

1.3.3 Streptococcosis in Fish

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A. Name of Disease and Etiological Agent

Streptococcosis is primarily caused by the Gram-positive bacteria *Streptococcus iniae*, *S. agalactiae*, *S. dysgalactiae*, and *S. ictaluri*. *Streptococcus iniae* emerged as a major pathogen of farmed and wild fishes in the 1990-2000's and has zoonotic potential (Agnew and Barnes 2007). *Streptococcus ictaluri* emerged in the catfish industry in the USA in the late 2000's and is phylogenetically most similar to *S. iniae* based on 16S rRNA sequencing with divergence ranging from 2-2.3 % (Shewmaker et al. 2007). *Streptococcus ictaluri* is not commonly reported and appears unique to channel catfish with low virulence (Camus et al. 2008; Pasnik et al. 2009). *Streptococcus iniae* and *S. ictaluri* are non-groupable with the Lancefield method (Shoemaker et al. 2017a; Shewmaker et al. 2007). Non-hemolytic Lancefield Group B *S. agalactiae* capsular type Ib is most commonly reported from freshwater and brackish water fishes especially in North, Central and South America (Plumb et al. 1974; Soto et al. 2015; Shoemaker et al. 2017a; Shoemaker et al. 2017c). *Streptococcus agalactiae* capsular type Ia and III are common in Asia and the Middle East (Delannoy et al. 2013). In some areas, *S. agalactiae* Ia, Ib and III have been reported to co-occur. A capsular type III isolate with an antibiotic resistant phenotype has recently emerged in tilapia culture in Brazil (Chideroli et al. 2017). *Streptococcus dysgalactiae* is Lancefield Group C and was initially described from marine fish (Nomoto et al. 2006) but has since emerged in tilapia in Brazil (Netto et al. 2011). Several other *Streptococcus* spp. have been reported to cause disease in fish and shrimp (Romalde et al. 2008; Hasson et al. 2009); however, this chapter will focus on the species most often associated with disease in finfish (*S. iniae*, *S. agalactiae*, *S. dysgalactiae*, and *S. ictaluri*).

B. Known Geographical Range and Host Species of the Disease

1. Geographical Range

It is generally assumed that streptococcosis has a worldwide distribution, having been described in fishes from Europe, the Americas, the Middle East, throughout Asia and Australia (Agnew and Barnes 2007; Chou et al. 2014; Shoemaker et al. 2017a).

2. Host Species

Streptococcosis has been reported globally in wild and cultured fishes (Agnew and Barnes 2007; Delannoy et al. 2013; Osman et al. 2017). The pathogens impact fishes in fresh, brackish and marine waters, including aquarium fishes. Farmed tilapia (*Oreochromis* sp.) are greatly impacted worldwide (Shoemaker et al. 2017a).

C. Epizootiology

The epizootiology of streptococcosis is complex. Fish stress and unfavorable environmental conditions enhance the probability and severity of infection, especially higher temperatures ($\geq 30^{\circ}\text{C}$) in the case of *S. agalactiae*. External parasites, such as *Gyrodactylus* spp., also increase the susceptibility of fish to streptococcal disease (Xu et al. 2007).

The most likely mode of transmission is horizontally via water with fish (i.e., carriers) as a source of infection. Ingestion of infected material is likely a mode of oral transmission as it has been observed that healthy fish fed dead carrier fish became infected (Kim et al. 2007). Furthermore, healthy tilapia cannibalizing the eyes and internal organs of *S. iniae* infected fish displayed signs of disease and died (Shoemaker et al. 2000). Alternative routes of infection via nares, skin and gill are also probable. The bacteria may survive for extended periods in water and sediment (Nguyen et al. 2002). Vertical transmission of both *S. iniae* and *S. agalactiae* (Pradeep et al. 2016) has been reported in tilapia.

Streptococcus iniae, *S. agalactiae*, and *S. dysgalactiae* may present zoonotic concerns (Weinstein et al. 1997; Delannoy et al. 2013; Efstratiou et al. 1994; Nishiki et al. 2011). *Streptococcus iniae* was isolated from immunocompromised patients who handled live fish (Gauthier 2015). Comparative genomic analysis of *S. agalactiae* isolated from fish suggests that human strains of *S. agalactiae* are present in fish, frogs, and aquatic mammals, thus posing a potential risk for human disease (Delannoy et al. 2013; Liu et al. 2013).

D. Disease Signs

1. Behavioral Changes Associated with the Disease

Some of the first behavioral changes associated with disease are lethargy and loss of appetite. Central nervous system involvement often leads to tail-chasing, spiral swimming due to spinal curvature (Figure 1), and buccal paralysis in the case of *S. agalactiae* Ib infections.



