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Histopathological Lesions of Some Microbial Infections in Tilapia Fish at El Salam Canal, Egypt

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Abstract

Microbial infections in tilapia aquacultures cause severe economic losses in Egypt. They appear in summer with other stress factors such as low oxygen and high ammonia levels in aquaculture. Streptococcosis lead to severe losses in tilapia farming, especially in third countries. Microbiological and histopathological examinations are very important diagnostic tools in distinguishing fish microbial infections. This study aimed to isolate, identify, and characterize the most common bacterial fish pathogen recovered from Nile tilapia private farm around El Salam Canal, at Ismailia governorate. In addition to detection the main histopathological lesions were done. The highest rate of bacterial isolation was *Streptococcus agalactiae* with 29.16% from all samples. Grossly the infected fish showed hemorrhagic septicemia, and exophthalmia. Histopathological examination revealed severe hyperemia of gill filaments and gill arch, chronic enteritis, hydropic degeneration of the liver, edema and hemosiderosis of the spleen, necrosis of muscle and hemorrhage and myocardiolysis of heart.

Keywords: *Streptococcus*; Nile Tilapia; Bacteriology; Histopathology

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1. Introduction

The fish pathogen, *Streptococcus*, increased in different fish diseases and caused serious economic losses due to high outbreaks **(Abdel Aziz et al., 2016)**. In South-East Asia, numerous incidences of streptococcosis have been reported in intensive tilapia culture systems with tremendous financial damage since tilapia farming is the most nutritional protein source **(Kayansamruaj et al., 2020).** Although a range of bacterial etiological agents have been identified from fish infections, the greatest causes of these diseases are the Gram-positive, *Streptococcus iniae* and *Streptococcus agalactiae* **(Mishra et al., 2018).** Streptococcal species were isolated as *Streptococcus faecalis* and *Streptococcus faecum* from *Oreochromis niloticus* (freshwater) and *Mugil cephalus* (marine water), respectively **(Badran, 1994)**. Generally, Environmental stresses such as low dissolved oxygen levels, water hardness, crowding and high nitrite concentration are the main factors to induce Streptococcosis as *Streptococcus* spp is considered as opportunistic pathogen in aquaculture **(Bunch and Bejerano, 1997 and Wedemeyer, 1997)**. All groups of Streptococcus infected fish at 30°C in 0 ppt or 15 ppt salinities had significantly higher mortalities than the corresponding groups held at 25°C. **(Chang and Plumb, 1996).** Infection with *Streptococcus* spp. includes all vital organs of fish, and mortality becomes massive (50-60 %) **(Miyazaki et al., 1989)**. The infected fish with *Streptococcus* is mainly suffered from loss of appetite, corneal opacity, hemorrhage in the eye, exophthalmia, distention of the abdomen, stiffness, and the spinal cord curvature, swimming with erratic motion and base of the fins associated with bleeding **(Yanong and Francis-Floyd, 2002).** Experimentally infected *O. niloticus* with *S. agalactiae* revealed; extreme congestion in the gills, liver, kidney, and intestine, distention of the gall bladder, and an excess of bloody hemorrhagic ascetic fluids filled the abdominal cavity **(Hanan et al., 2021).** Moreover, *Streptococcus* spp has recently emerged as a public health hazard due to its zoonotic importance, as it was isolated from a human with accidental injuries while handling fresh infected fish **(Lau *et al*., 2006).**

2. Materials and Methods

2.1.Fish samples

One hundred and twenty fish samples were freshly collected aseptically from a private Nile tilapia (*O. niloticus*) aquaculture around El Salam Canal, at Ismailia governorate with variable size transported in a cleaning bag in an ice box with cooled ice bags to the laboratory and processed as soon as possible.

2.2.Bacterial examination

Swabs from the ascites, kidney, liver, intestine and gill were streaked on brain heart infusion agar (BHIA, Oxoid), Trypticase soya agar (TSA, Difco) and blood agar (SBA, Difco) containing 5 % sheep blood and incubated at 26 ± 1 ˚ C for 48-72 h **(Garrity, 2001; Austin and Austin, 2007).** A single colony from each plate was examined by gram reaction, oxidase, catalase, hemolysis, and other conventional biochemical tests. Following the criteria presented by those described in Bergey's Manual of Determinative Bacteriology **(Garrity, 2001),** pure isolates were identified and confirmed using the API 20 STREP System (Biomerieux) according to the manufacturer's protocol.

