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CASE STUDY ON MASS MORTALITIES IN CAGED CULTURED MONOSEX NILE TILAPIA (*OREOCHROMIS NILOTICUS*) IN EL-FAYUOM GOVERNORATE, EGYPT.

Walaa T. El-Ekiaby[1]; Abou El-Atta, M. E.[1]; Moustafa, M. S. [1]and Lamees S. Abd El-Fattah[2]

[1] *Fish Health and Management Department, Central Laboratory For Aquaculture Research, Agricultural Research Center, Egypt.*

[2] *National Institute of Ocheanography and Fisheries.*

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Abstract

A total number of 100 naturally infected monosex Nile tilapia *(Oreochromis niloticus)* were collected alive with an average body weight 200 ±5g per fish from cages suffered from heavy mortalities in El Rayan valley in El Fayuom Governorate in the period of April to October 2012. The physicochemical analyses of water were recorded. Clinical signs and postmortum findings of naturally infected fish were done.

The bacteriological examination revealed the presence of 160 isolates of *Enterococcus fecalis* with prevalence 45.6 %. Isolation and identification was done by traditional methods then confirmed using regular PCR technique which revealed that the isolated bacteria strain was *Enterococcus fecalis*. Isolates were isolated from fins, skin ulcers, liver, spleen, kidney and brain with percentage of 11.25, 25, 21.25, 15, 18.12 and 9.94% respectively. *Enterococcus fecalis* gave 733 bp product size specific for 16S rRNA. The antibiogram sensitivity test of isolated *Enterococcus fecalis* showed highly sensitive to Amoxicillin (Ax25) and Penicillin (P10). The experimental infection of *Oreochromis niloticus* with *Enterococcus fecalis* was associated with mortality rate 80 % in case of intraperitoneal injection (I/P) while intramuscularly injection (I/M) cause 60% mortality. Histopathological changes were studied.

INTRODUCTION

Cage culture practices have numerous advantages over other culture systems. By integrating the cage culture system into the aquatic ecosystem, the carrying capacity per unit area is optimized because the free flow of current brings in freshwater and removes metabolic wastes, excess feed and fecal matter Beverdge (1983).

Nowadays, with an increase in water pollution and intensive aquaculture expansion, there are increasingly numerous of fish diseases appear in that freshwater fishes and cause severe economic losses every year from countries around the world. Fish diseases outbreak are problems in cage cultured fish, increased production, pressure on faster growth, high density and structure efficiency can create conditions conductive to outbreak of infectious diseases. Infectious diseases in fish culture are not only accentuated by waste pollution, but exacerbated by crowding, improper harvest or handling as trauma and transportation, high fish densities accompanied by high feeding rates and poor water quality. So, tilapia under one or more of these stressful factors can become more severely affected by bacterial causes which the most serious disease problem in tilapia production causing 80% of fish mortalities. Plumb (1999) and Shoemaker *et al*. (2000).

Streptococcosis is a major problem affecting wild and cultured freshwater and marine fish throughout the world caused by different Streptococcus species and other closely related genera Enterococcus. Stress is usually one of the predisposing factors resulting in streptococcosis outbreaks such as poor environmental condition; rising in the environmental temperature, harvesting, bad handling, transportation, and poor water quality Francis-Floyd and Yanong (2013).

The interest in studying streptococcosis in fish has been increased due to the dramatic economic losses results from its outbreaks which is estimated hundred million dollars annually or exceeding Haghighi *et al*.

(2010). Streptococcosis attacking both freshwater and marine fishes Kusudaand and Komatsu (1978); Kitao *et al*. (1993) and Austin and Austin (1999) causing high mortality rate which may reach more than 50% within 3 to 7 days in acute cases, or few mortalities over a long period of time “several weeks” in the chronic cases Francis-Floyd and Yanong (2013). Mortalities may reach 100 % within 2-4 days, if the bacterial concentration in aquaculture is high Ferguson *et al*. (1994).

Streptococcal disease in fish was first reported in 1957, affecting cultured rainbow trout in Japan by Hoshina *et al*. (1958). It has a wide host range and can affect different fish species including rainbow trout, seabream, tilapia, yellowtail, catfish species, killifish, menhaden species, mullet, and silver pompfret, sturgeon, sea bass, eel, sea trout, striped bass, mullet, salmon, bottle nose dolphin and tilapia Inglis *et al*. (1993), Evans *et al*. (2005) and Abdullah (2013). In Egypt streptococcosis was recorded among Nile tilapia by Badran (1994); Ebtesam (2002); Dena (2004); Torky *et al*. (2006) and Khafagy *et al*. (2009).

Conventional microbiological methods needed to identify bacteria from fish are often limited by the length of time required to complete the assays. In recent years, PCR-based molecular techniques overcome problems associated with culture-based techniques and preferred in fish diagnostic laboratories because of their reliability and ability to identify the genetic structure of bacteria directly in clinical samples without the need for previous culturing Jafar *et al*. (2009).

