Prevalence of *Streptococcus iniae* in tilapia, hybrid striped bass, and channel catfish on commercial fish farms in the United States

Craig A. Shoemaker, PhD; Phillip H. Klesius, PhD; Joyce J. Evans, PhD

**Objective**—To determine the prevalence of *Streptococcus iniae* in tilapia (*Oreochromis* spp), hybrid striped bass (*Morone chrysops* × *M saxatilis*), and channel catfish (*Ictalurus punctatus*) on commercial fish farms in the United States.

**Animals**—1,543 fish (970 tilapia, 415 hybrid striped bass, and 158 channel catfish).

**Procedures**—The dry-swab technique was used for collection of specimens for streptococcal isolation. Specimens were shipped by overnight delivery and processed by use of standard bacteriologic techniques. 

**Results**—*Streptococcus iniae* was not isolated from market-size channel catfish. Prevalence in tilapia and hybrid striped bass was 37 of 970 (3.81%) and 30 of 415 (7.23%), respectively. Prevalence by farm ranged from 0.0 to 21.6% for hybrid striped bass. In tilapia, prevalence was lowest in market-size and nursery fish (3 of 429 [0.70%] and 3 of 339 [0.89%], respectively), with an increase in prevalence for fish in the grow-out stage (30 of 337 [9.03%]). For hybrid striped bass, prevalence was lowest in nursery and market-size fish (3 of 96 [3.12%] and 1 of 47 [2.12%, respectively) and highest in fish in the grow-out stage (26 of 272 [9.56%]). 

**Conclusions and Clinical Relevance**—Results of this study do not support the contention that *S iniae* is a serious public health threat associated with commercially raised fish; rather, it represents a limited risk for older or immunocompromised people who incur puncture wounds while handling and preparing fish. 

6 The objective of the study reported here was to determine the prevalence of *S iniae* in tilapia, hybrid striped (sunshine) bass (*Morone chrysops* × *M saxatilis*), and channel catfish (*Ictalurus punctatus*) on commercial fish farms that were not having an epizootic of streptococcal-induced disease.

**Materials and Methods**

**Fish farms**—The prevalence study was conducted for 1 year (April 1997 through March 1998). Prior to initiation of the study, it was determined that we would not identify fish farms that had streptococcal problems or *S iniae* isolates and that records would not identify specific farms, owners, or localities. All of the producers contacted agreed to participate in the study. The study involved 24 farms throughout the United States. Specimens were obtained from fish on 12 tilapia farms, 6 hybrid striped bass farms, and 6 channel catfish farms. One farm that raised both hybrid striped bass and tilapia was included as a hybrid striped bass farm; however, the tilapia and hybrid striped bass from that farm were allocated to the appropriate species for determination of overall prevalence and prevalence by species.

**Fish and specimen collection**—Prior to collection of specimens, it was determined that detection of *S iniae* (with 90% confidence) would require collection of specimens from 22 fish/farm per farm. Fish were randomly collected, using a dip net or seine, on farms that agreed to participate in the study. Nursery fish ranged from <1 to 15 g. Fish in the grow-out stage ranged from 15 to 454 g, and market-size fish were >454 g. After collection, fish were euthanatized with an overdose of tricaine methane sulfonate. Prior to dissection or collection of swab specimens, fish were swabbed with alcohol-soaked wipes to eliminate the chance of bacterial contamination from the skin. Fish were dissected, and swab specimens of the brain and kidneys were obtained. On some farms, skin (mucus) samples were obtained from fish; these were collected prior to swabbing of the skin with alcohol-soaked wipes. Swab specimens were obtained from the brain of each fish in the nursery.

Swab specimens were placed in individually identified silica gel transportation envelopes, as described by Reyes et al, for transport of specimens to a laboratory for streptococcal isolation. 

Received Dec 3, 1999. 
Accepted Apr 17, 2000.

From the USDA-Agricultural Research Service, Aquatic Animal Health Research Laboratory, PO Box 0952, Auburn, AL 36830 (Shoemaker, Klesius); and the USDA-Agricultural Research Service, Aquatic Animal Health Research Laboratory, 151 Dixon Dr, Suite 4, Chestertown, MD 21620 (Evans). 

