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Fish diseases

Streptococcus agalactiae Isolation and Characterization in Nile Tilapia (*Oreochromis niloticus*) with Histopathological Studies

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ABSTRACT

Streptococcus agalactiae has been described as one of the most serious bacterial diseases in tilapia culture. This study investigated the isolation and characterization of *Streptococcus agalactiae* from a condition of mass mortality in farmed *Oreochromis niloticus* at Baltim region, Kafr El-Sheikh governorate, Egypt during the summer of 2019, and the effectiveness of locally prepared bacterin for prevention of streptococcal disease. Infected fish showed corneal opacity, congestion of spleen, liver, and kidney, the abdominal space filled with watery and bloody ascites**,** and light-colored nodules on the kidneys, spleen, or liver. Water qualities were measured parallel to the fish sample. *S. agalactiae* isolates were identified by phenotypic and biochemical VITEK II methods. Ampicillin, Colistin sulfate, would appear as the best antibacterial agents to use in the treatment. The relative level of protection value was 70% in *Oreochromis niloticus* challenged with a virulent strain of *S. agalactiae* (10^7 CFU /1 ml) and vaccinated with locally prepared killed bacterin. Histopathological investigations revealed congested blood vessels in all organ's specimens examined, inflammatory cells infiltration, degenerative changes, different stages of necrosis of the liver, and generalized meningoencephalitis of the brain. The current study recommended that *S. agalactia* induced many problems in tilapia culture in presence of bad water quality during the summer season, and the vaccination program gives good results and high protection which may be a useful method for the prevention and control of streptococcal infection.

Keywords: *Streptococcus agalactiae, Oreochromis niloticus*, Histopathology.

INTRODUCTION

Nile tilapia culture is one of the fastest growing aqua-culture practices in the world (Conroy *et al.*, 2008). Tilapia's contribution to global food security is 4.5 million metric tons annually and acts as a relatively less costly source of protein (Senapin *et al.*, 2018). At present the fourth most common fish species in aquaculture is tilapia (FAO., 2019). Nevertheless, stress resulted as a consequence of the intensification of fish production, which increases the susceptibility to disease outbreaks such as bacteria, parasites, nutritional deficiencies, and fungi (Amal and Zamri-Saad, 2011). Last but not least, tilapia is a member of the food chain, making it ideal for low-priced consumption and a wide variety of food sources with limited environmental effects (Beveridge and Baird, 1998). Streptococcus agalactiae, also known as Group B Streptococcus, (GBS) in many fish species causes septicemia, meningoencephalitis, exophthalmia, anorexia and ascites, and is recognized around the world as a major tilapia pathogen (Verner-Jeffreys *et*

al., 2018). Streptococcus genus is one of the most important Gram-positive bacteria affecting tilapia cultivation, and the most widespread species in the world is *S. agalactiae* species (Conroy, 2009 and Jimenéz, 2010). *S. agalactiae* is a coccus, organized in pairs or short, catalase and oxidase negative and positive CAMP chains; it may or may not be hemolytic (Ali *et al.,* 2010).The clinical signs are characterized by loss of appetite, exophthalmia, hemorrhage in eye, corneal opacity, distention of abdomen, curvature of the spinal cord, erratic swimming, stiffness, and bleeding on the base of the fins (Yanong and Francis-Floyd, 2002). Streptococcosis usually causes acute outbreaks that affect many fishes, with mortality ranging from 10% to 80%. Mass mortality was caused by the disease between tilapia farms (Ali *et al.,* 2010). Vaccines are considered to be an alternative approach for streptococcosis prevention or control. without adverse effects from chemotherapy, such as high cost, drug-resistant pathways, ogenic strains and drug residues followed by environmental and public health hazards. (Dehghani *et al.,* 2012), The administration of vaccines remains the most viable method of fish disease control. Reliable and efficient vaccines are therefore essential for the sustainable development of the aquaculture industry (Evensen and Leong, 2013), Not only to greatly reduce mortality and use of antibiotics in aquaculture, but also induction and generation of resistance against many pathogenic micro-organisms. So, this study was aimed to investigate the isolation and characterization of *Streptococcus agalactiae* from diseased fish from farms with histopathological studies, and the evaluation of relative level of protection to locally prepared bacterin against a virulent strain of *S. agalactiae* in *Oreochromis niloticus.*

