Pathogenicity of *Streptococcus ictaluri* to Channel Catfish

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Abstract.—The pathogenicity of a *Streptococcus ictaluri* isolate in channel catfish *Ictalurus punctatus* at the fry (0.5 g), fingerling (15 g), and juvenile (55 g) stages was determined by experimental bath immersion and injection experiments. Channel catfish were exposed in 1-L immersion baths containing 10⁶, 10⁷, 10⁸, or 10⁹ colony-forming units (cfu) of *S. ictaluri*. Fish were also injected intraperitoneally with 0.1 mL of bacterial solution for final doses of 10⁴, 10⁵, 10⁶, 10⁷, or 10⁸ cfu of *S. ictaluri* per fish. *Streptococcus ictaluri* caused mortality in fry, fingerling, and juvenile channel catfish within 21 d postinfection. When mortalities were calculated based on size and challenge route, the cumulative percent mortalities were 11% for fry and 0% for fingerlings by the bath immersion route and 14% for fingerlings and 6% for juveniles by the injection route. Isolation of *S. ictaluri* from moribund and dead catfish was confirmed by the newly established BIOLOG profile (MicroLog systsem). The results indicate that channel catfish were only susceptible to high concentrations of *S. ictaluri* and that juvenile channel catfish were less susceptible, possibly explaining why little mortality has been attributed to *S. ictaluri* infection in catfish aquaculture.

Streptococcal pathogens affect a wide variety of saltwater and freshwater fish (Evans et al. 2006), but there are few reports of *Streptococcus* infecting channel catfish *Ictalurus punctatus*. Robinson and Meyer (1966) reported that channel catfish were not susceptible to group B *Streptococcus* sp. (GBS), but Plumb et al. (1974) indicated that channel catfish were susceptible to GBS upon intramuscular injection. This GBS isolate was obtained during a streptococcal outbreak in several marine fish species in Gulf of Mexico inland waters (Plumb et al. 1974). Clinical signs were observed in channel catfish exposed to aquatic animal and human GBS isolates (Teska and Shotts 1994). Chang and Plumb (1996) studied streptococcal disease in channel catfish using a *Streptococcus* sp. isolated from other channel catfish. These authors disrupted the skin of the catfish by scraping their lateral body surface and then immersing the catfish in water containing the bacteria. The subsequent streptococcal disease was characterized by inflammatory lesions, including meningoencephalitis, myocarditis, and splenitis. However, the lesions were not as severe as in Nile tilapia *Oreochromis niloticus* similarly exposed to the isolates. Perera et al. (1997) found that channel catfish exposed to *S. iniae* though oral or immersion administration were resistant to infection, though hybrid tilapia (Nile tilapia × blue tilapia *O. aureus*) were susceptible. Shoemaker et al. (2001) sampled aquaculture sites throughout the USA and did not isolate *S. iniae* from sampled channel catfish, whereas approximately 4% of tilapia of the genus *Oreochromis* and 7% of hybrid striped bass (striped bass *Morone saxatilis* × white bass *M. chrysops*) were *S. iniae*-positive. Channel catfish were susceptible to experimental *S. iniae* and *S. agalactiae* infection when fish were parasitized with *Trichodina* spp. (Evans et al. 2007).

The natural infection of channel catfish broodstock with a novel streptococcal species, subsequently named *S. ictaluri* has recently been reported (Shewmaker et al. 2007; Camus et al. 2008). This species was defined as a Gram-positive, catalase-negative, nonhemolytic cocci that was distinguished from other streptococcal species by biochemical testing, DNA–DNA hybridization, and 16S rRNA gene sequencing data (Shewmaker et al. 2007). Clinical signs with broodstock natural infections included low-grade mortalities, emaciation, arched backs, gross lesions of the fins and jaws, and diminished reproduction. Camus et al. (2008) experimentally injected 50–75-g channel catfish with *S. ictaluri* and reisolated bacteria from jaw and fin lesions. However, no mortalities were reported. Given that mortalities have been reported in broodstock (fish approximately 2–3 kg) and only clinical signs were observed with experimentally infected juveniles (50–75 g), this study examined whether morbidity and mortality could be produced by experimental infection of fry, fingerling, and juvenile channel catfish.
Methods

