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Impacts of streptococcal infection on Nile tilapia *(Oreochromis niloticus)* in Egyptian farms with special reference to diagnosis and prevention

Amira El-daim*, Eman A. Abdel Gawad, Amel M. El Asely, Hiam Elabd, Aya F. Matter, Hadeer Youssuf, Adel A. Shaheen, Amany A. Abbass

Department of Aquatic Animals Medicine, Faculty of Veterinary Medicine, Benha University, Egypt

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ABSTRACT

Streptococcosis is a serious bacterial disease responsible for significant economic losses in wild and farmed fishes It is the second disease threat to *O. niloticus*. Several species of *Streptococcus* have been reported worldwide as etiological agents of the disease. Also, other related bacterial species such as *Lactococcus garvieae, Vagococcus salmoninarum,* and *Enterococcous faecalis* with varied degrees of pathogenicity have been implicated in streptococcal infection. Streptococcosisis characterized by hemorrhagic septicemia, pop eye, nervous manifestation, abnormal swimming behavior, and high mortalities. Control of Streptococcosis is principally achieved by implementing some preventive measures and treatment with antibiotics and to a lesser extent vaccination. Probiotics and immunostimulants can be used to enhance host immunity against the infection with some success. The majority of streptococcal species exhibited multiple antimicrobial resistance; thus, vaccination appears to be the most effective method of controlling in aquaculture. This present review summarizes some aspects of Streptococcosis such as history, epidemiology, diagnosis, and possible control measures in cultured *O. niloticus* and its status in Egypt

1. INTRODUCTION

Aquaculture intensification raises the risk of disease outbreaks, particularly bacterial infection, resulting in a high mortality rate and considerable economic losses (Shoemaker et al., 2017). Streptococcosis is one of the most bacterial diseases affecting both wild and cultured tilapia. Among the bacteria that typically cause infection are *Streptococcus sp.*, *Lactococcus garvieae*, *Enterococcus faecalis*, and *Vagococcus salmoninarum* (Anshary et al., 2014; Hanol-Bektaş et al. 2017).

Streptococcal control in fish farms is achieved by combining management techniques with oral antibiotic administration (Abu-Elala et al., 2019). The development of an efficient, environmentally safe, and affordable vaccine against streptococcus species is the key focus of disease control research at present. The reason why we used vaccine is emergence of antimicrobial resistance and jeopardizes public health (Zhang et al., 2020). We have compiled information on the epidemiology, and diagnosis of the common streptococcus species in fish farms worldwide, including Egyptian Nile tilapia farms.

2. HISTORY AND TAXONOMY

Streptococcal infection was first detected in cultured rainbow trout, *Oncorhynchus mykiss* (Hoshina et al., 1958). Streptococcus is non-spore-forming, Gram-positive cocci (0.5–2.0 μm diameter), facultative anaerobic bacteria which form groups in pairs and chains. The criteria for early classification were phenotypic characteristics, hemolytic reactions, Gramstain, sugar fermentation, growth temperatures, and tolerance to sodium chloride or heat. Based on the hemolysis on blood agar, Streptococcus genus is categorized into three groups: α-hemolytic (Foo et al., 1985), β-hemolytic (Minami et al., 1979), and γ-hemolytic (Rasheed and Plumb, 1984).

Lancefield described the serological categorization of beta-hemolytic bacteria based on group-specific polysaccharides (Lancefield, 1933). The Lancefield groups are based on these cell wall-associated antigens, which are classified as A-H and K-V (Iregui et al., 2016). The majority of pathogenic streptococci have a carbohydrate sheath that is serologically reactive and antigenically unique to each species of streptococcus.

Sherman established a four-category system (pyogenic, viridans, lactic, and enterococci) for streptococci based on: hemolytic reaction, Lancefield serology, and phenotypic testing (Sherman, 1937). One of Sherman's four divisions was the pyogenic division, which included beta-hemolytic organisms with group antigens (A, B, C, and G). The viridans group included non-hemolytic streptococcal species that could not withstand salt or high pH and did not grow at 10°C.