So, the aim of this study to describe the isolation, biochemical and biophysical characterization, examination of histopathological alternations and the antimicrobial sensitivity patterns of Streptococcus from Nile tilapia (*Oreochromis niloticus*). In addition to using of polymerase chain reaction (PCR), as a rapid and sensitive method for identification of streptococcus with potential diagnostic value.

MATERIAL AND METHODS

Water analysis:

Samples of water were collected from examined cages to determine the water parameters (e.g temperature, dissolved oxygen, pH, nitrite, ammonia, hardness, alkalinity and salinity) according to APHA (2005).

Fish samples:

A total number of 100 diseased monosex Nile tilapia (*Oreochromis niloticus*) with an average body weight 200 ±5g were collected from 8 floating cages, each cage was 8x8x4 m in El Rayan valley that located in El Fayuom Governorate. The mortality rate ranged from 70 to 110 fish /day and the mortality rate persist for 12 days. The stocking densities for each cage were recorded as shown in Table (1). The fish were transferred to the Central Laboratory for Aquaculture Research, El-Abbassa, Sharkia, Egypt.

Table (1): The stocking densities of *Oreochromis niloticus* for each cage.

Cage No.	1	2	3	4	5	6	7	8
Stocking densities (fish)	30000	30000	40000	40000	30000	30000	50000	6000

Clinical and postmortem examination of naturally infected fish:

All naturally infected fish were subjected to full clinical and postmortem examinations as described by Austin and Austin (2007).

Bacteriological examination:

Samples were taken from diseased fish (fins, skin ulcer, liver, spleen, kidney and brain) under complete aseptic conditions for bacteriological examination according to Noga (1996). A sterile loopfull were taken from sterilized organs then inoculated onto Tryptic Soy Broth

(TSB) and incubated at 29-30 ºC for 24 hours, then streaked onto streptococcus selective agar media and incubated at 29-30 ºC for 24 hours. The suspected pure colonies were picked up and streaked onto the same specific media for further purification. Identification of the isolated pure colonies was done by cultural, morphological and biochemical characters according to Ravelo *et al*., (2001).

Molecular identification:

1. Bacterial DNA Extraction:

DNA Extraction was done by boiling method Bansal (1996). The bacterial pellets were washed once with 300 µl phosphate buffered saline (PBS), pH 7.4, centrifuged at 12.000 RPM, and resuspended again in PBS, incubated in hot plate at 105°C for 10 min. Then rapidly cooled at freeze for another 10 min. The clear supernatants obtained after a 5 min centrifugation at 12000g is the DNA used for PCR reaction.

2. Molecular identification of the bacterial using Polymerase Chain Reaction Technique (PCR):

PCR was done according to Deasy *et al*. (2000) which were unique to *Enterococcus* species by multiple sequence alignments of published 16S rRNA sequences using two types of primers (Enter-F and Enter-R) Table (2). Amplification was performed with the following thermal cycler profile: an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 seconds, primer annealing at 45°C for 30 seconds and extension at 72°C for 30 seconds and Final extension at 72 ºC for 5 min. The PCR products were resolved by 1.5% agarose gel electrophoresis and photographed under UV light using standard procedures.

Table (2): Oligonucleotide used as primers in PCR reaction.

Name of oligonucleotide	Sequence	Primer length	Primer location	Product length (bp)
Enter-F	5'-TCA ACC GGG GAG GGT-3'	15	623-646	733
Enter-R	5'-ATT ACT AGC GAT TCC GG-3'	17	1353-1369	

Antibiogram sensitivity test:

Identified strain of streptococcus was tested for their susceptibility aganist different antibiotics disks using disc diffusion method according to Quinn *et al*. (2002).

Pathogenicity test:

Thirty apparently healthy *Oreochromis niloticus* with average body weight 75 ±5g collected from Abbassa fish farm of Central Laboratory for Aquaculture Research acclimatized and divided into 3 groups each group contain 10 fish (10 fish/group). The fishes kept in glass aquaria (80×60×50 cm of each one) at 27 °C for two weeks aerated by electric aerator and fed with 25% protein in a rate of 5% body weight. 1st group injected intra peritoneum (I/P) with isolated strain in a dose 0.5 ml 5×10^{6} cfu/ml. 2nd group injected intramuscularly (I/M) with same dose and the 3rd group was served as a control group and injected with 0.5 ml of sterile saline. Injected fish were observed daily for 10 days to record any clinical signs abnormalities and mortalities according to Qunine *et al*. (1999).

Histopathological examination:

Tissue specimens from gills, liver, spleen, kidney, fin and skin of naturally infected fish were taken and fixed in 10% neutral buffer formalin and processed using a standard histological technique. After 24 h fixation, the tissue was trimmed before dehydrated in an ethanol series,

followed by embedding in paraffin, and finally serial sectioning at 4 µm. The sections were stained routinely with haematoxylin and eosin (H&E) according to Roberts (2012).

RESULTS

Results of the water analysis:

The results of physico-chemical analysis of water holding *Oreochromis niloticus* were recorded in Table (3).

Table (3): Water quality parameters measurements.

