين وارتفعت اليوريا وكذلك مستوى السكر في الدم.وقد أحدثت العدوى التجريبية تغييرات مرضية على شكل وتخثر بخلايا الكبد والبنكرياس والطحال مع تغييرات هدامه وانحلال بخلايا الامعاء بالاضافة لتهتك موضعى بالخياشيم وضمور بمراكز الميلانومكروفاج. وقد تباينت هذه الصور باختلاف نوع الميكروب وفترة العدوى.