Figure 1. Tilapia with spinal curvature characteristic of some streptococcal infections which can result in spiral swimming. Photographs taken from Shotts and Plumb (1994) with permission.

2. External Gross Signs

Externally, fish often exhibit a darkening of the skin in color; however, acutely infected fish may die due to septicemia with few clinical signs. Some affected fish may have numerous raised, hemorrhagic, inflamed areas on the skin including the operculum, around the mouth, at the bases of fins, and along the dorsolateral portions of the body (Figure 2). Unilateral or bilateral exophthalmia (pop eye) is common with or without hemorrhage in the eye (Figure 3), and the abdomen can be distended because of fluid accumulation and ventral reddening. Fecal casts or strings are also an indicator of streptococcal infection. Dead fish as well as survivors of recent infections may have jaw and caudal pustules (LaFrentz et al. 2016; Shoemaker et al. 2017b) (Figure 4).



Figure 2. Atlantic Menhaden with streptococcosis. Photographs taken from Shotts and Plumb (1994) with permission.

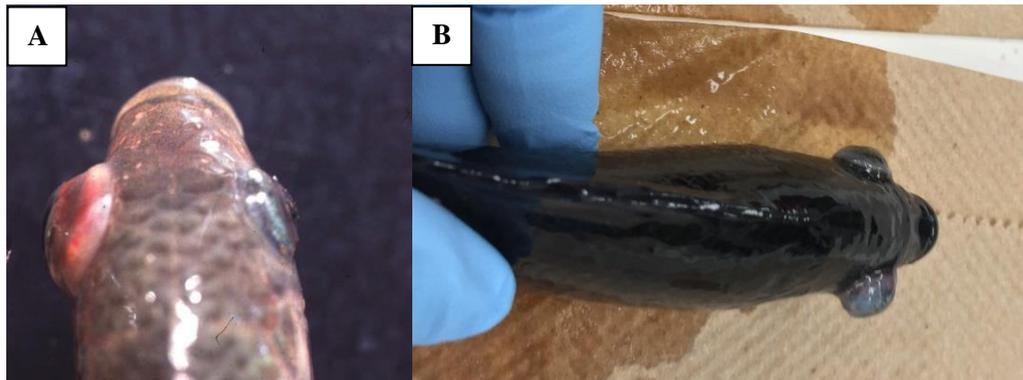


Figure 3. Unilateral and bilateral exophthalmia with hemorrhage in tilapia (*Oreochromis* spp.) with streptococcosis. Photo on right shows bilateral exophthalmia caused by *S. agalactiae*. Photograph taken from Shotts and Plumb (1994) with permission.

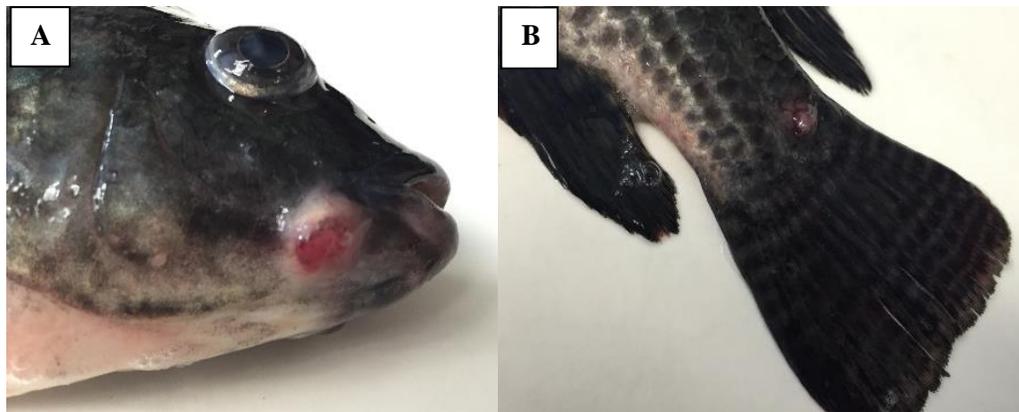


Figure 4. Nile tilapia surviving a *Streptococcus iniae* challenge (by intraperitoneal injection) showing jaw pustules (A) and pustules on the caudal peduncle (B). Tilapia challenged by intramuscular injection with *S. agalactiae* Ib exhibit similar lesions.

3. Internal Gross Signs

The peritoneal cavity may contain a straw to bloody colored fluid (Figures 5 and 6). The liver may appear pale and the spleen dark red. In the case of *S. agalactiae* infection, the spleen is also often enlarged. Posterior kidneys are not a major target organ for clinical pathology; however, the bacteria can be recovered from the organ upon culture. A hemorrhagic enteritis with bloody fluid present in the intestinal lumen may also be seen.