MATERIALS AND METHODS

Fish sampling

Two hundred *Oreochromis niloticus* were collected from Baltim region, Kafr El-Sheikh governorate during the summer season and were classified into two sections. The first section was fifty *Oreochromis niloticus* with an average body weight 100 ±10 gm and was collected showing clinical signs and transported to the private lab at Alexandria governorate for full clinical, postmortem (PM) lesions and bacteriological examinations according to the methodology outlined by (Austin *et al.,* 2012). The second section were 150 apparently healthy *O. niloticus*, weighting 50 ± 2 g and were selected for determination of lethal dose fifty (LD_{50}), and evaluation of the relative level of protection value.

Physico-chemical analysis of water sample

Water samples were taken parallel to fish samples and subjected to complete water analysis according to the standard method described by (Boyd, 1990)**.**

Bacterial isolation

Bacterial culture: Bacterial inoculums were taken under complete aseptic condition from the liver, Kidney, spleen, and heart and cultured on tryptic soy broth (Difco, Detroit, MI, USA) moreover supplementation with 3 % NaCl and incubation at 25 ^{0}C for 24 – 48 hrs. then sub cultured on Tryptic soy agar supplemented with 3 % NaCl; Blood agar media using 5 % sheep RBCs supplemented with 3 % NaCl. The inoculated plates were incubated at 25 ^{0}C for 24 – 48 hrs.

Biochemical characterization

The biochemical characters investigated by conventional tests in accordance with the technique defined by (Quinn *et al.,* 2002). Moreover, using VITEK® 2 compact (BioMérieux) biochemical Identification (VITEK2 Compact System, BIOMERIEUX, and France). The criteria used to detect the isolates are based on the morphology of colonies which characterized by gram staining of the microorganisms

Anti-bacterial sensitivity test:

For the antibacterial sensitivity experiment, antibiotic discs used were: Gentamycin (10 Mg), Flumequine (30 MG) Enrofloxacin (5Mg), Oxytetracycline (30 Mg), Doxycycline (30 Mg), Ciprofloxacin (5 Mg) Sulphamethoxazole / Trimethoprim (23.7+1.25 Mg), , Amoxycillin (10 Mg) and Erythromycin (15 Mg). Such antimicrobial discs are derived from (Oxoid, England) at the end of incubation, Inhibition zones were measured using measure caliber.

Determinations of lethal dose fifty (LD_{50}):

The most prevalent bacterial isolate, which was *S. agalactiae* was selected as the inocula. and prepared for I/P injections. The isolate of bacteria was sub-cultured on trypticase soy agar plates and incubated at 25 - 28 ^{0}C for 24hr. typically isolated colony was picked up and inoculated into trypticase soy broth and incubated at 25 – 28 ^{0}C for 24hr. Then

centrifugation of broth culture, also the supernatant removed. The sediment was resuspended in sterile saline and the optical density of MacCforland No. 2 was standardized (each ml contains approximately 10^6 bacterial cells). An overall number of 70 apparently healthy *O. niloticus*, weighting 50 ± 2 g. After the acclimation period, about two weeks, were selected and then divided into 7 equal groups; each group contained 10 fish. The first six groups were consistently inoculated I/P with a bacterial suspension of *S. agalactiae* suspension at a dose rate of 0.2 ml of concentration ranging from 1.0×10^3 to 1.0×10^8 cfu/ml while the control group (group 7) was injected I/P with 0.2 ml of sterile saline and act as a control group as illustrated in Table (1). In accordance with the method defined by (Reed and Muench, 1938). , LD50 was calculated using the following formula:

$$LD_{50} = \frac{\text{Mortalities above 50\% - 50}}{\text{Mortalities above50\%- Mortalities below 50\%}}$$

For about one week the clinical signs and PM lesions were reported daily, and specimens were collected for histopathological studies.