The family streptococcaceae was recently divided into three genera i.e., genus *Streptococcus* (pyogenic and viridans streptococci); genus *Lactococcus* (Lancefield Group N streptococci) (Schleifer et al., 1985); and genus Enterococcus (Lancefield Group D *streptococci*) (Sherman, 1937; Schleifer and Kilpper-Balz 1984) after systematic bacteriology Bergey's Manual publication (Hardie, 1986).

* Correspondence to: amira.mohamed@fvtm.bu.edu.eg

3. COMMON STREPTOCOCCUS SPECIES

3.1. Streptococcus iniae

Pier and Madin (1976) initially isolated *Streptococcus iniae* from external skin lesions in captive Amazon River fish, *Inia geoffrensis*. Thereafter, it was isolated from a variety of marine, estuarine, and freshwater sources (Daneshamouz et al., 2020; Legario et al., 2020; Piamsomboon et al., 2020; Pierezan et al., 2020). A bacterial infection outbreak in Israel in 1986 was initially attributed to a novel species called *S. shiloi* (Eldar et al., 1994). The nomenclature was changed to *S. iniae* after it was discovered to be similar to *S. iniae* strain which isolated from a freshwater dolphin in 1976 (Eldar et al., 1994, 1995). *S. iniae* is divided into two serotypes; serotype-I strain was first defined by Pier and Madin (1976) and serotype II was originally isolated from the golf ball disease case in a dolphin (Pier et al., 1978), which differed in arginine dihydrolase activity where serotype-I give a positive result (Bachrach et al., 2001).

Table (1) showed the most common and harmful Streptococcus species infecting Nile tilapia in Egypt. Due to the detrimental economic effect on Nile tilapia cultivation in numerous regions in Egypt (Radwan, 2003), from which *S. iniae* showed to be a major problem (Younes et al., 2019). Streptococcosis has also been reported from Egypt's Lake El-Ibrahimia (Ebtsam, 2002).

Table 1 List of the most common serious streptococcus species affecting Nile tilapia from different localities in Egypt.

Identified species	Location	References
Streptococcus iniae	Fayoum Governorate	Radwan (2003)
	Kafr El-Sheikh Governorate	Mahfouz et al. (2016), Saleh et al. *(*2019), Younes et al. (2019)
	Beheira Governorate	Ebtsam (2002)
Streptococcus agalactiae	Kafr El-sheikh governorate	Abu-Elala et al. (2020), Ghetas et al. (2021)
	Aswan Governorate	Hamouda et al. (2019)
Streptococcus dysgalactiae subsp. *Dysgalactiae*	Giza, Bani-sweif and Ismailia Governorates	Osman et al. (2017)
	Ismailia Governorate	Fawzy et al. (2014)

3.2. Streptococcus agalactiae

Worldwide *S. agalactiae* (synonym *S.difficile*) causes a major pathogenic species responsible for high mortality in cultured fish (Bowater et al., 2012; Lusiastuti et al., 2014; Barato et al., 2016; Al-Harbi, 2016). *S. agalactiae* serotype Ia has been isolated from cultured Nile tilapia in China (Zhang et al., 2013) and Taiwan (Sudpraseart et al., 2020).

Biochemical and hemolytic identifications have distinguished *S. agalactiae* under two biotypes i.e., biotype-I (β-hemolytic) in Asian countries and biotype-II (γ-hemolytic) in Indonesia and China (Soto et al., 2015). Originally believed to be a non-hemolytic variety of *S. agalactiae*, the etiology has been reclassified as a non-hemolytic Group B Streptococcus (Sun et al., 2016). The first case of *S. agalactiae in* captive golden shiners, *Notemigonus crysoleucas* was reported in 1966 by Robinson and Meyer (1966). The capsular polysaccharide (CPS), which has ten different serotypes is used for molecular serotyping of *S. agalactiae* (Poyart et al., 2007), among which, only five serotypes i.e., Ia, Ib, II, III, and IV known for aquatic species (Li et al., 2013).