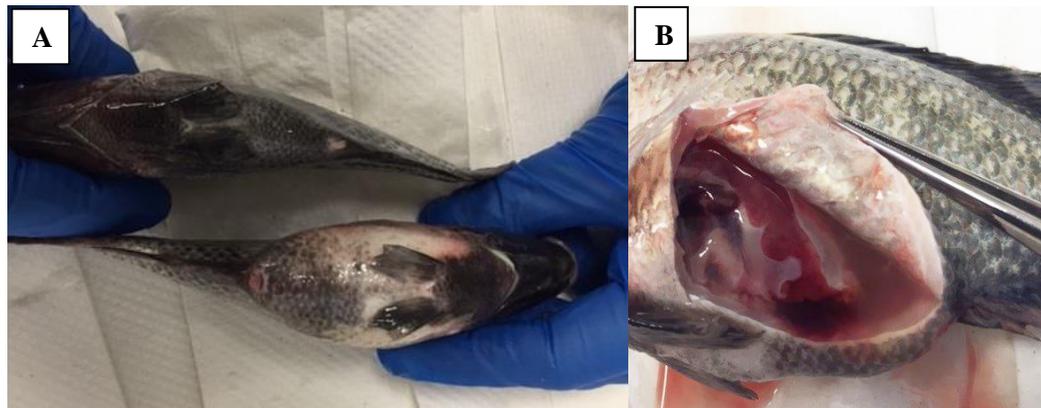


Figure 5. Nile tilapia infected with *Streptococcus agalactiae* Ib showing abdominal distention (A) and bloody fluid in the peritoneal cavity (B).



Figure 6. Hemorrhage from the vent of Atlantic menhaden with a streptococcosis. Photographs taken from Shotts and Plumb (1994) with permission.

4. Histopathological Changes

Histopathological findings vary depending on the pathogen and host species involved. The pathological changes were recently reviewed in Shoemaker et al. (2017a). Infections of the head and brain often produce a granulomatous encephalitis and meningitis. Infection of the eye results in granulomatous or lymphohistiocytic choroiditis. Tilapia infected with *Streptococcus* sp. also show polyserositis, granulomatous splenitis, ovaritis, granulomatous or lymphohistiocytic epicarditis, pericarditis and myocarditis.

E. Disease Diagnostic Procedures

Diagnosis is based on clinical signs consistent with bacteremia (although not pathognomonic) and the isolation and identification of the causative bacterium. Readers are referred to Section 1.1.1 General Procedures for Bacteriology. Primary isolation may be made from fish tissue on 5% sheep blood agar, tryptic soy agar, and/or brain heart infusion agar and incubated at 25 to 35°C for 24 to 48 hours. If isolating from marine species, salt may be added when preparing the sheep blood agar. The brain is often the best organ for bacterial isolation but other tissues such as the spleen, kidney, etc. are also suitable for culture. Columbia CNA (colistin and nalidixic acid) agar (Ellner et al. 1966) can be used to enhance recovery of streptococci by limiting growth of most Gram-negative bacteria.

1. Presumptive Diagnosis

See Section 1.1.2 Flow Chart for the Presumptive Identification of Selected Bacteria From Fishes. For Streptococci, organisms isolated in culture are Gram-positive, cocci in pairs or chains, non-motile, negative for cytochrome oxidase, and negative for catalase. The colonies are pinpoint to pinhead in size and convex (Figure 7). In liquid culture, such as tryptic soy or Todd-Hewitt, the broth may appear viscous. On sheep blood agar, colonies of Group B streptococci can be beta-hemolytic and/or non-hemolytic depending on capsular type. *Streptococcus iniae* may exhibit alpha, beta or gamma hemolysis (Chou et al. 2014). *Streptococcus dysgalactiae* is usually alpha hemolytic (Nomoto et al. 2006).



Figure 7. Beta-hemolytic *S. iniae* (A) and non-hemolytic *S. agalactiae* Ib (B) on sheep blood agar plate.

2. Confirmatory Diagnosis

Diagnosis is confirmed by the isolation of catalase-negative, Gram-positive cocci in chains. Biochemical tests including rapid test strips (e.g., API® 20 Strep strips) can be used to identify the bacteria to genus; however, phenotypic heterogeneity is noted (Chou et al. 2014; Shoemaker et al.

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2017c). Lancefield grouping can also be used to identify hemolytic or non-hemolytic group B *S. agalactiae* and group C *S. dysgalactiae*. Commercial kits are available (e.g., Streptex kit, Remel, Lenexa, KS).