Cage No	Temp. °C	D.0 mg/L	pH	NH3 mg/L	N02 mg/L	Hardness mg/L	Alkalinity mg/L	Salinity mg/L
1	21.9	4.5	8.6	0.7	0.02	650	160	3
2	22.8	4.4	9.5	0.6	0.03	340	180	2
3	21.0	3.9	9.2	0.6	0.03	410	200	2.4
4	24.3	3.9	8.6	0.6	0.03	290	175	2
5	24.6	4.2	9.0	0.4	0.02	740	175	4.2
6	24.7	4.1	8.0	0.4	0.03	510	200	3.2
7	24.8	3.9	8.6	0.6	0.1	466	180	2.75
8	24.7	4.2	9.0	0.5	0.1	390	200	2.1

(Te) Temperature, (D.0) Dissolved oxygen, (NH3) Ammonia, (NO2) Nitrite.

Results of clinical signs:

The clinical signs revealed changes in the behavior concluded in loss of appetite, sluggish movement, swimming near the surface of the water, screwed, lateral side or upward swimming, small hemorrhages, erosion and ulcers 2-3mm distributed on different parts of the skin, base of the tail, fins, mouth, and anal opening Plate (1, B and C). Ophthalmic lesions as unilateral or bilateral opacity, hemorrhage of the eye and some cases showed exophthalmia (pop eye) Plate (1, D).

Results of postmortem lesions:

Naturally infected fishes showed sever congestion in mouth lips; the anal opening protruded and showed congestion and ulceration, pin point hemorrhagic ulcers in the ventral aspect of the gill cover. The gills were pale and showed small white necrotic foci. The brain showed congestion and the superficial veins were prominent and engorged with blood. The liver was enlarged, friable, pale or gray in color, with white pin point necrotic foci on the peripheral margin and the gall bladder enlarged and engorged with bile. The spleen was enlarged, hemorrhaged or dark brown in color with pin point white necrotic foci. The intestine was hemorrhagic and filled with fluids. The abdomen in some cases enlarged and filled with fluids (Ascites). The kidney was congested and enlarged size Plate (1, E).

Results of bacteriological examination:

The results of bacteriological examination according to morphological, culture and biochemical characters revealed the presence of 160 *Enterococcus fecalis* with a percentage of 45.6 %. The colonies on Streptococcus selective agar at 29-30 ^{o}C for 48hrs was dew drops like, white colonies, very small, rounded with entire edges or creamy rounded, large colonies (2-3mm). All bacterial isolates were characterized as oxidase negative, catalase negative, glucose fermentative, vogus proskauer positive, esculine hydrolysis positive. It was negative for starch hydrolysis, arabinos and indole tests. While lactose fermentation and sucrose were positive, Growth at 0-6.5 % NaCl positive, grew at temperatures 10-45 ^{o}C as shown in Table (4).

Concerning the distribution of isolates in different organs of naturally infected *Oreochromis niloticus* the bacteria was isolated from fins, skin ulcers, liver, spleen, kidney and brain with percentage of 11.25, 25, 21.25, 15, 18.12 and 9.94% respectively as shown in Table (5).

Table (4): Morphological and biochemical characters of bacterial isolates.

Character	Result
Shape	Cocci pairs and short chain
Gram's stain	+ve
Motility	-ve
Oxidase test	-ve
Catalase test	-ve
Glucose fermentation	+ve
Vogus proskauer	+ve
Esculine hydrolysis	+ve
Starsh hydrolysis	-ve
Lactose fermentation	+ve
Sucrose	+ve
Arabinose	-ve
Indole test	-ve
Growth at 0 %Nacl	+ve
Growth at 6.5 %Nacl	+ve
Growth at 10°C	+ve
Growth at 45°C	+ve

Table (5): Distribution of isolates in different organs of naturally infected *Oreochromis niliticus*.

Examined organ	No. of isolates	Percentage %
Fins	18	11.25
Skin ulcers	40	25
Liver	34	21.25
Spleen	24	15
Kidneys	29	18.12
Brain	15	9.94
Total	160	

Results of molecular identification of *Enterococcus fecalis*:

Bacterial isolate representing morphology and biochemical profiles of streptococcus spp. were further confirmed by polymerase chain reaction which revealed that the electrophoresis of *Enterococcus fecalis* PCR product with the specific band at733bp. PCR based on the 16S rRNA that provides rapid, simple, and reliable identification of *Enterococcus fecalis*. Plate (1, F).

Results of antibiogram test:

The antibiogram sensitivity test of the isolates was being done and revealed that *Enterococcus faecalis* was highly sensitive to Amoxicillin (Ax25) and Penicillin (P10), while it showed medium sensitivity to Ciprofloxacin (Cip5), Tetracycline (Te30) and Streptomycin (S10) but it was resistant to Nalidixic acid (NA30) as recorded in Table (6). From this result Amoxicillin(Ax10) and Penicillin(p10) were the drug of choice against *Enterococcus fecalis*.

Table (6): Antibiogram sensitivity test.