3.3. Streptococcus dysgalactiae

The first incidence of α-hemolytic *S. dysgalactiae* (GCSD) was observed in 2002 among Japanese cultured yellowtail, *Seriola quinqueradiata,* and amberjack, *Seriola dumerili* (Nomoto et al., 2004).

The nomenclature *S. dysgalactiae* was initially termed by Diernhofer (1932) but accepted formally in 1983 (Garvie et al., 1983). Five streptococci ecovars i.e., GLS, *S. dysgalactiae* bovine, *S. equisimilis* human, *S. equisimilis* animal, and human GGS ecovars using serotyping and biotyping (Devriese, 1991). However, *S. dysgalactiae*, according to phenotypic and genotypic characteristics was divided into four varieties: GCS α-hemolytic *S. dysgalactiae subsp. dysgalactiae* (GCSD), GGS β-hemolytic *S. dysgalactiae subsp. equisimilis*, GCS β-hemolytic *S. dysgalactiae* subsp. equisimilis, and GLS β-hemolytic *S. dysgalactiae subsp. equisimilis* (Vieira et al., 1998).Human alpha-hemolytic streptococci with Lancefield Group C or G antigen are *S. dysgalactiae subsp. equisimilis*. Later, infections were found worldwide (Abdelsalam et al., 2013; Zhao et al., 2020). In Egypt, *S. dysagalactiae subsp. dysgalactiae* was recorded from cultured and wild tilapia (Osman et al., 2017), and experimentally fish (Fawzy et al., 2014).

Raw fish handling patients with ascending cellulitis and upper limb bacteremia have been observed to develop GCSD (Koh et al., 2020).

3.4. Other related bacteria causing streptococcosis

Lactococcus garvieae, previously named *E. seriolicida,* infects both fresh and marine water fishes, resulting in acute hemorrhagic septicemia with higher mortality (Abu-Elala et al., 2018). *L. garvieae* was isolated from naturally and experimentally infected Nile tilapia in Egypt (Osman et al., 2017; Abu-Elala et al., 2020).

4. EPIDEMIOLOGY OF THE DISEASE

Streptococcus species can be found in water and mud throughout the year in the aquatic environment (Kitao et al., 1979). The environmental factors such as high-water temperature (more than 28°C), diminished oxygen, high pH, ammonia, and high earthen ponds' organic content contributed to the emergence of streptococcal infection in aquaculture (Abu-Elala et al., 2019). Warm water streptococcosis is primarily caused by *S. iniae*, *L. garvieae, S. agalactiae,* and *S. parauberis* at temperatures beyond 15°C. While cold water streptococcosis is caused by *V. salmoninarum* and *L. piscium* at temperatures below 15°C (Domenech et al., 1996).

Streptococcus spp. exist as a complex bacterial group that can infect a wide range of hosts, including but not limited to rainbow trout, gray mullet, *Mugil cephalus*, and Nile tilapia (Liu et al., 2016; Ismail et al., 2017; Halimi et al., 2019).

Many scientific studies have shown that Streptococcus spp. can spread horizontally in tilapia (Hossain et al., 2012; Amal et al., 2013). *S.agalactiae* and *S. iniae* were reported from red tilapia collected from apparently healthy broodstock, implying that vertical transmission of streptococcus pathogen to progeny may potentially be possible (Pradeep et al., 2016).