Experimental design for evaluation of the relative level of protection to locally prepared bacterin against a virulent strain of S. agalactiae in O. niloticus:

S. agalactiae was modified by the plate count process to 4×10 9 and used for vaccine preparation. The isolates were separately inoculated in TSB at 27 °C for 72 h in a shaker water bath at 70 RPM. In culture procedures, ten percent neutral buffered formalin was used at 27 °C for 24 h, resulting in a final concentration of 3%. The cultures treated with formalin were centrifuged for 30 minutes at 7000g according to the method defined by (Klesius *et al.*, 1999). The cultures were then washed three times with sterile saline and resuspended in 10 ml of sterile saline, and the prepared bacterium was stored at 4 °C (Sakai *et al.*, 1984). According to Anderson and Conroy (1970) the safety measures of the prepared vaccines were carried out by inoculation in the TSA at 30 °C for 24 hours. A total number of 80 apparently healthy *O. niloticus*, after an acclimatization period of approximately two weeks, 50 ± 2 g were selected and then divided into 2 equal groups; each group contained 40 fish (2 replicates 20 per each). The first group was consistently inoculated I/P with bacterin against *S. agalactia*e of concentration 10^7, Vaccination by bacterin occurs at zero-day and a booster at 14 days. the challenge by a virulent strain of *S. agalactiae* occurs after 28 days post-vaccination. The mortality rate and the postmortem changes were registered after challenge, and the 2nd control group was intraperitoneally injected with 0.1 ml of sterile saline and considered a control group. In accordance with Newman and Majnarich (1982) the relative level of protection was calculated: RLP = 1 – (% mortality of vaccinated fish / % mortality of control).

Histopathological examination:

Samples of kidney, spleen, liver, eye, heart and brain were taken from naturally and experimentally infected fish with *S. agalactiae* for histopathological examination according to the methodology outlined by (Roberts, 2001)

Statistical analysis:

Data were analyzed statistically using Chi-square and the ANOVA one-way test (SPSS 1997). Results were reported as mean ± standard deviation (SD). To indicate statistical significance, the value of $P < 0.05$ was used.

Table (1): Experimental design of LD_{50} of *S. agalactiae* in *O. niloticus*:

Group number	Group (1)	Group (2)	Group (3)	Group (4)	Group (5)	Group (6)	Control group (7)
***S. agalactiae* dilution**	10^3	10^4	10^5	10^6	10^7	10^8	Sterile saline
No. of *O. niloticus*	10	10	10	10	10	10	10

RESULTS

Results of naturally collected fishes and PM lesions:

Naturally examined *O. niloticus* showed darkness raised area over the dorsal region and hemorrhage over the pectoral fins and different degree of corneal opacity. The Post-mortem (PM) lesions revealed that severe congestion with petechial hemorrhage of the liver, kidney and spleen, gonads and intestinal tract. The liver edematous and became flabby as showed in Fig (1)

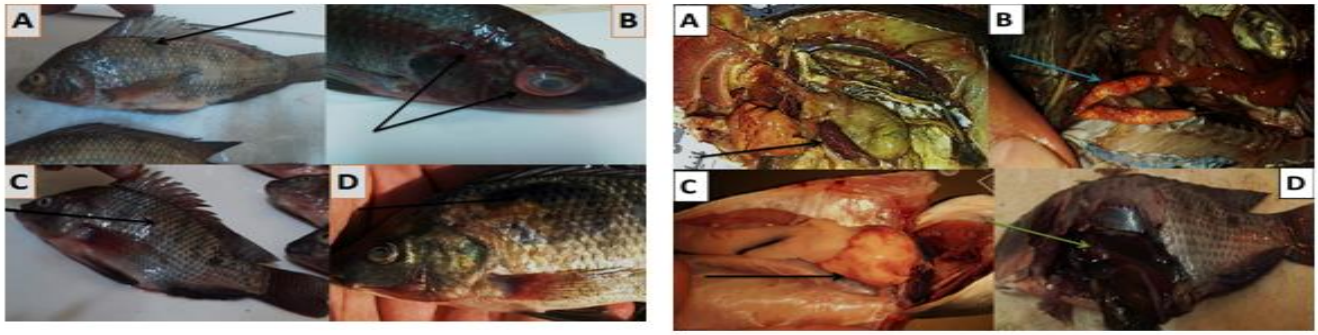

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Fig. (1): *Showed the clinical signs and postmortem lesions of naturally examined O. niloticus.*

Results of water quality in cultured O. niloticus

The dissolved oxygen value was lower than the optimum level of cultured *Oreochromis niloticus*. Water temperature and pH were 29.7ºC and 8.2 respectively in summer. There were a higher significant ($p \leq 0.05$) levels of un-ionized ammonia, nitrite, and nitrate in the summer 0.74, 0.061 mg/l, and 6.8 ppm, respectively (Table 2).