Antbiotic disc	Code symbol	Concentration (μg)	Interpretation zone of inhibition			Reaction
			R ≤	I	S ≥	
Penicillin	P10	**10**	**11**	**12-21**	**22**	+++
Amoxicillin	AX25	**10**	**22**	**22-30**	**31**	+++
Ciprofloxacin	Cip5	**5**	**18**	**19-20**	**21**	++
Nalidixic acid	NA30	**30**	**13**	**14-18**	**19**	++
Tetracycline	T30	**30**	**14**	**15-18**	**19**	++
Streptomycin	S10	**10**	**11**	**12-14**	**15**	+

S: Sensetive.R: Resistant. I: Intermediate.

Results of pathogenicity test:

The results of pathogenicity test of *Enterococcus fecalis* revealed that it was pathogenic for *O. niloticus* as showed in Table (7).Intraperitoneal injection (I/P) was high effect than intramuscularly injection (I/M) route. I/P cause 80% mortality while I/M cause 60% mortality as showed in Table (7). The pathogens were reisolated as a pure culture from the freshly dead fish. The clinical signs and postmortem changes were similar to naturally infected fish.

Table (7): Pathogenicity and mortality rate of experimentally infected *Oreochromis niloticus* with *Enterococcus fecalis*.

Group	Dose of injection	Route of injection	No. of injected fish	No. of dead fish	Mortality rate %
1st	0.5 ml 5×10^{6} cfu/ ml.	I/P	10	8	80%
2nd	0.5 ml 5×10^{6} cfu/ ml.	I/M	10	6	60%
3rd	Sterile saline	I/P	10	0	0

Results of histopathological examination:

Specimens which were taken from skin, fins, kidney and liver of naturally infected *Oreochromis niloticus* with *E. fecalis* revealed histological alterations. Concerning skin examined tissue it was vacuolated superficial layer of the epidermis with increase mucus secreting cells and alarm substance cells together with dilation of dermal capillaries and hemorrhage Plate (2, A).

In regards to the histopathological changes that observed in the examined sections of fin it showed hyperplasic marginal epidermal layer with increased of alarm substance cells and the basement membrane is under lined with melanomacrophages and congetion of blood vessels Plate (2, B).

Regarding the renal alterations it revealed; congestion of the glomerular and intertubular blood vessels of variable severity together with inter tubular pockets of hemorrhage Plate (2, C). Hydropic degeneration in many renal tubules and alternative areas of depletion and activation of hemopoietic elements Plate (2, D).

Concerning the examined liver tissue specimens it was characterized by highly congested central vein with aggregation of leucocytes around vessels Plate (2, E).

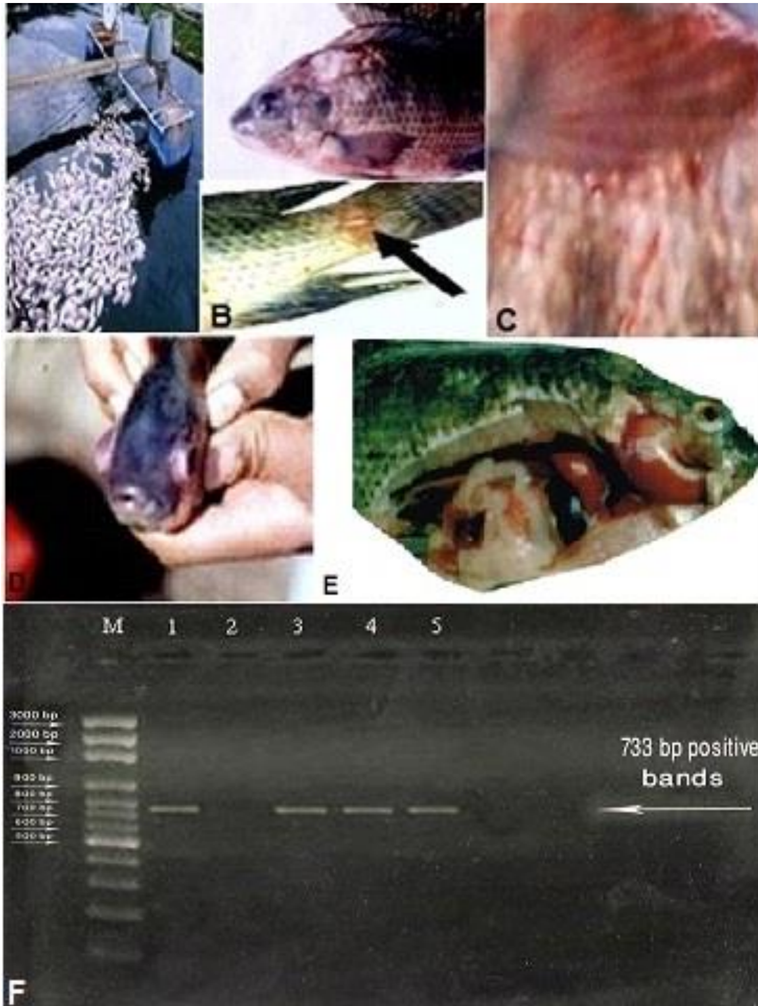

Plate (1).