5. PATHOGENESIS

Capsular polysaccharide (CPS) is a pathogenic component found in many streptococcal serotypes, related to the evasion of host immune defense mechanisms via sialic acid

(Xia et al., 2015). CPS inhibits phagocytosis by host cells by suppressing the collection of the complement factor C3b. The Christie–Atkins–Munch–Peterson factor, also known as co-hemolysin, that promotes pathogenesis by creating discrete pores on host membranes and attaching to GPI-anchored proteins (Lang and Palmer, 2003).
Hyaluronidase enzyme secreted by Group B Streptococcus can break the glycosidic bond between N-acetyl-D-glucosamine and D-glucuronic acid destroys the host's connective and nervous tissues (Mello et al., 2002). The phosphotransferase system (PTS) regulates the pathogenicity of GBS bacteria by phosphorylating sugar substrates (Sutcliffe et al., 2008). Cellobiose-PTS (cel-PTS) deficient bacteria had lower colonization capacity (McAllister et al., 2012). Furthermore, Ma et al. (2017) recorded that the quorum sensing signaling molecule was defective in streptococcus strains lacking the luxS gene.

6. DIAGNOSTIC METHODS

6.1. Presumptive diagnosis

6.1.1. Clinical signs

Several studies have reported that fish may showed lethargy, erratic swimming, skin depigmentation, uni- or bilateral exophthalmia, ocular opacity, hemorrhages on all body surfaces, and skeletal deformity irrespective of the etiological agents (Abdel Gawad et al., 2007; Mahfouz et al., 2016; Saleh et al.,2019; Younes et al., 2019). In addition, ascitic serosanguineous fluid, hemorrhage in the liver, muscle, and intestine, Splenomegaly and meningitis have also been reported (Sudpraseart et al., 2020).
In the course of our ongoing research, cultured Nile tilapia obtained from several locations in Egypt had the same clinical signs and lesions (Fig. 1, Unpublished data).

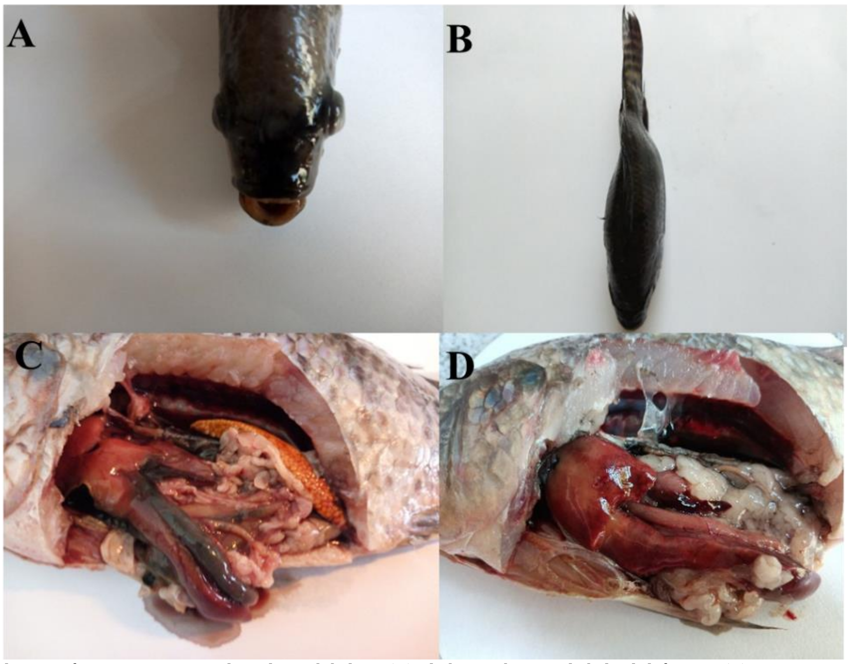

Fig. 1 Nile tilapia showed signs of streptococcosis unilateral exophthalmia (A), darkness skin and skeletal deformity (B), congestion and enlargement in all internal organs (C, D) (Unpublished data).