Groups of National Warmwater Aquaculture Center (NWAC) NWAC-103-descendant channel catfish fry were originally shipped from the center in Stoneville, Mississippi, to the Aquatic Animal Health Research Laboratory (AAHRL), U.S. Department of Agriculture, Agricultural Research Service laboratory in Chestertown, Maryland. The fish were housed in 57-L aquaria, supplied with water flow-through dechlorinated tap water and maintained on a 12 h light : 12 h dark photoperiod. The fish were fed daily to satiation with Aquamax Starter 300 or Grower 400 (Purina Mills, Brentwood, Missouri). During the study, mean daily water temperature was 30.03°C (SD, 0.43), mean daily dissolved oxygen was 3.69 mg/L (SD, 0.90), mean ammonia concentration was 0.08 mg/L (SD, 0.03), and mean pH was 7.47 (SD, 0.17).

We kept Streptococcus ictaluri (ATCC BAA-1300) frozen at −78°C in 2 mL of tryptic soy broth (TSB) aliquots until thawed, cultured on 5% sheep blood agar (SBA; Remel, Lenexa, Kansas), and incubated at 30°C for 24 h. The isolate was passed through channel catfish fingerlings (mean, 15 g) five times via intraperitoneal injection at 10^7 colony forming units (cfu) and isolated from the brain following each passage. The isolate from the fifth passage was used for the experimental challenge. A characteristic colony of S. ictaluri was picked with a sterile loop, inoculated 8°C in 2 mL of tryptic soy broth (TSB) and incubated at 30°C for 24 h. Triplicate 50-μL samples from each culture were spiral plated with an Autoplate 4000 (Spiral Biotech, Norwood, Massachusetts) and cultured to determine density (cfu/mL) according to the manufacturer’s instructions. We exposed 30 channel catfish fry (mean weight = 0.5 g; SD = 0.1) and 10 fingerlings (15.1 g; SD = 0.5) per immersion treatment in 1-L baths of 10^4, 10^5, 10^6, 10^7, or 10^8 cfu of S. ictaluri for 15 min at 30°C. We also injected (intraperitoneal) 10 fingerlings (16.5 g; SD = 0.6) and 10 juveniles (55 g; SD = 10.5) per treatment group: 0.1 mL of bacterial solution for a final dose of 10^4, 10^5, 10^6, 10^7, or 10^8 cfu of S. ictaluri per fish. Fish were then housed in 38-L (fry and fingerlings) or 57-L (juveniles) tanks. Control fish for each group were similarly immersed in or injected with TSB. Single tanks were used for each dose and group: 30 fry per tank or 10 fingerlings or juveniles per tank.

The challenged fish were monitored daily for signs of disease and mortality for 21 d postchallenge. Moribund and dead fish were removed three times daily. Bacterial samples were obtained from 10% of moribid and dead fish from each tank; brain, kidney, nares, and intestine samples were taken from fingerlings and juveniles. Whole fry were homogenized to obtain bacterial samples. Samples were cultured at 30°C for 24 h on SBA to isolate S. ictaluri. Identification of prechallenge and recovered postchallenge S. ictaluri isolates was confirmed using select previously established biochemical results (Shewmaker et al. 2007; Camus et al. 2008) and using a newly established ATCC isolate BIOLOG carbon utilization pattern. For the MicroLog3 system (BIOLOG, Hayward, California), BIOLOG Gram-positive microplates were inoculated according to manufacturer instructions and incubated for 24 h at 30°C. The BIOLOG carbon utilization pattern results were compared with the manufacturer’s MicroLog and AAHRL fish and marine mammal Streptococcus isolate user databases, and a similarity index (SI) of less than 0.500 yielded an identification. No S. ictaluri profile is in the MicroLog database because it is a newly characterized isolate. Serogrouping of the ATCC S. ictaluri isolate was completed using the SlideX Strepto Kit for groups A, B, C, D, F, and G (bioMerieux, Durham, North Carolina) and following the manufacturer instructions. The methods of MacFaddin (2000) were also used to determine the Christie, Atkins, and Munch–Petersen (CAMP) and pyrrolidonyl arylamidase reactions. Starch hydrolysis was tested by the activity of the ATCC S. ictaluri isolate on starch agar plates according to the methods of Evans et al. (2004).

Cumulative percent mortalities (CPM) data were statistically analyzed using the SAS program (SAS Institute, Cary, North Carolina, USA) for the Lifetest procedure (Kaplan-Meier method); significant differences were accepted at P < 0.05.