A: Mass mortality of *O. niloticus* in the floating cages.

B: *O. niloticus* naturally infected with *Enterococcus fecalis* showing skin darkness, erosion and ulceration.

C: *O. niloticus* naturally infected with *Enterococcus fecalis* showing hemorrhage of skin.

D: *O. niloticus* naturally infected with *Enterococcus fecalis* showing hemorrhage of the eye with sever exophthalmia (pop eye).

E: *O. niloticus* naturally infected with *Enterococcus fecalis* showing enlarged and hemorrhaged liver, distended gall bladder, enlarged spleen and congested kidney.

F: Showing the electrophoresis of *Enterococcus fecalis* PCR product with the specific band at733bp**.**

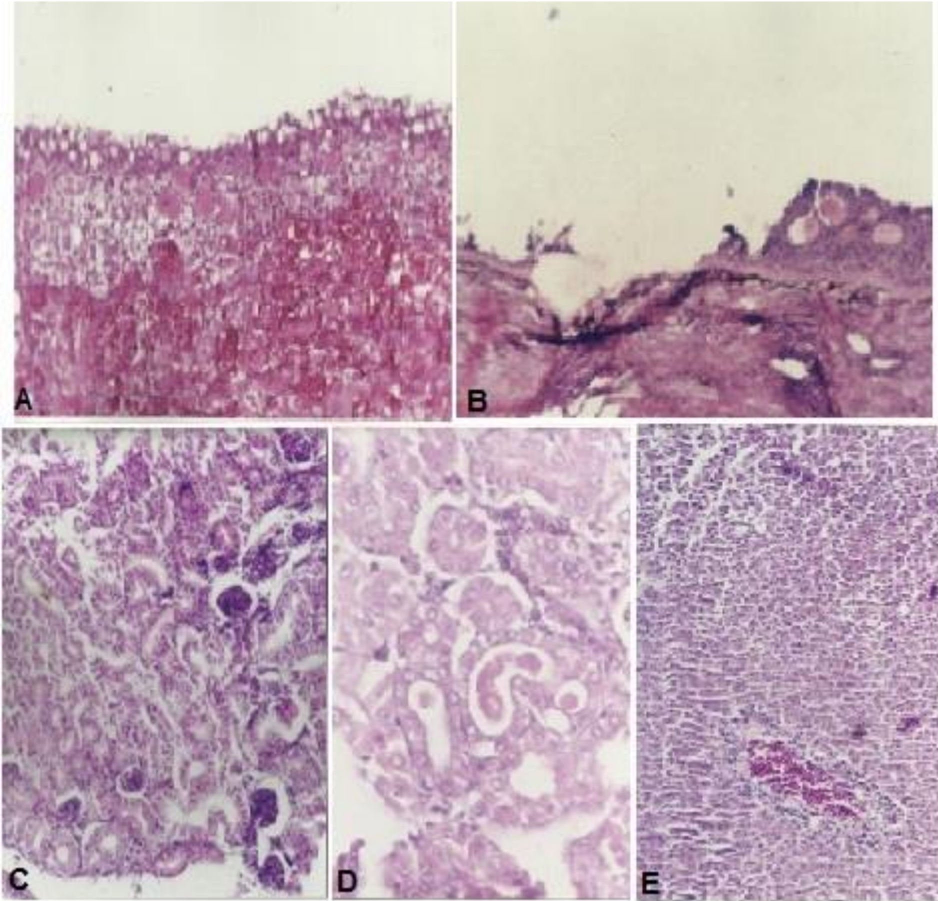

Plate 2.

A: Section of skin of *Oreochromis niloticus* infected with *E. fecalis* showing vacuolated superficial layer of epidermis with increased mucus secreting cells, alarm substance cells together with dilation of dermal capillaries and hemorrhage (H&E X400).

B: Section of fin of *Oreochromis niloticus* infected with *E. fecalis* showing hyperplasic marginal epidermal layer with increased of alarm substance cells and the basement membrane is under lined with melanomacrophages and congestion of blood vessels (H&E X400).

C: Section of kidney of *Oreochromis niloticus* infected with *E. fecalis* showing dilated glomerul cavities in the presence of glomerul hyper cellularity (H&E X400).

D: Section of kidney of *Oreochromis niloticus* infected with *E. fecalis* showing hydropic degeneration and hyaline casts beside depletion of hemopoietic elements(H&E X400).

E: Section of liver of *Oreochromis niloticus* infected with *E. fecalis* showing highly congested central vein with aggregation of leucocytes around vessels (H&E X400).

DISCUSSION

Throughout the past two decades, it has been documented that Egyptian aquaculture is in an uprising state of growth FAO (2005). The intensification of fish farming often leads to the emergence of infectious diseases Diab (2004). Bacterial pathogens are among the most critical causes of mass mortalities in Egyptian aquaculture Eissa *et al*. (2010). Despite the fact that most of fish pathogens were primarily water inhabitants, yet, they can be extremely pathogenic to immune-compromised fishes.