2.3.Pathological assessments

Necropsy was performed for moribund for freshly dead and sacrificed fish. Following the necropsy, tissue specimens were collected from the gills, liver, spleen, muscle, heart and intestine. Tissue specimens were rapidly fixed in 10% neutral buffered formalin solution for at least 24 hrs. The routine paraffin embedding technique processed the fixed specimens. Followed by prepared and stained with hematoxylin and eosin, according to **Bancroft and Gamble (2002).**

3. Results

3.1.Clinical signs and lesions of naturally infected fish

Clinical signs of lethargy, eye opacity, exophthalmia, petechial hemorrhages on the body surface, particularly around the mouth, gill, and fins bases and ulceration on the body surface,

especially at the caudal peduncle were seen. Internally, the fish had ascites, the abdominal cavity filled with dark exudates, and the spleen, kidney, and liver hypertrophy. These clinical signs and lesions were more apparent among infected tilapia.

3.2.Bacteriological examination

Examination of gram stained which showed gram positive cocci (short and long chains). The colonies on blood agar were small with beta hemolysis (**Table 1**) by using API 20 STREP System with bacterial isolates from diseased tilapia, *S. agalactiae* was identified. The isolation rate was 35 positive fish with *S. agalactiae* from 120 fish (29.16%), and the rate of isolation were 25%, 26.66 %, 12%, 18.33 % and 15% from ascites, kidney, liver, intestine and gill respectively as mentioned in **Table 2**. Also, other bacteria were isolated but in low recovery rate than *S. agalactiae*, which were *Ps. aeruginosa*, *Ps. fluorescens Rhizobium radiobacter*, *Enterobacter cloacae*, and *Salmonella* spp with recovery rates of 22.21%, 20.7%, 14.83 %, 10 % and 3.1%, respectively.

3.3.Histopathological Examination

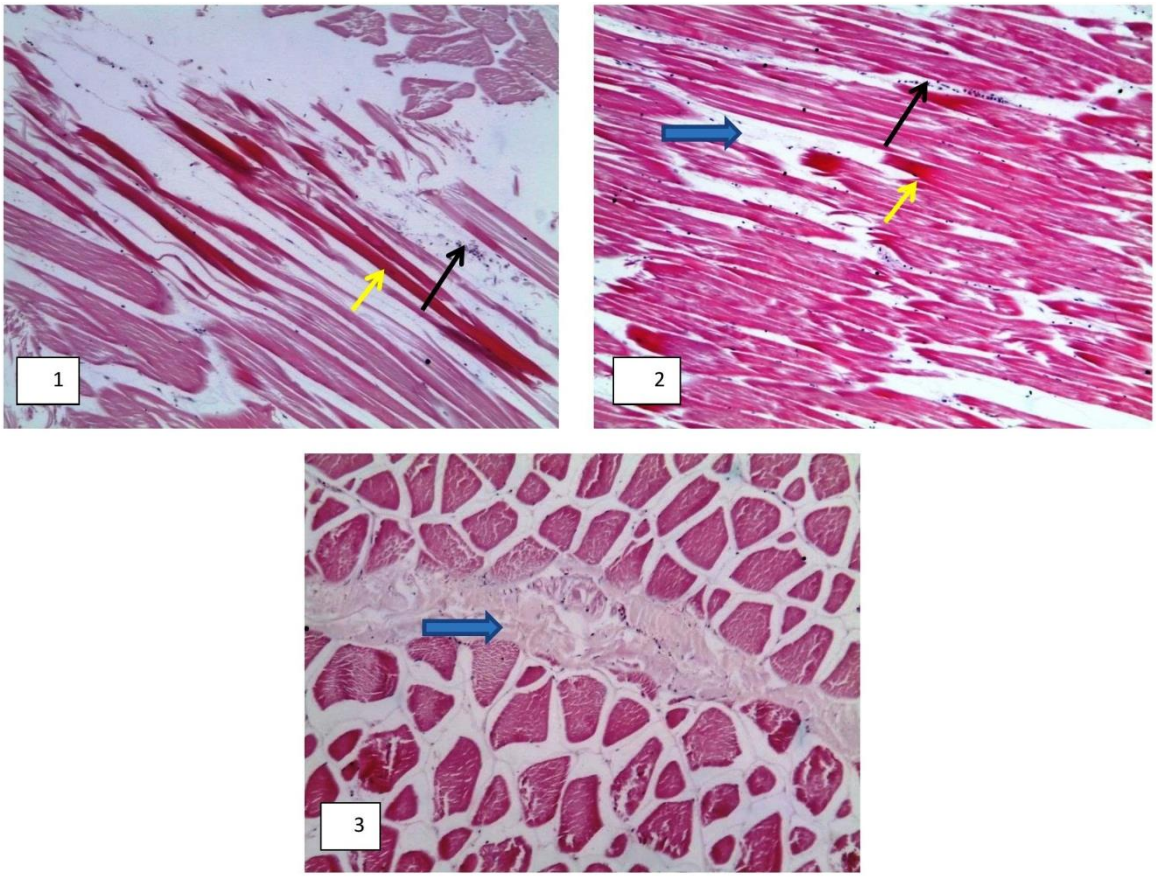
The main histopathological lesions detected from infected fish were severe hyperemia of gill filaments and gill arch, epithelial desquamation of the gill filament, and epithelial hyperplasia at the base, epithelial lifting of the gill filament and shortening of the gill filament as shown in **Figures 1**, **2**, and **3**. The intestine of fish shows chronic enteritis and atrophied villi as shown in **Figure 4**, while liver shows chronic enteritis and atrophied villi while liver shows chronic enteritis and atrophied villi while liver shows fatty change, infiltration of chronic inflammatory cells, and hydropic degeneration of the liver (**Figures 5**, **6**, and **7**). The spleen of fish shows hemosiderosis, edema and depletion of white pulp was clearly appeared in **Figures 8** and **9**. Also, muscle necrosis was detected, and infiltration of inflammatory cells as shown in **Figures 10**, **11**, and **12**. Severs hemorrhage and myocardiolysis of heart was detected as in **Figures 13** and **14**.