Table (2): Results of water quality of cultured *O. niloticus*.

Water parameters	PH	Dissolved oxygen (mg/l)	Salinity (ppt)	Nitrite NO_{2-} (mg/l)	Nitrate (NO_{3-} (mg/l)	Ammonia NH_3 (mg/l)	Temperature °C
Results Mean ± SD	8.2 ± 0.5	3.8 ± 0.13 ^{B}	3.2 ± 0.3	0.061± 0.029 ^{A}	6.8 ± 1.58 ^{A}	0.74 ± 0.13 ^{A}	29.7 ± 1.2
Optimum ranges	7.5-8.5	5.1-5.9 ^{A}	-	0.01^{B}	<1 ^{B}	0.01 ^{B}	-

Means within the same row of different litters are significantly different at ($P < 0.01$).

Results of Phenotypic and biochemical characteristics of S. agalactiae

Morphological and biochemical characterization of the isolates were summarized in (Table 3)

Fig. (2).: Raised, grayish-white colonies were observed on TSA after 24 hrs from culture.

Table (3): Comparison of phenotypic characteristics of the isolates to the reference strains of ***S agalactiae***: -

General characters of *S. agalactiae*					
Gram staining reaction	+	Catalase test	-	Utilization of: -	
Cell morphology	Cocci in chain	Cytochrome oxidase test	-	Lactose	-
Motility	-	Voges Proskauer test	-	Maltose	+
Swarming on TSA (2.0% NaCl)	+	H_2S production	-	Mannitol	+
Growth on TCBS agar	-	Indole (peptone H2O)	-	Sorbitol	-
NaCl tolerance: -		Urease	-	Sucrose	+
TSA + 1 % NaCl	+	Catalase test	-	Haemolysis (5% sheep RBCs)	β haemolysis
TSA + 8 % NaCl	-	Cytochrome oxidase test	-		
TSA + 10 % NaCl	-				

S. agalactiae isolates prevalence in naturally infected *O. niloticus* after identification by conventional methods is illustrated in **Table (4)**. Ten isolates using Vitek2 compact have been identified. Nine isolates were identified as *S. agalactiae*, with a probability of 99%. **Table (4)**.

Table (4): Prevalence of different *S. agalactiae* isolates in different internal organs of *O. niloticus* samples after identification by conventional methods and by the Vitek2 system.

Organs examined	No. of isolates by conventional methods	*P -Value* by conventional methods	No. of isolates by the Vitek2 system	*P -Value* by the Vitek2 system
Liver	31	0.001*	13	0.001*
Kidney	12	0.001*	3	0.001*
Spleen	10	0.001**	5	0.001**
Brain	7	0.001**	5	0.001**
Total isolates	60		26	

Table (4) showed a significant difference (P< 0.001) of the prevalence of *S. agalactiae* isolates from the different organs. The number of *S. agalactiae* isolates isolated showed higher number in liver (31 isolates) and kidney (12 isolates) by conventional methods and the lower bacterial isolates observed in spleen and brain. And by the Vitek2 system revealed 13 isolates by the liver followed by spleen and brain then the kidney (3 isolates).

Results of susceptibility and resistance as well as intermediate of *S. agalactiae* to different antibiotics:

The results of the sensitivity of the isolates of *Streptococcus agalactiae* to the different antimicrobial agents were summarized in table (5).

Table (5): Diameter of zone inhibition to nearest mm in different isolates of *S. agalactiae* to different Antimicrobial agents.

Disc content in μg	10	5	30	30	30	5	30	10	30	30
Antimicrobial agents	Aml	Cipro	Colistin sulphate	Doxycycline	Enrofl	Erythro	Flumequine,	Genta	Oxyt	Sulphametho and Trimetho
Sensitivity of *S. agalactiae*	34 (S)	20 (S)	31 (S)	25 (S)	22 (S)	14 (R)	11 (R)	10 (R)	11 (I)	8 (R)

S: Sensitive; I: Intermediate susecptible; R: Resistance.