6.1.2. Isolation and Identification

The major Streptococcus species in Egypt are *S. agalactiae*, *S. iniae,* and *S. dysgalactiae* subsp. *dysgalactiae* (Table 1). *S. iniae* is a Gram-positive encapsulated coccus grows in long chains. The organism fills the bottom of the broth tube with a rough, white, granular substance. Small white colonies with an entire opaque border are produced on blood agar and a moderate zone of β-hemolysis surrounds colonies, with a diffuse outer zone of α-hemolysis (Rahmatullah et al., 2017). It is a facultative anaerobe and produces acid, grow well at 37°C and it did not grow well at 4, 12, and 55°C. Additionally, the *S. iniae* isolates were also found resistant to oxolinic acid (Legario et al., 2020). Heckman and Soto (2021) reported that *S. iniae* can form a biofilm that unable microorganisms to resist the widely used disinfectant.
S. agalactiae, a type of Gram-positive cocci, exhibits a variety of traits. The colonies of non-hemolytic and hemolytic cells ranged between 0.591-0.748 mm and 0.787–1.231 mm in diameter, respectively. Both bacteria grew well at 28 and 37 °C, were catalase and oxidase negative, and weren't able to grow on sulfide indole motility medium but did on 6.5% NaCl and 40% bile salt media (Suhermanto et al., 2019).

On TSA agar, colonies of *S. agalactiae* were elevated and glossy, with a diameter of 1.5-2.0 mm. Biochemically, oxidase-negative, catalase-negative, and lysine decarboxylase-positive non-motile bacteria were found on blood agar in small white colonies, transparent or pigmented, slightly convex (Hamouda et al., 2019). The pure strain of *S. dysgalactiae* on Colombia blood agar demonstrated the existence of pinpoint white colonies (0.5 mm).

6.1.3. Biochemical and Serological characterization

Other methods for detecting streptococcus bacteria, such as the RAPID, VITEX, API 20 STREP, and Rapid Strep 32 systems can also be applied (Facklam et al., 2005; Lau et al., 2006).Serological tests, such as the enzyme-linked immunosorbent assay is also used for identification purpose (Liu et al., 2014). For quick detection and identification of *S. iniae*, a monoclonal antibody-based indirect fluorescent antibody method was also used (Klesius et al., 2006; Souter et al., 2021).

6.2. Confirmative diagnosis

Molecular diagnosis using the PCR based on the selective amplification of the 16S rRNA gene is used for confirmative identification of streptococcus species (Kayansamruaj et al., 2014; Mishra et al., 2017; Yoon et al., 2017; Deng et al., 2019). The loop-mediated isothermal amplification method (LAMP) is employed to quickly discover the *fbsB* gene in *S. agalactiae* (Wang et al., 2012). Multi Locus Sequence Typing was also utilized for identification purposes (Lusiastuti et al., 2013; Heckman et al., 2020). Identification of streptococcus species is also done by the MALDI-TOF-MS technique (Kim et al., 2017; Souter et al., 2021).

7. CONTROL MEASURES

Many studies have shown that improving conditions e.g., reducing fish stocking rates and removing moribund fishes are effective strategies for disease prevention (Shoemaker et al., 2000; 2001). Other characteristics that can reduce the infection rate are improving water quality (Mishra et al., 2018).

7.1. Chemotherapy

Oxytetracycline is found to be effective for controlling *S. iniae* at 75 and 100 mg (Faria et al., 2013). Dietary application of 1.5 g erythromycin and 80 mg amoxicillin are effective against Streptococcosis in tilapia (Darwish and Hobbs, 2005). Florfenicol was added in tilapia diet to control *S. iniae* (Gabriel et al., 2014) and *S. agalactiae* (Oliveira et al., 2018).

Although streptococcal infection responds to a little of antibiotic treatment, it cannot be controlled completely with antibiotics because the withdrawal period is longer than the time it takes to develop antibiotic resistance (Darwish and Hobbs, 2005).

7.2. Prevention

7.2.1. Probiotics and immunostimulants

Probiotics and immune-stimulants are valuable tools to prevent many bacterial infections in aquaculture (Gültepe et al., 2014). β-glucans have a protective effect against Streptococcus sp. infection (Matsuyama et al., 1992). Probiotics and immune-stimulant were shown to be effective in preventing *S. iniae* infection in rainbow trout (Po-Tsang et al., 2020). A Study in Egypt investigated the role of probiotics as immune-stimulants and growth promoters (El-Kady et al., 2022).