Table 1. Biochemical characteristics of *S. agalactiae* isolated from naturally infected hybrid tilapia (*Oreochromis* spp).

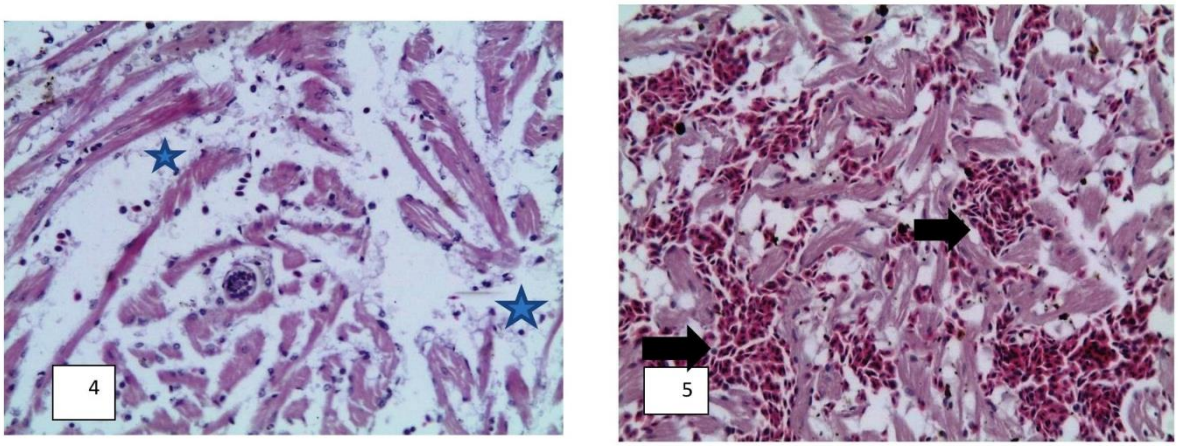
Biochemical test	Results
Gram-stain	G+ve Cocci in pair or short chain
Motility	-
Oxidase	-
Catalase	-
Blood hemolysis	Beta hemolysis
Urease	-
Esculin hydrolysis	-
Fermentation of:	+
Glucose	-
Raffinose	+
Sucrose	-
Lactose	-
Arabinose	-
Mannitol	-