Aml: Ampicillin; **Cipro**: Ciprofloxacin; **Enrofl:** Enrofloxacin; **Erythro**: Erythromycin

Genta: Gentamicin; **Oxy**: Oxytetracycline; **Sulphametho and Trimetho**: Sulphamethoxazole and Trimethoprim. Table **(5)** showed that *S. agalactiae* susceptible to Ampicillin, Colistin sulfate, Enrofloxacin, Doxycycline, and Ciprofloxacin and not susceptible to any other antibiotics.

6. Results of lethal dose fifty (LD_{50}): -

The LD_{50} results of *S. agalactiae* have been listed in Table (6).

Table (6): LD_{50} of *S. agalactiae* in *O. niloticus*: -

Conc. of bacteria	10^3	10^4	10^5	10^6	10^7	10^8	control
No. of injected fish	10	10	10	10	10	10	10
Mortality ratio of *O. niloticus.*	0/10	2/10	3/10	4/10	**5/10**	10/10	0/10

The results demonstrated that the LD50 of *S. agalactiae* from *O. niloticus* were 10^7

$LD_{50} = (10^7)$

- During the LD50 assessment, clinical signs began to appear on the 3rd day after infection and continued until the end of the experimental period (one week). Fish showed generalized erythematic hemorrhages spread over various parts of the surface of the body and abdominal distension.

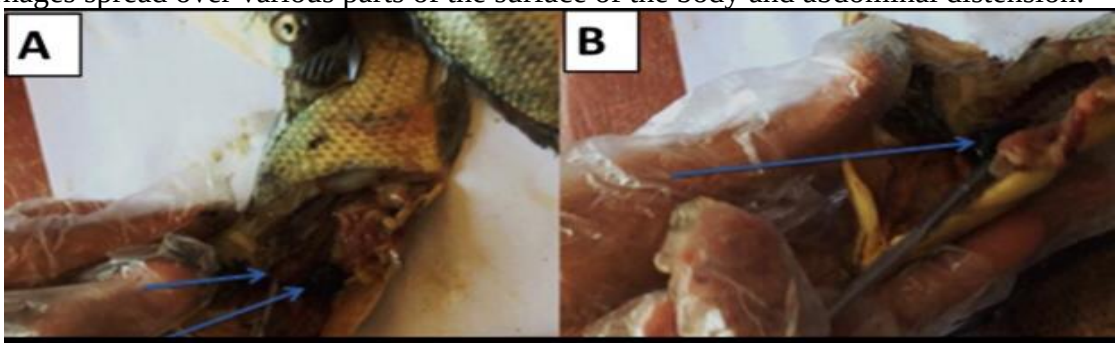

Fig. (3): The postmortem lesions of I/P experimentally infected *O. niloticus* with *S. agalactiae* revealed; extreme congestion in the gills, liver, kidney, and intestine, distention of gall bladder, an excess of bloody hemorrhagic ascetic fluids filled the abdominal cavity. (Photo, A) and (Photo, B).

Results of the relative level of protection to locally prepared bacterin against a virulent strain of S. agalactiae in O. niloticus: -

The results of the relative level of protection to locally prepared bacterin against a virulent strain of *S. agalactiae* in *O. niloticus* (intraperitoneally, I.P. injection was described in Table **(7).**

Table (7): Results of the relative level of protection to locally prepared bacterin against a virulent strain of *S. agalactiae* in *O. niloticus*: -

Groups	No. of fish	Number of Mortality	RLP
Group 1 injected intraperitoneally with 0.1 ml bacterin of concentration 10^{-7}	40 (2 replicates 20 per each)	6/20 8/20	70 60
Control injected intraperitoneally with 0.1 ml of sterile saline	40 (2 replicates 20 per each)	20/20 20/20	0.0 0.0

The data gained demonstrated that the relative level of protection was 70 %. in vaccinated *O. niloticus*.