In the present study the physico-chemical analysis of water holding *Oreochromis niloticus* as recorded in Table (3) showed slight increase in nitrite and ammonia which leads to immune suppression and enhance the bacterial infection. Also slight decrease in dissolved oxygen which act as a stress on cultured Tilapia in cages with high in stocking density and leades to mortality in caged cultured of *Oreochromis niloticus***.** These results in coincidence with Manal *et al.,* (2011) who demonstrated that the poor water quality of a certain aquaculture facility (high ammonia, high nitrite and pH fluctuations) usually synergize with other viable components (bacteria, fungi, virus and parasites) in the water system of such facility to produce an eminent case of diseases in fish and other aquatic species. Immunologically, compromising animmune system of a certain fish due to an initiator stressor may enhance the environmental selection of certain type of microorganisms in certain type of rearing water pond.

Regarding the clinical signs it was observed changes in the behavior concluded in loss of appetite, sluggish movement, swimming near the surface of the water, screwed, lateral side or upward swimming, darkening of the skin, small hemorrhages, erosion and ulcers 2-3mm distributed on different parts of the skin, base of the tail, fins, mouth, and anal opening. Ophthalmic lesions as unilateral or bilateral opacity,

hemorrhage of the eye and some cases showed exophthalmia (pop eye). This signs may be attributed to the haemolytic effect of the exotoxin produced by bacteria. Pop eye may be due to the accumulation of mucopurulant exudates around the eye. These results were supported by those of Badran (1994); Plumb (1994); Shoemaker and Klesius (1997); Romalde and Toranzo (1999); Roberts (2001); El-Bouhy (2002); Zeid (2004) and Torky *et al*. (2006) who mentioned that some signs that may distinct for streptococcal disease, include erratic swimming, ulcers and haemorrhage in the base of the fines and of the operculum and sometimes with cloudy eyes and pop eye.

In addition, the postmortem lesions observed in naturally infected fishes were sever congestion in mouth lips; the anal opening protruded and showed congestion and ulceration, pin point hemorrhagic ulcers in the ventral aspect of the gill cover. The gills were pale and showed small white necrotic foci. The brain showed congestion and the superficial veins were prominent and engorged with blood. The liver was enlarged, friable, pale or gray in color, with white pin point necrotic foci on the peripheral margin and the gall bladder enlarged and engorged with bile. The spleen was enlarged, hemorrhaged or dark brown in color with pin point white necrotic foci. The intestine was hemorrhagic and filled with fluids. The abdomen in some cases enlarged and filled with fluids (Ascites). The kidney was pale and enlarged size. The previous observations were more or less similar to those observed by Badran (1994); Plumb (1994); Shoemaker and Klesius (1997); Romalde and Toranzo (1999); Roberts (2001); El-Bouhy (2002); Zeid (2004) and Torky *et al*. (2006) who reported that the liver was enlarged and pale, kidney and spleen were enlargement and congestion, the brain was congested, clear or hemorrhagic ascites and enlarged dark brown spleen.

In the present research, the results of bacteriological examination revealed the isolation of the species *Enterococcus faecalis* from

Oreochromis niloticus. These results agree with those of Torky *et al*., (2006); Eman (2007) and Khafagy *et al*. (2009) who recorded *Enterococcus faecalis* from *Oreochromis niloticus*.

The results of bacteriological examination Table (4) showed that the isolates were gram-positive cocci, non-motile. These findings were agreed to that recorded by Al Harbi (1994); El-Bouhy (2002); Zeid (2004); Torky *et al*. (2006) and Khafagy *et al*. (2009). Concerning the biochemical tests in this study *Enterococcus faecalis* was negative for oxidase, catalase, starch hydrolysis and indole these findings were agreed to that recorded by Ravelo *et al*. (2001); El-Bouhy (2002) and Torky *et al*. (2006). Also, Shoemaker and Klesius (1997) who recorded that two of the most important tests for identification of streptococcal pathogens from fish were catalase and starch hydrolysis test. Lactose fermentation was negative this was agreed with Facklam and Carly (1985); Ravelo *et al* (2001) and Torky *et al*. (2006) but disagree with Eman (2007). The isolates were positive for euscolin hydrolysis that agree with El-Bouhy (2002); Torky *et al*. (2006) and Khafagy *et al*. (2009) and disagrees with Facklan and Moody (1970) and Zeid (2004). Positive for lactose fermentation and sucrose and negative for arabinos these result agree with Ravelo *et al*. (2001) and Torky *et al*. (2006) and disagree with Khafagy *et al*. (2009) who reported that the isolates were lactose fermentation negative.

The isolates grew at 45 ^{o}C and this agree with Perera *et al.* (1997); El-Bouhy (2002); Zeid (2004); Torky *et al*. (2006) and Khafagy *et al*. (2009) and grew well at media with 6.5% NaCl, which agree with Carson *et al.* (1993); El-Bouhy (2002); Zeid (2004); Torky *et al*. (2006) and Khafagy *et al*. (2009) while, disagree with Bragg and Broere (1986) who stated that the isolated *Streptococcus spp.* did not grow on medium containing 6% NaCl.