Table 2. Rate of isolation according to fish sites

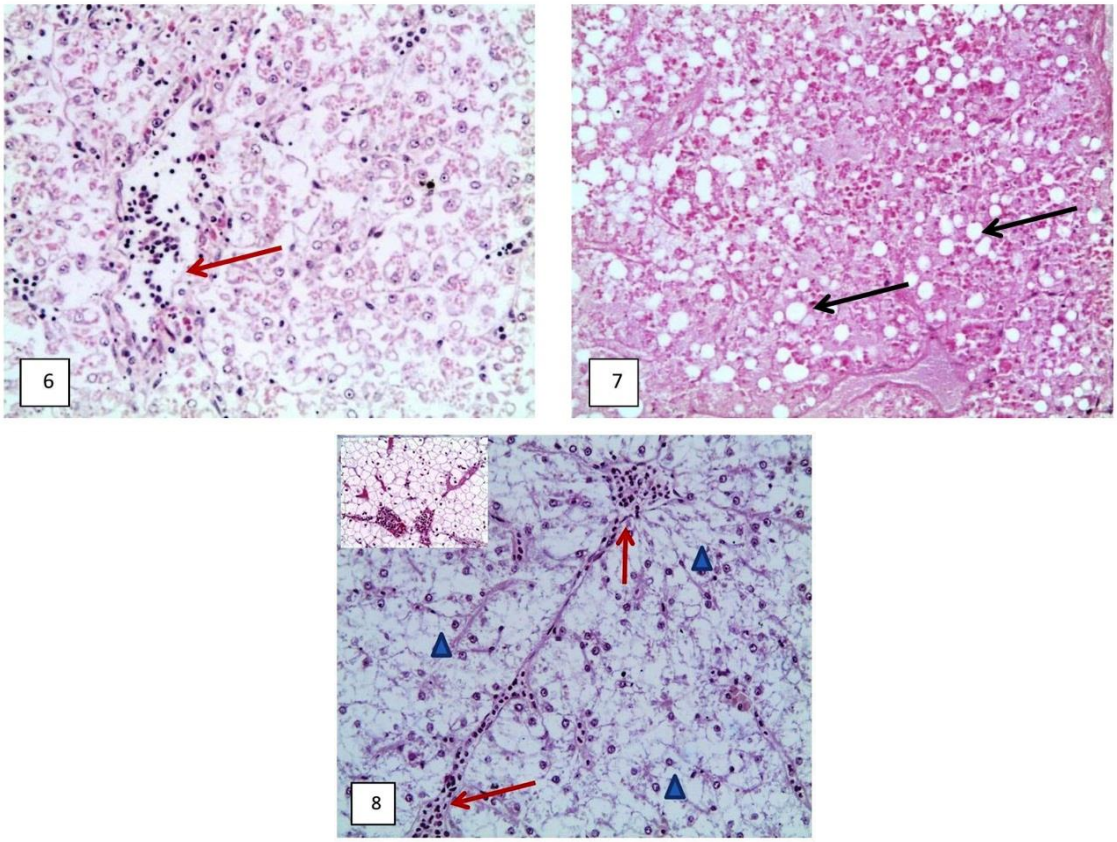
Site of isolation	Rate of isolation	%
Ascites	30/120	25
kidney	32/120	26.66
Liver	10/120	12
intestine	22/120	18.33
Gill	18/120	15



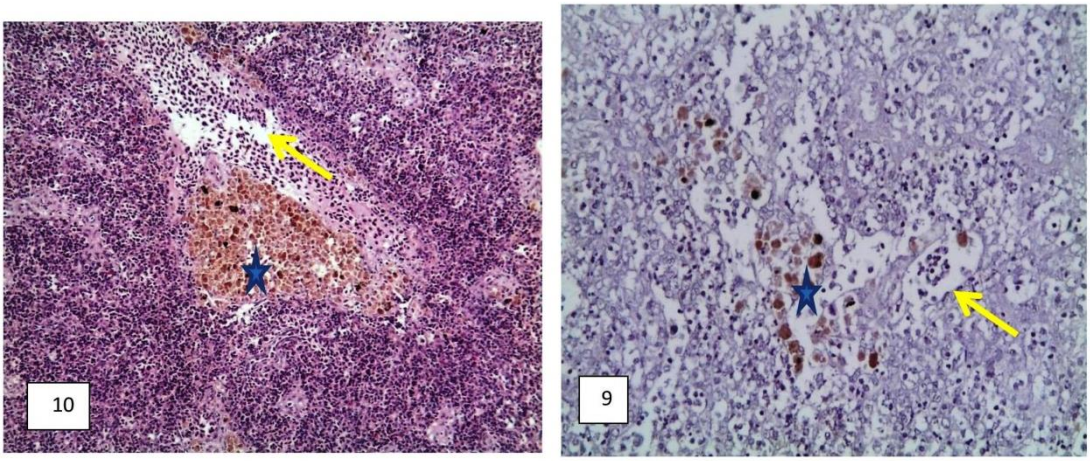
Figures 1, **2**, and **3**. Muscle of fish: showing myolysis, (blue arrow), necrosis (yellow arrow) and infiltration of inflammatory cells (black arrow). H&E stain, X20.