The results of histopathological alterations of naturally as well as experimentally O. niloticus infected with S. agalactiae were summarized as the following: -

Liver of naturally and experimentally infected *O. niloticus* with *S. agalactiae* showed blood vessel congestion, portal blood vessel thrombosis and infiltration of inflammatory cells, and vacuolar (fatty) degeneration of hepatocytes. Kidney of naturally and experimentally infected *O. niloticus* with *S. agalactiae* showed hemorrhage, thrombosis in glomeruli and tubules, mononuclear cell infiltration, and tubular hyaline degenerations. Spleen of naturally and experimentally infected *O. niloticus* with *S. agalactiae* displayed large thrombus in the splenic blood vessel, deposition of multifocal hemosiderin, increased melanomacrophage cells and inflammatory cells infiltration. The heart displayed increased pericardial epithelial layer thickness and moderate edoema in the epicardium. The eye showed damage of lens capsule, choroid edema. The brain of *O. niloticus* which is naturally infected with *S. agalactiae* showed inflammatory cell infiltration, perivascular and pericellular edema.

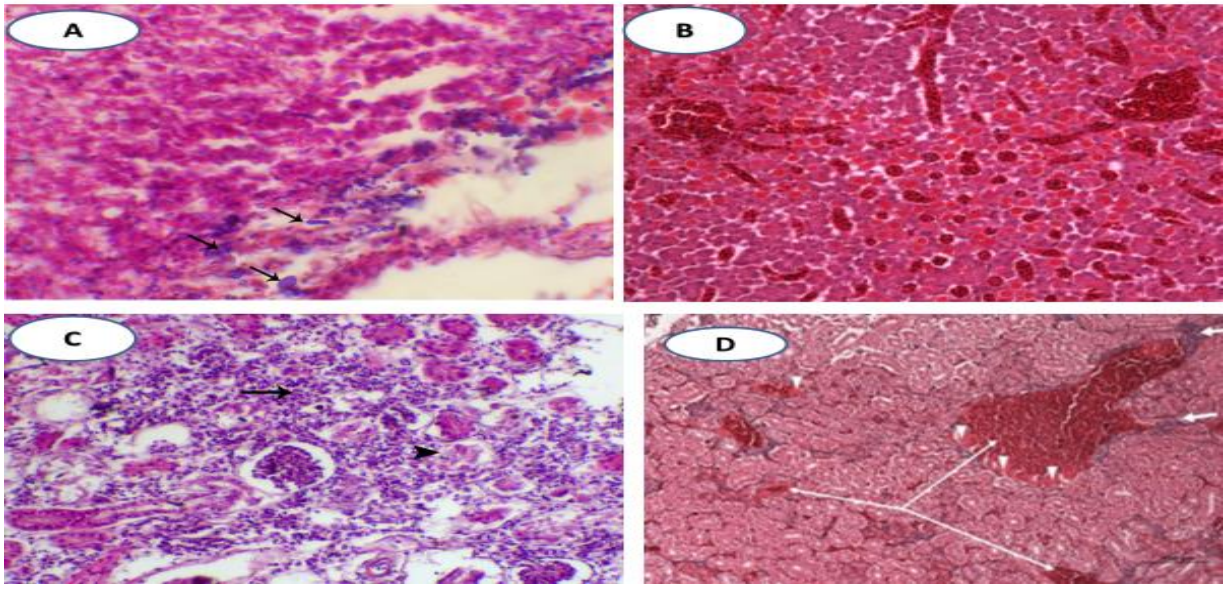

Fig. (A): Liver of *O. niloticus* experimentally infected with *S. agalactiae* exhibited hepatic necrosis with perivascular and peripancreatic infiltration of macrophages laden bacteria H&E X200.

Fig. (B): Liver of naturally infected *O. niloticus* with *S. agalactiae* exhibited severe liver blood capillaries congestion and formation of hyaline droplets. H&E, X 150.

Fig. (C): Kidney of *O. niloticus* experimentally infected with *S. agalactiae* displayed focal necrotic area associated with tubular necrosis and mononuclear cells infiltration, H&E X200.

Fig. (D): Kidney of naturally infected *O. niloticus* with *S. agalactiae* exhibited hemorrhage, and inflammatory cell infiltration. H&E, X 150.