Regarding the prevalence of streptococcus infection among *Oreochromis niloticus*, the percentage of infection was 45.6%. This result was higher than recorded by El-Bouhy and Megaheed (1994) the prevalence was (9.2%), Ebtsam (2002) was 10% and Khafagy *et al.* (2009) was 23.76% among cultured *Oreochromis niloticus*. The results may increase because of the increase in the biological pollution, usage of manure and wastewater in the area of the study.

With the respect to distribution of *Enterococcus faecalis* in infected *O. niloticus* Table (5) revealed that the highest percentage were isolated from skin (25%) followed by liver (21.25%), kidney (18.12%), spleen (15%), fins (11.25%) and brain (9.94%). These results in coincidence with Khafagy *et al.* (2009) who recorded that the highest prevalence of *Enterococcus faecalis* was in skin ulcers (49.5%) then the liver (16.83%), kidney (15.84%), spleen (11.88%) and brain (5.94%). While disagree with Torky *et al.* (2006) who isolated *Streptococcus spp.* with high prevalence from kidneys (31.72%) followed by liver (29.85%). The results in this study also disagree with those of Eman (2007) who revealed that there were no isolates in brain. The difference between authors in tissue distribution may be due to the different number of diseased fishes, different number of isolated samples and different culturing methods of the bacteria.

In the current study, the molecular identification revealed that the electrophoresis of *Enterococcus fecalis* PCR product with the specific band at733bp. This result is simulating that of Deasy *et al.* (2000) and Özer *et al.* (2011) who demonstrated that 16S rRNA-based PCR assays is successfully used to detect the presence of Enterococcus. Also, this agree with Mata *et al.* (2004) and Pourgholam *et al.* (2011) who use the 16S rRNA gene as target molecule for Streptococcus species.

The steps of PCR protocol were done as follows; an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at

94°C for 30 seconds, primer annealing at 45°C for 30 seconds and extension at 72°C for 30 seconds and Final extension at 72 ºC for 5 min. These steps were partially similar to the steps done by Özer *et al*. (2011) who demonstrated the steps as 3 min at 94°C for initial denaturation; 30 cycles of 1 min at 94°C, 1 min at 54°C, and 1 min at 72°C; 7 min at 72°C for final extension. The difference between the present study methods and the anther were according to (Rychlik and Rhoads, 1989) who reported that the melting temperature (Tm) is calculated as the following simple rule is: Tm= 4 (G+C) + 2 (A+T) and according to Innis and Gelfand (1990) who reported that the annea-ling temperature (Ta) chosen for a PCR about 5oC below the lowest Tm of their pair of primers to be used as follows: Ta = 4 (G+C) + 2 (A+T) - 5. By applying this formula on the primer taken by Özer *et al*., 2011The results of melting temperature (Tm) after calculation of the forward primer sequence were: Tm = 4 (7+3) + 2 (3+2) – 5= (40+10) - 5= 45 ºC. The results of this study indicated that the using of PCR is a reliable, specific and sensitive method for accurate identification of isolated bacteria and in diagnosis of bacterial fish pathogens in aquaculture. These agreed with that recorded by Bader *et al*. (2003); Mata *et al*. (2004); Khafagy *et al*. (2009) and Pourgholam *et al*. (2011).

In this study the results of antibiogram sensitivity Table (6) revealed that *Enterococcus faecalis* was highly sensitive to Amoxicillin, Penicillin, and showed medium sensitivity to Ciprofloxacin, Tetracycline, Streptomycin and resistant to Nalidixic acid this results were agreed with recorded with Al-Harbi (1994) and Khafagy *et al.* 2009 but partially disagree with those of Dena (2004) who revealed that the bacteria were sensitive to Tetracyclin and resistant to Streptomycin, Nalidixic acid and Torky *et al*. (2006) who recorded that the bacteria was sensitive to Penicillin and Nalidixic acid and resistant to Amoxicillin, Ciprofloxacin and Tetracycline; while it was medium sensitivity to Streptomycin. Foulquie Moreno *et al*. (2006) reported that this variation may be related

to the extracellular surface protein (Esp) which is a cell-wall-associated protein of Enterococcus species. It is thought to promote adhesion, colonization and evasion of the immune system, and to play some role in antibiotic resistance. Extracellular surface protein also contributes to enterococcal biofilm formation, which could lead to resistance to environmental stresses.

Results of virulence test of *Enterococcus fecalis* Table (7) revealed that it was pathogenic for *O. niloticus*. Similar observations was reported by Shoemaker *et al*. (2000), Ebtsam (2002); El-Bouhy (2002) and Zeid (2004). Also supported Woo (1999) who mentioned that the pathogenicty of streptococci may be attributed to the effect of exotoxins produced by the bacterium. Intraperitoneal injection (I/P) was more dangerous and cause 80% mortality, while intramuscularly injection (I/M) cause 60% mortality. These results were similar to that recorded by Torky *et al*. (2006) who mentioned that the *O. niloticus* injected I/P with *Enterococcus fecalis* in dose 5×10^{6} cfu/ml cause 80% mortality. While these results disagree with those reported by Perera *et al*. (1997) who recorded that the intrapretonial injection of *Streptococcus iniae* produced no mortalities and could not be isolated from fish 4 days post-injection. The possible explain for these differences in the results may be due to fish species and dose of infection.