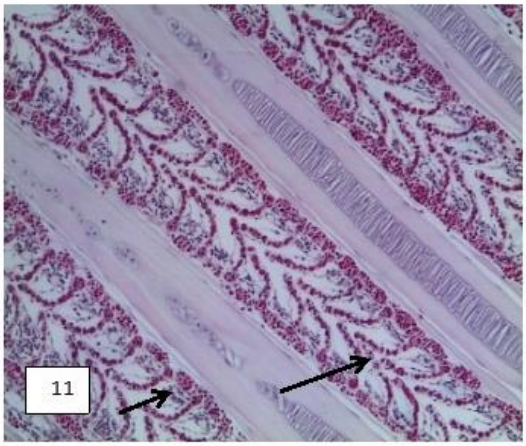
Figures 4 and **5**. Heart of fish: showing myocardiolysis (star shape), sever hemorrhage (black arrow) and infiltration of chronic inflammatory cells H&E stain, X20.

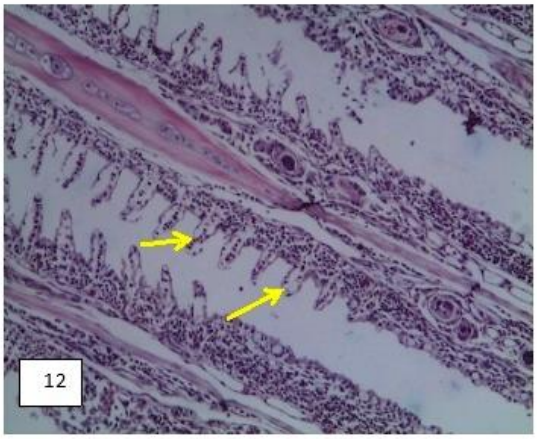


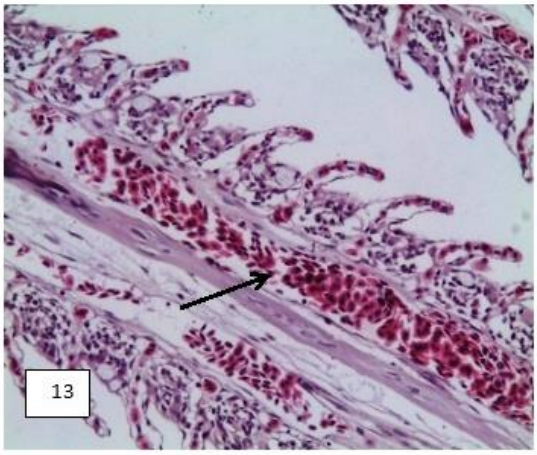
Figures 6, **7**, and **8**. Liver of fish: showing fatty change (black arrow), infiltration of chronic inflammatory cells (red arrow) and hydropic degeneration of liver (triangle). H&E stain, X10and X20.



Figures 9 and **10**. spleen of fish: showing hemosiderosis (star), edema (yellow arrow) and depletion of white pulp. H&E stain, X20.







Figures 11, **12**, and **13**. Gills of fish: showing sever hyperemia of gill filaments and gill arch (black arrow), epithelial desquamation of the gill filament, epithelial hyperplasia at the base of the gill filament, sever hyperemia, epithelial lifting of the gill filament and shortening of the gill filament (yellow arrow). H&E, X10.