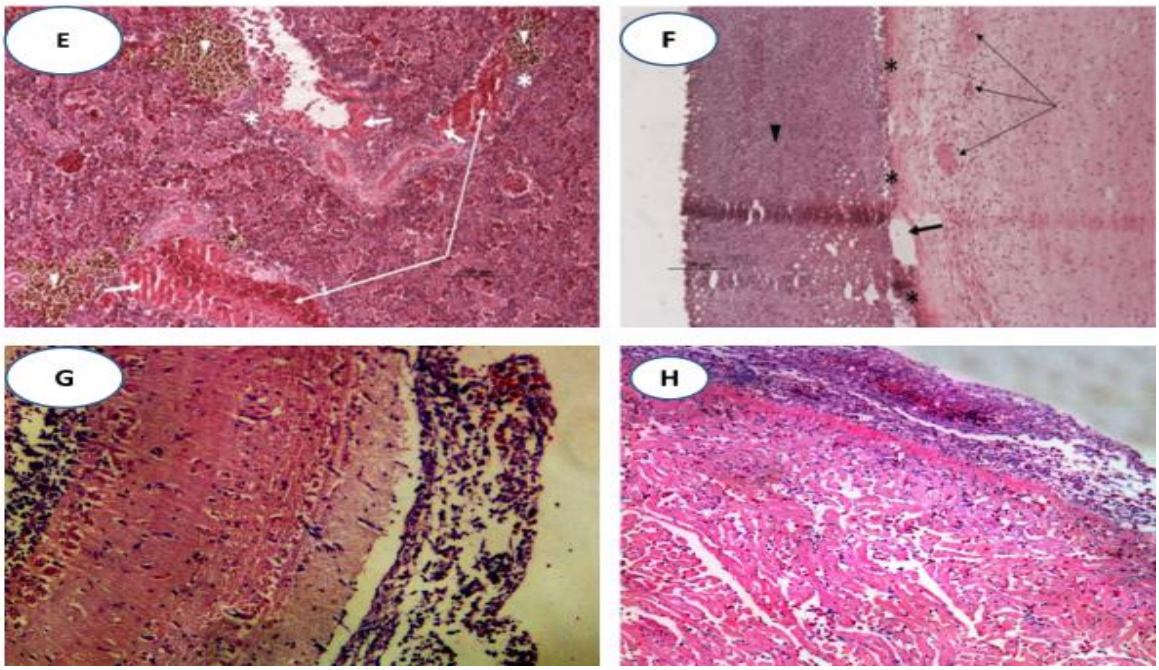

Fig. (E): Spleen parenchyma of naturally infected *O. niloticus* with *S. agalactiae* showed large thrombus in the splenic blood vessel, deposition of multifocal hemosiderin, H&E, X 200.
Fig. (F): Eye of experimentally infected *O. niloticus* with *S. agalactiae* showing retina hyperplasia, choroid hemorrhage with inflammatory cellular infiltration. H&E, X 200.
Fig.(G): Brain of experimentally infected *O. niloticus* with *S. agalactiae* showing haemorrhages, and oedema in the meninges. H&E, X 150.
Fig. (H): Heart of naturally infected *O. niloticus* with *S. agalactiae* showed pericardial epithelial layer thickness and moderate edoema in the epicardium. H&E, X 400.