The authors thank Ning Qin, W Paige Hendrix, Holly Carter, Jeannie L. Hatfield, Bill Hemstreet, and Drs. John Grizzle, Chhorn Lim, John Hawke, Andrew Goodwin, and David Wise for technical assistance.
Table 1—Isolation of *Streptococcus iniae*, by species of fish

<table>
<thead>
<tr>
<th>Fish species</th>
<th>No. of positive farms/No. of fish</th>
<th>No. of fish for <em>S. iniae</em></th>
<th>Percentage of fish positive for <em>S. iniae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tilapia</td>
<td>6/12</td>
<td>970</td>
<td>37</td>
</tr>
<tr>
<td>Hybrid striped bass</td>
<td>2/6</td>
<td>415</td>
<td>30</td>
</tr>
<tr>
<td>Channel catfish</td>
<td>0/6</td>
<td>158</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>8/24</strong></td>
<td><strong>1,543</strong></td>
<td><strong>67</strong></td>
</tr>
</tbody>
</table>

*Values represent No. of samples positive for *S. iniae* / total No. of samples from that location. *Mean ± 90% confidence interval.*

Table 2—Prevalence of *S. iniae* in tilapia, hybrid striped bass, and channel catfish, by farm

<table>
<thead>
<tr>
<th>Fish species</th>
<th>No. of farms</th>
<th>No. of samples positive for <em>S. iniae</em></th>
<th>No. of fish for <em>S. iniae</em></th>
<th>Prevalence (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tilapia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feral</td>
<td>24</td>
<td>2,012</td>
<td>78</td>
<td>1.543</td>
</tr>
<tr>
<td>Hybrid striped bass</td>
<td>24</td>
<td>1.543</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>24</strong></td>
<td><strong>2,012</strong></td>
<td><strong>78</strong></td>
<td><strong>1.543</strong></td>
</tr>
</tbody>
</table>

*Values reported are median (range). *Mean ± 90% confidence interval.*

Table 3—Prevalence of *S. iniae* in tilapia and hybrid striped bass from nursery, grow-out, or market groups

<table>
<thead>
<tr>
<th>Fish species</th>
<th>No. of fish for <em>S. iniae</em></th>
<th>Prevalence (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tilapia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nursery</td>
<td>3</td>
<td>0.88 ± 0.8</td>
</tr>
<tr>
<td>Grow-out</td>
<td>377</td>
<td>7.96 ± 2.3</td>
</tr>
<tr>
<td>Market</td>
<td>239</td>
<td>1.67 ± 1.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>955</td>
<td>3.87 ± 1.0</td>
</tr>
</tbody>
</table>

*Mean ± 90% confidence interval.*

Table 4—Prevalence of *S. iniae* in tilapia and hybrid striped bass, by type of tissue sample

<table>
<thead>
<tr>
<th>Species</th>
<th>Type of tissue sample*</th>
<th>No. of fish for <em>S. iniae</em></th>
<th>Prevalence (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tilapia</td>
<td>Brain</td>
<td>16/603 (2.65)</td>
<td>32/451 (50–261)</td>
</tr>
<tr>
<td>Hybrid striped bass</td>
<td>Brain</td>
<td>14/318 (4.00)</td>
<td>2/267 (2.91)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>30/921 (3.25)</strong></td>
<td><strong>44/680 (5.11)</strong></td>
</tr>
</tbody>
</table>

*Values represent No. of samples positive for *S. iniae* / total No. of samples from that location. *Mean ± 90% confidence interval.*

Results

Prevalence of *S. iniae* among farms and by fish species—Of specimens obtained from the 12 tilapia farms, 6 had positive results for *S. iniae*. Specimens from 2 of 6 hybrid striped bass farms were positive when tested for *S. iniae*. However, *S. iniae* was not isolated from specimens obtained from any of the 6 channel catfish farms. Overall, specimens from 27.4% of fish farms, including the channel catfish farms, yielded *S. iniae* (Table 1). For tilapia, *S. iniae* was isolated from 37 of 970 (3.81%) specimens, whereas *S. iniae* was isolated from 30 of 415 (7.29%) specimens from hybrid striped bass. None of the 158 specimens obtained from channel catfish yielded *S. iniae*. Of 1,543 specimens collected from farm-raised fish, only 67 (4.34%) yielded *S. iniae*.

Prevalence of *S. iniae* by farm—Prevalence of *S. iniae* was determined for each fish farm (Table 2). On tilapia farms, prevalence ranged from 0.0 to 27.4%, and prevalence on hybrid striped bass farms ranged from 0.0 to 21.6%. *Streptococcus iniae* was not isolated from specimens obtained from any of the channel catfish. Furthermore, *S. iniae* was not isolated from any of the specimens we collected from 15 feral tilapia.