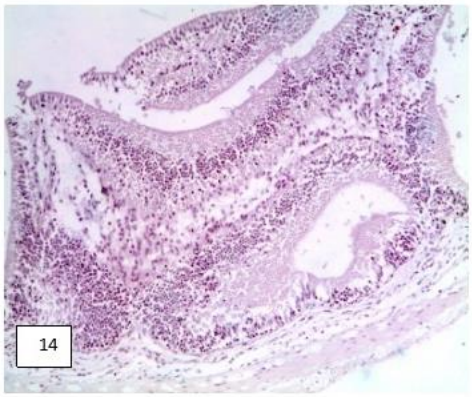


Figure 14. Intestine of fish: showing chronic enteritis and atrophied villi. H&E, X10.

Figure 15. Macroscopic lesion of dead fish with exophthalmia

Figure 16. Dead tilapia fish with skin erosion

4. Discussion

S. agalactiae is the most fish pathogen in many regions of the world. These bacteria are also zoonotic, and tilapia fish is considered a perfect host for this infection **(Amal and Zamri, 2011** and **Ma et al., 2016).** Mortalities were observed in the summer season among Tilapia fish in a private farm at Ismailia governorate. Fish were exposed to environmental fluctuationsdue to global warming and climate change as sudden rise in water temperature up to > 29.8 ˚C with poorly flushed water that leads to lowering levels of dissolved oxygen and accumulating high levels of ammonia. These factors seem to contribute to the rapid spread of *Streptococcus* infections **(Eldar et al., 1994; Chang and Plumb, 1996).** All diseased tilapias collected from the study area showed typical clinical signs of streptococcosis as; lethargy, exophthalmia, petechial hemorrhages on the operculum, body peduncle, ascites, intestine, and abdominal cavity filled with bloody exudates and hypertrophy of spleen and kidney. These clinical signs go hand to hand with observations previously reported by **Badran (1994)** in Egyptian fish; *O. niloticus*. **Austin and Austin (2007)** noted that S*treptococcus* strains were isolated from different geographical areas depending on the water environment. Also, many investigators, as **Eldar et al. (1999)** and **Badran (1994)** found that the fish pathogenic Streptococci have been linked with *S. faecalis*, *S. faecium*, *S. equisimilis*, *S. agalactia*, *S. dysgalactia*, *S. pyogenes*, *S. milleri*, *S. iniae*, *S. lactis*, and *Lactococcus garvieae* in many parts of the world. **Miyazaki *et al* (1989)** mentioned that *β*- and *γ*-hemolytic strains of *Streptococcus* caused severe outbreaks. **Chang and Plumb (1996)** found that the incidence of *Streptococcus* infection increases with increased salinity at a temperature of 25 to 30 ˚C, which is the same study area condition. The identification in the laboratory is based on colony characteristics, hemolytic properties, sugar fermentation and other biochemical reactions. The cell wall-associated antigens are designated A-H and K-V are the basis of the Lancefield groups **(Iregui et al., 2016).** *S. agalactiae* contains the cAMP factor, a pore-forming toxin first identified in this bacterium **(Jin et al., 2018).** The biochemical characteristics of all isolates with streptococcosis showed non-motile, *β*-hemolytic, catalase and oxidase negative, fermentative positive. The characteristics in the confirmation test using API 20 STREP which go hand to hand with the result of **Suhermanto et al., (2019).** The bacteriological results in this study were nearly the same obtained by **Donia et al., (2018)** who isolated *S. agalactiae* and *S.iniae* with recovery rate of 32.9%. *S. agalactiae* is supposed to acquired additional mechanisms to make invasions of tilapia and successfully multiply in tilapia tissues **(Chen et al., 2007).** A range of histopathology changes was observed from the moribund tilapia infected with *S. agalactiae*. The histological changes included muscle necrosis, myocardiolysis, hydropic degeneration and fatty change of liver following **Asencios et al** (**2016)** they recorded myonecrosis, endocarditis, mmyocarditis, and pericarditis. Histiocytosis and increased melanomacrophage centers were noted in the spleen, while we recorded hemosiderosis of the spleen and edema. The liver presented separation of hepatocytes, perivascular inflammation, necrosis and vacuolation. Mononuclear and bacterial infiltration was seen in most of the sampled organs. Most research recorded that the greatest histological changes were meningoencephalitis or meningitis, which was observed in almost 80% of the diseased fish with streptococcosis **(Francis et al., 2020).**

5. Conclusion

Streptococcosis caused severe economic losses in tilapia aquaculture in Egypt in addition to its zoonotic effect. Consequently, many studies are needed to overcome this problem.

Conflict of interest: The authors report that they have no conflict of interest.

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