DISCUSSION

Stress is often one of the predisposing factors that contribute to streptococcosis outbreaks such as poor environmental conditions; increasing ambient temperatures, harvesting, mismanagement, transportation, and poor water quality (Francis-Floyd and Yanong, 2013). Amal et al. (2008) reported that increasing and low water temperatures, high salinity and alkalinity (pH>8), low dissolved oxygen content, poor water quality (such as high concentrations of ammonia or nitrite), as well as impacts on harvesting and handling are some of the stressors associated with Streptococcal outbreaks. The most prominent clinical signs, of the infected tilapia fish in the current study were; the different degree of corneal opacity; darkness raised area over the dorsal region and hemorrhagic over the pectoral fins. These results may be due to exotoxin secreted by the *S. agalactiae* during high temperatures. The preceding findings were more or less identical to those found by Ali et al. (2011) This current study revealed that from routine bacterial isolation using tryptone soy agar media , round- shaped, gray pinpoint colonies were isolated. And the isolates were β-hemolytic, gram-positive cocci. The results obtained were in line with Delannoy et al. (2013) who identified that Streptococcosis caused by *S. agalactiae* of cultured fish based on biochemical and serological characteristics. In addition, Auzureen et al. (2016) stated that the *Streptococcus agalactiae* were a Gram-positive, translucent pinpoint colony, and has oxidase and catalase-negative.The characterization of S. agalactiae have been screened and validated by VITEK 2 using biochemical methods. The obtained results were parallel to that obtained by (Laith *et al.*, 2017)**.** *S. agalactiae* isolates in the present study were sensitive to Ampicillin, Colistin sulfate, Enrofloxacin, and Ciprofloxacin, and not susceptible to any other antibiotics. Darwish and Hobbs (2005) stated that *S. agalactiae* are vulnerable to amoxicillin and roxithromycin also, sensitive only to cefoxitin, the isolates show a narrower antibiotic susceptibility, which indicated bacterial resistance to antibiotics was more serious. Differences in resistance and susceptibility measurement to the same antibiotics among results could be due to differences in antibiotics given since in fishpond. The prevalence of bacterial isolates in the internal organs of the naturally infected *O. niloticus* samples showed the liver had a high incidence of *S. agalactiae*, followed by the gills; and the lowest incidence occurs in the spleen and heart. Also, the results of epicarditis and meningitis found in this study

are similar with those reported by (Zamri-Saad *et al.*, 2010) in naturally infected *O. niloticus* with *S. agalactiae*. The LD_{50} from *S. agalactiae* in this research were (10^7). These results nearly agreed somewhat to that obtained by (Wang *et al.*, 2013) who determined the median lethal doses (LD_{50}) of *S. agalactiae* were 6.8×10^6 and 5.3×10^6 CFU/fish thruogh intraperitoneal injections. Histopathological alterations of naturally and experimentally *O. niloticus* infected with *S. agalactiae* in the present study revealed septicemic lesions in different organs. This explanation is agreed with that of Mian et al. (2009) who found that Streptococus infection in fish caused by *S. agalactiae* is distinguished with septicemia and meningoencephalitis, and this was reinforced by the clinical signs and symptoms in this outbreak of streptococcosis in tilapia. All histopathological findings in this study were evidence for septicemia occurrence resulted from streptococcosis which is detected in experimental and natural infection (Moustafa *et al.*, 2010). Furthermore Ferguson et al. (1994) also estimated that the spleen and kidney are the target organs of the pathogens that cause streptococcosis due to the severe histopathological changes that occurred in them. *O. niloticus* intraperitonially injected with locally prepared bacterin showed effective resistance against *S. agalactiae* infection in *O. niloticus* in this study. And the survival rate was high in vaccinated groups. Klesius et al. (2006) mentioned that a vaccine is a prevention method used to combat infectious diseases in a health management strategy, and that killed vaccines are considered safer than adjusted live vaccines, which may return to virulence. Also, Iregui et al. (2016) recorded that the vaccination of *Streptococcus agalactiae* by different route induce good protection. Diab et al. (2019) studied the effect of autogenous bacterins cross-protection for protection against streptococcosis in *Oreochromis niloticus*, and concluded that autogenous bacterins of *S. agalactiae* or *S.iniae* induced cross-protection against *S. agalactiae* and *S. iniae*, in *O. niloticus*. Which considered a useful method for the prevention and control of streptococcosis. Moreover, Ismail et al. (2017) studied the influence of vaccination with a feed-based vaccine on naturally occurring streptococcosis in tilapia farm experiencing endemic streptococcosis, and vaccinated by bacterin vaccine at 4% body weight. And concluded that the survival rate for unvaccinated groups was 45.2 ± 2.45%, for single booster was 65.3 ± 4.8% and for double booster groups was 75.1 ± 2.1%.

CONCLUSION

The findings of this study indicated that *S. agalactiae* cause serious pathogenic effects in tilapia culture and the vaccine administration yields excellent protection against *S. agalactiae* infection. For better Streptococcosis control and prevention in Nile tilapia, additional studies are necessary on rapid diagnostic techniques and other control measures against streptococcosis.

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