Prevalence of *S. iniae* in fish—Prevalence of *S. iniae* was 3 of 339 (< 1%) for tilapia in the nursery (Table 3). Prevalence increased to 30 of 377 (7.96%) for tilapia in the grow-out stage. For market-size tilapia, *S. iniae* developed on the basis of the original description of *S. iniae* by Pier et al. On arrival at the laboratory, specimens were enriched by placing them into 5 ml of tryptic soy broth that had been supplemented with 3 or 4 drops of defibrinated sheep blood; specimens then were incubated (27 C for ≥ 4 hours). After enrichment, bacterial cultures were streaked and stabbed onto tryptic soy agar containing 5% sheep blood for colony differentiation and hemolysis testing. Blood agar plates were incubated in a 5% CO2 incubator (27 C for 24 hours). Thereafter, single β-hemolytic colonies were picked for gram staining and inoculation onto tryptic soy agar slants without sheep blood. After incubation overnight, gram-positive cultures were tested for catalase. All catalase-negative gram-positive cultures were inoculated on starch hydrolysis agar and were used in other biochemical tests needed to identify *S. iniae*. Analysis included results for sorbitol, mannitol, arabinose, bile-esculin, CAMP test, Mann, Rogosa and Sharpe broth, Methyl Red-Voges Proskauer, motility, hirupate, leucine aminopeptidase, and pyrrolidonylarylamidase. Vancomycin and bacitracin susceptibility also was evaluated.
was isolated from 4 of 239 (1.67%). For hybrid striped bass, *S inia* was isolated from 3 of 96 (3.12%) fish in the nursery. Prevalence of *S inia* increased to 26 of 272 (9.56%) for hybrid striped bass in the grow-out stage, with a decrease in prevalence to 1 of 47 (2.12%) for market-size hybrid striped bass. *Streptococcus inia* was not isolated in 158 market-size channel catfish.

**Prevalence by type of tissue sample**—A total of 2,541 tissue samples was collected from tilapia and hybrid striped bass (Table 4). The percentage prevalence by type of tissue sample was similar for brain, kidneys, and skin samples obtained from tilapia (2.65, 2.83, and 2.78%, respectively). Percentage prevalence (0.68%) was lower in miscellaneous samples, which included whole fish (fry), eye, and skin ulcers. A similar pattern was observed for the hybrid striped bass, with the prevalence for samples of brain, kidneys, and skin being similar (4.40, 5.15, and 4.17%, respectively). Prevalence also was lower (1.11%) for miscellaneous samples for hybrid striped bass. The combined total prevalence by type of tissue sample was similar to that observed for each species.

**Discussion**

Overall prevalence of *S inia* was 4.34% in specimens collected from the 1,543 fish, and it was 1.73% in the 286 market-size tilapia and hybrid striped bass. *Streptococcus inia* was cultured from 67 of 1,385 (4.84%) tilapia and hybrid striped bass on 24 commercial fish farms in the United States. To our knowledge, infections attributable to *S inia* were not reported in workers on the study farms. Also, *S inia* was not cultured from channel catfish. Prevalence for each species of fish revealed that 30 of 415 (7.23%) hybrid striped bass yielded *S inia*, whereas 37 of 970 (3.81%) tilapia yielded *S inia*. Weinstein et al. isolated *S inia* from 42 skin samples obtained from 73 fish (62 tilapia, 4 perch, 6 striped bass, and 1 green bass). Of the fish included in that study, 33 skin samples were collected by personnel employed by the Canadian Department of Fisheries and Oceans from live tilapia obtained from fish suppliers in the greater Toronto area, and 27 *S inia* isolates were cultured (prevalence, 81.8%). The other *S inia* isolates in that study were obtained by researchers from the Vancouver Hospital Health Science Center in skin samples obtained from fish bought from retailers in the greater Toronto and Vancouver areas. Samples for the greater Toronto area included 23 tilapia, 4 perch, and 7 bass (6 striped and 1 green bass), and the prevalence in each group of fish was 32, 0, and 42.9%, respectively. Testing for the Vancouver area included skin samples from 4 tilapia, all of which yielded *S inia*. In that study, they did not perform microbiological analysis for *S inia* on US farms. Prevalence of *S inia* in samples of tilapia collected by the Canadian Department of Fisheries and Oceans was 18-fold higher than that observed in the study reported here. Prevalence was 40-fold higher, comparing market-size fish of that study and our study (81.8 vs 1.75%).

The large difference in prevalence suggests that the methods for housing of fish prior to distribution to retailers in Canada may favor colonization or contamination of fish skin with *S inia*. This is further supported by the high percentage of samples of fish skin from which the Canadian researchers were able to isolate *S inia*. In the samples obtained from fish sampled from retail outlets (ie, fish bought at a market) in Toronto and Vancouver, prevalence was 18- to 50-fold higher than that observed in the market-size tilapia and hybrid striped bass from farms in the United States. We reported that fish density had a significant effect on streptococcal-induced disease in tilapia and that a high density of fish favors transmission of *S inia*. The prevalence in tilapia and hybrid striped bass from suppliers and retail outlets in Canada was about 10- to 20-fold higher than that observed in market-size fish from commercial US fish farms.