Regarding histopathological examination of the tissue sections taken from skin showing vacuolated superficial layer of the epidermis with increase mucus secreting cells and alarm substance cells together with dilation of dermal capillaries and hemorrhage such lesions due to production of extra cellular enzymes and toxins which had hemolytic and proteolytic activities these results are similar to that recorded by Austin and Austin (1987). The dilatation of dermal capillaries and hemorrhage occur due to the process of acute inflammation was initiated by action of the released vaso-active amines on the microcirculation of the area and

the release of cell break down products similar results were demonstrated by Roberts (1978) and Saker and Abou El-Atta (2006). Fin showing hyperplasic marginal epidermal layer with increased of alarm substance cells and the basement membrane is under lined with melanomacrophages. Depletion and destruction of the host defense system may favor the bacteria to have a direct action on the epithelial covering of the epidermis of the fins resulting in the degenerative and necrotic changes of the upper covering epithelium and hyperplasia of alarm substance cells and mucous cell as defense mechanism against the toxins produced by infect bacteria these results were also recorded by Easa *et al*. (1985) and Saker and Abou El-Atta (2006). The kidney is a target organ in many diseases due to affinity of the organ for circulating particular antigen; the trapping is mainly performed by the large number phagocytes lining the renal sinusoids and peritubular capillaries of anterior and posterior portion Ferguson *et al*. (1994). Regarding the renal alterations it revealed; congestion of the glomerular and intertubular blood vessels of variable severity together with inter tubular pockets of hemorrhage. Hydropic degeneration in many renal tubules and alternative areas of depletion and activation of hemopoietic elements. The hemopoietic activities are a general response throughout the kidney to the incitation of the infection Roberts (1978). The contraction of the capillary tufts could be attributed to the pressure of the edematous fluid which accumulated in Bowman's capsules. These results were agreed with Faisal and Easa 1987 and Saker and Abou El-Atta (2006).

Concerning the examined liver tissue specimens it was characterized by highly congested central vein with aggregation of leucocytes around vessels. Such hepatic lesions are indication of septicemia as the liver was damaged by both pathogenic bacteria and its toxins. These lesions were also described by Ventua and Gizzle (1988) and Saker and Abou El-Atta (2006).

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دراسة حالات النفوق فى الاقفاص المستزرعة بالبلطى النيلى وحيد الجنس بمحافظة الفيوم ـ مصر

ولاء طلعت الإكيابى[١]، محمد السيد ابو العطا[١]،
محمد مصطفى سيد احمد[١]، لاميس شوقى عبد الفتاح[٢]

١- قسم بحوث صحة الأسماك و رعايتها- المعمل المركزى لبحوث الثروة السمكية –مركز البحوث الزراعية.
٢- معهد علوم البحار بالسويس .

الملخـص العربــى

تم تجميع عدد ١٠٠ سمكة من البلطى النيلى وحيد الجنس المصابة بشكل طبيعى متوسط وزن ٢٠٠±٥ جرام للسمكة من الاقفاص التى تعانى من نسبة نفوق عالية بوادى الريان محافظة الفيوم فى الفترة من ابريل حتى اكتوبر ٢٠١٢. وكذلك تم قياس بعض الصفات الفيزيائية والكيميائية للمياة. وقد تم الفحص الاكلينيكى و الصفة التشريحية للأسماك المصابة. وكشف الفحص البكتريولوجي وجود عدد ١٦٠ عزلة لبكتريا الإنتيروكوكس فيكالز بنسبة ٤٥.٦٪ من البلطي النيلي. وقد تم العزل والتعريف بالطرق التقليدية العادية ثم أكد باستخدام تقنية انزيم البلمرة المتسلسل الذى اكد وجود بكتريا الإنتيروكوكس فيكالز. وقد تم العزل من الزعانف، التقرحات الجلدية، الكبد، الطحال، الكلى والمخ بمعدل اصابة ١١.٢٥، ٢٥، ٢١.٢٥، ١٥، ١٨.١٢، ٩.٩٤٪ على التوالي. وأكدت النتائج وجود الرابط المخصص لبكتريا الإنتيروكوكس فيكالز عند BP ٣٧٧ وذلك بناءا علي وجود الجين المخصص لها .16S rRNA. وبإجراء اختبار الحساسية وجد أن ميكروب الإنتيروكوكس فيكالز شديد الحساسية للأموكسيسيلين والبينيسيلن. وقد احدثت العدوى التجريبية نفوقا بنسبة ٨٠% للحقن البريتونى، ٦٠% للحقن العضلى. كذلك تم دراسة التغيرات الهستوباثولوجية للأسماك المصابة.