Molecular methods should be used for definitive identification of the *Streptococcus* sp. by sequencing the 16S rRNA gene and/or using polymerase chain reaction (PCR) assays specific for streptococcal pathogens. *Streptococcus iniae* and *S. ictaluri* are non-groupable, and to the authors knowledge, no PCR assays have been developed for *S. ictaluri*. Thus, confirmatory diagnosis should be made through sequencing of the 16S rRNA gene. Molecular identification of *S. iniae* is possible using the method of Mata et al. (2004), in which PCR primers were designed to be specific to a portion of the lactate oxidase (*lctO*) gene of *S. iniae*, resulting in an 870 bp product. The primers are LOX-1F (5'-AAGGGGAAATCGCAAGTGCC-3') and LOX-2R (5'-ATATCTGATTGGGCCGTCTAA-3'). A PCR assay is also available for the identification of fish *S. dysgalactiae* (Nomoto et al. 2008). Primers (fish-dys-sodAF: 5'-ATGCGACTTATGTTGCCA-3'; fish-dys-sodA R:5'-GTGTATCTTGATTGCTG-3') were designed to be specific to a portion of the *sodA* gene and positive amplification results in a 350 bp product (Nomoto et al. 2008).

Upon identification of group B *S. agalactiae*, it is useful to determine the capsular type of the isolates, as capsular type Ib isolates are extremely virulent (injection of tilapia with 10-1000 colony forming units results in $\geq 50\%$ mortality). A multiplex PCR, adapted from Imperi et al. (2010), can be used to determine the capsular type of *S. agalactiae* and includes a set of primers (*cpsG*) diagnostic for *S. agalactiae*. Table 1 list the primer pairs for determining the capsular type of *S. agalactiae* from aquatic animals and fish. A capsular serotyping kit (Denka Seiken Co., Ltd., Japan) was also effective in determining the capsular type of isolates associated with fish (Shoemaker et al. 2017c).

Table 1. Primer pairs obtained from Imperi et al. (2010) used for molecular typing of Group B *Streptococcus agalactiae* from aquatic mammals and fish.

Primer Name	Sequence (5' ----> 3')
cpsL-F	CAATCCTAAGTATTTTCGGTTCATT
cpsL-R	TAGGAACATGTTTCATTAACATAGC
cpsG-F	ACATGAACAGCAGTTCAACCGT
cpsG-R	ATGCTCTCCAAACTGTTCTTGT
cpsG-2-3-6-R	TCCATCTACATCTTCAATCCAAGC
cpsJ-2-4-F	CATTTATTGATTTCAGACGATTACATTGA
cpsJ-2-R	CCTCTTTCTCTAAAATATTCCAACC
cpsJ-Ib-F	GCAATTCTTAACAGAATATTCAGTTG
cpsJ-Ib-R	GCGTTTCTTTATCACATACTCTTG

A real time quantitative PCR (qPCR) specific for *S. agalactiae* was developed (Su et al. 2016) and validated (Tavares et al. 2016). The primers (IGS-s 5'-GGAAACCTGCCATTTGCGTCT-3' and IGS-a 5'-AATCTATTTCTAGATCGTGGAAT-3') are specific for the 16S-23S rRNA intergenic spacer region (7 copies in *S. agalactiae*), and the limit of detection was determined to be 8.6 genome equivalents (Su et al. 2016). Su et al. (2016) used SYBR green real time PCR master mix to design the assay and Tavares et al. (2016) used GoTaq qPCR master mix in validation. Recently, another qPCR, based on the *groEL* gene of *S. agalactiae* was developed and validated with a limit of detection of 1.7 copies per μL (Leigh et al. 2019). To the authors knowledge, no qPCR assays have been developed and validated for *S. iniae* and *S. dysgalactiae* in aquaculture.

F. Procedures for Detecting Subclinical Infections

To the authors knowledge, there are not any extensively studied and validated procedures for detecting subclinical infections. Subclinical infections will likely be best assessed via quantitative PCR versus culture as the gold standard, but much more work is needed in this area. Non-lethal sampling and qPCR was effective in the detection of *S. agalactiae* (Tavares et al. 2016). The authors utilized kidney aspiration, venipuncture, gill mucus swabbing and fecal collection. The best results were seen with kidney aspiration and venipuncture (93 percent accuracy) using qPCR (Tavares et al. 2016).

G. Procedures for Determining Prior Exposure to the Etiological Agent

Serological methods including ELISA and western blot (Shelby et al. 2002; LaFrentz et al. 2011) may be effective at indicating prior exposure; however, they have not been validated.

H. Procedures for Transportation and Storage of Samples to Ensure Maximum Viability and Survival of the Etiological Agent

See Section 1, 1.1.1 General Procedures for Bacteriology. Dry swabs (Shoemaker et al. 2001) and commercially available swabs (Chou et al. 2014) have been used to effectively transport and isolate *Streptococcus* sp. from the field.

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