7.2.2. Vaccination

Vaccine development and application in aquaculture is now evolving at a rapid pace to achieve effective disease prevention. Injection (Abu-Elala et al., 2019), immersion (dip or bath) (Kole et al., 2021), and oral vaccine (Yaoa et al., 2019)are various administration methods that have been tried in fish, depending on the type of vaccination and the fish's developmental stage (Munang'andu et al., 2016).

The injection approach for vaccination delivery induces a more robust immunological response when compared to spray and immersion. Oral immunization has a lesser efficacy and a shorter protection time (Ismail et al., 2016). So, immune-stimulants and probiotics are required until the fish can be vaccinated (Kole et al., 2021).

Firdaus-Nawi et al. (2012) developed a feed-based adjuvant vaccine that, four weeks after initial immunization, gave red tilapia remarkable protective effectiveness against *S. agalactiae* infection. Furthermore, feeding tilapia with a formalin-killed *S. agalactiae* vaccination increased immunological response and survival rate (Ismail et al., 2017). *S. agalactiae* that had been formalin-inactivated were used to immunize fish, which significantly increased the phagocytic activities and IgM antibodies, guarding against virulent strains (Wang et al., 2018; 2020).

The α-enolase subunit vaccine, which is a virulence gene in GCSD, exhibited a significant level of protection (Thu Nguyen et al., 2020).

The development of a streptococcal vaccine received little attention in Egypt. According to Abu-Elala et al. (2019), immunization of Nile tilapia with *S. agalactiae* and *S. iniae* in Montanide adjuvant resulted in Relative Percent Survival (RPS) values ranging from 56% to 95%. The key characteristics of any ideal vaccine are its safety, durability, and capacity to elicit a robust immune response with a minimal number of doses (Beverley et al., 2002).

The use of a nanocarrier-based delivery method can help deliver tailored immunogen to antigen-presenting cells, boost vaccine durability, and prevent vaccines from premature degradation (Means et al., 2003). Nanoscale (20–100 nm) vaccination particles known as nano-immuno stimulators can increase vaccine effectiveness more than bulk molecules (Irvine et al., 2013). Compared to bigger NPs, smaller NPs are more effective at altering cell signaling mechanisms (Lim et al., 2012). Inorganic NPs (iron and silica) (Pusic et al., 2013) and polymeric NPs (chitosan, PLGA) (Prego et al., 2010) are some of the nano-immuno stimulators that have been used in vaccine delivery. Oral vaccination of Channel catfish *(Ictalurus punctatus*) with *S. iniae* in chitosan adjuvant resulted in a higher RPS (Wang et al., 2018).

7.2.3. An alternative control method

Bacteriophages represent a new class of antimicrobials. Intraperitoneal injection with bacteriophage had no adverse effects but increase survival rates in tilapia (Luo et al., 2018). The Ilongot-Eǵongot crude extract and synthesized gold nanoparticles inhibit quorum sensing and can reduce bacterial virulenceand resistance (Fernando et al., 2020). Ozone nanobubble technology has been reported to involve injecting nanobubbles made of ozone, and it reduces the concentration of pathogens like *S. agalactiae* (Jhunkeaw et al., 2021).

8. CONCLUSION

Streptococcosis has become a major issue in Egypt as a result of direct effects and high mortalities among farmed Nile tilapia in several geographical areas. The most prevalent streptococcus species found in Egypt are *S. agalactiae*,*S. iniae*, and *S. dysgalactiae* subsp. *dysgalactiae*. To provide an extensive defense against streptococcus species, it is necessary to develop a vaccine that efficiently manages outbreaks. The use of nanotechnology in immunizations is a novel development that will help treat several bacterial illnesses, including Streptococcosis.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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