Prevalence of *S inia* on farms varied greatly and ranged from 0.0 to 27.4% on tilapia farms and 0.0 to 21.6% on hybrid striped bass farms. This wide range suggests that *S inia* is overdispersed (ie, aggregated or heterogeneous) on farms in the United States. However, it is difficult to make any assumptions as to why this result was observed because of the extremely wide range of fish-raising practices. Facilities consisted of tanks, raceways, cages, and ponds with water systems ranging from closed systems (ie, only replacing water lost as a result of evaporation) to open systems (ie, brackish-water cage system). Potential changes in *S inia* prevalence could be associated with season; however, most farms in the study used closed systems with water temperatures averaging 25°C.

Prevalence of *S inia* differed among nursery, grow-out, and market-size tilapia and hybrid striped bass. Tilapia from the grow-out stage had the highest prevalence (30 of 337, 7.96%), whereas a lower prevalence of *S inia* was observed for nursery (3 of 339, 0.88%) and market-size fish (4 of 239, 1.67%). In hybrid striped bass, fish in the grow-out stage also had the highest prevalence (26 of 272, 9.56%), with nursery and market-size fish yielding less *S inia* (3 of 96 [3.12%] and 1 of 47 [2.12%, respectively). Analysis of these findings suggests that young fish are not colonized as heavily or that the methods of detection (ie, standard bacterial culture) in young fish are not as sensitive as in older fish. Prieta et al. suggested that young fish rarely had signs of streptococcal-induced disease on fish farms in the Mediterranean. Research on diseases in rainbow trout attributable to infection caused by *Lactococcus garvieae* suggests that younger fish are more sensitive to artificially induced infection (intraperitoneal injection) than older fish, which seem to have the ability to eliminate bacteria. Thus, the decrease in overall prevalence of *S inia* in market-size tilapia and hybrid striped bass observed in the study reported here may have been an indication of clearance of the bacteria by the larger (market-size) fish. It also is possible that in the market-size fish, a certain number of fish were carriers that harbored *S inia* in numbers that were not detectable by standard culture methods. Using a polymerase chain reaction, Zlotkin et al. documented a carrier state in older apparently healthy rainbow trout colonized with *L. garvieae* the previous year. Those results suggest that culture techniques may be inadequate to detect tilapia and hybrid striped bass.
that are carriers of \textit{S iniae}. Development of rapid diagnostic tests is needed to better understand disease caused by \textit{S iniae} in farm-raised tilapia and hybrid striped bass. Such tests would allow for early detection of \textit{S iniae} and, thus, earlier intervention with approved antibiotics. However, antibiotic treatment may not be effective against streptococcal diseases because of the ability of the bacteria to survive in macrophages\textsuperscript{12} and the failure of sick fish to eat medicated feed. Therefore, antibiotic treatment may suppress clinical signs of disease and favor the development of carrier fish, resulting in latter rounds of infection by the same streptococci.\textsuperscript{12,13}

Overall prevalence of \textit{S iniae} from samples of brain, kidneys, and skin (mucus) of tilapia and hybrid striped bass was 3.25, 3.65, and 2.91%, respectively. Miscellaneous samples that included whole fish, eye, and skin ulcers had a lower prevalence (1.11%). Analysis of these results suggests that the skin (mucus) is an appropriate location to noninvasively obtain samples, using the dry-swab technique.\textsuperscript{7} Overall prevalence in skin samples from 231 tilapia and 24 hybrid striped bass in our study was 2.78 and 4.17%, respectively. Prevalence of \textit{S iniae} was 60.3% for skin samples obtained from tilapia in Canada\textsuperscript{3}; however, samples of internal organs were not collected by Canadian researchers in that study.

We did not find evidence to support the contention that \textit{S iniae} is a serious public health threat associated with commercially raised fish. Rather, it appears to be a risk associated with older or immuno-compromised people who incur a puncture wound during handling or preparation of fish for cooking. Currently, similar cases attributable to infection with \textit{S iniae} resulting from handling of fresh live tilapia have not been reported in the United States.

The solution to the problem of streptococcal-induced disease in tilapia and hybrid striped bass appears to be vaccination and management. Klesius et al\textsuperscript{14,15} and Eldar et al\textsuperscript{16} documented the efficacy of vaccines against \textit{S iniae} in tilapia and rainbow trout, respectively. Management practices such as quarantine of new fish,\textsuperscript{10} reduction of fish density,\textsuperscript{6} maintenance of acceptable water quality, and rapid removal of dead and dying fish\textsuperscript{5} may reduce the impact of \textit{S iniae} in tilapia and hybrid striped bass. Sanitary management practices should be used by suppliers and retailers of fresh fish to prevent increased colonization of fish skin by \textit{S iniae}.

References