Results

When channel catfish mortalities were calculated based on size and challenge route, the CPMs were 11% for fry and 0% for fingerlings by the bath immersion route and 14% for fingerlings and 6% for juveniles by the injection route. Controls had 0% CPM in all groups, regardless of size or experimental challenge method, whereas immersion-challenged fingerlings also had 0% CPM at all S. ictaluri exposures. Significant increases (P < 0.05; Table 1) in CPM were observed among immersion-challenged fry (10^6, 10^7, and 10^8 cfu), injection-challenged fingerlings (10^6 and 10^7 cfu/fish), and injection-challenged juveniles (10^8 cfu/fish). All mortalities occurred between 3 and 16 d postchallenge. Immersion-challenged fry had the highest CPM among all the doses and treatment groups. The injection-challenged fingerlings and juveniles required the highest doses (10^6 and 10^8 cfu, respectively) to exhibit increased CPM, but the immersion challenged fry had signifi-
Significantly increased CPM at a relatively low immersion dose \((10^9 \text{ cfu})\) compared with other immersion doses. Challenged fish in all size-groups exhibited behavioral changes: lethargy, confinement to the bottom of the tank, slow or no feeding response, external body coloration changes, eye opacity, altered swimming pattern (swimming turned on the side), and mild, focal ulceration at the base of the dorsal and caudal fins (Table 2). No gross external lesions were noted in immersion-challenged fingerlings, and no internal lesions were noted in any of the fish. Bacteria were isolated from the brain, kidney, nares, and intestine of sampled dead fish. The BIOLOG results obtained from these postchallenge samples corresponded to those of the ATCC \(S. ictaluri\) isolate analyzed before challenge.

The ATCC \(S. ictaluri\) type isolate and isolates obtained from challenged fish formed small, pinpoint, white colonies, containing gram-positive, catalase-negative, oxidase-negative, and nonhemolytic, non-typable cocci. Isolates were negative for pyrrolidonyl arylamidase, CAMP, and starch hydrolysis. We used BIOLOG to characterize ATCC \(S. ictaluri\) isolates prechallenge and postchallenge from fingerling mortalities. Species identification made with the MicroLog and User databases indicated that the isolates most resembled \(S. agalactiae\) (Probability = 80%; Similarity Index [SI] = 0.53) after 24 h incubation. After \(S. agalactiae\), the isolates most resembled \(Bacillus amyloliquefaciens\) (Probability = 11%; SI = 0.07). Positive reactions for both the ATCC isolate and recovered fish isolates included dextrin, \(N\)-acetyl-\(D\)-glucosamine, \(D\)-fructose, \(\alpha-D\)-glucose, maltose, malto-triose, \(D\)-mannose, methyl pyruvate (pyruvic acid methyl ester), pyruvic acid, glycerol, adenosine, 2'-deoxy adenosine, inosine, thymidine, and uridine. All other reactions were negative.

**Discussion**

Experimental challenge with \(S. ictaluri\) caused clinical signs and mortalities among channel catfish in immersion-challenged fry, injection-challenged fingerlings, and injection-challenged juveniles but not in immersion-challenged fingerlings, Camus et al. (2008) experimentally challenged juvenile (50–75 g) channel catfish with \(S. ictaluri\) by intraperitoneal, intravenous, or intramuscular injection of \(1 \times 10^4\ \text{cfu/mL}\) and reported no mortalities within 28 d. As in Camus et al. (2008), our experimental challenge generated lesions at the base of the dorsal and caudal fins, but in contrast to Camus et al. (2008), we found (1) mortalities generated

### Table 1

Cumulative percent mortality among channel catfish fry, fingerlings, and juveniles challenged with *Streptococcus ictaluri*, by dose; \(n = \) number per dose. Significant differences from control results are denoted by asterisks (\(P < 0.05\)). See text for additional details.

<table>
<thead>
<tr>
<th>Immersion challenge</th>
<th>Injection challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cumulative percent mortality</strong></td>
<td><strong>Cumulative percent mortality</strong></td>
</tr>
<tr>
<td><strong>Dose (cfu)</strong></td>
<td><strong>Fry (n = 30)</strong></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>(10^8)</td>
<td>7*</td>
</tr>
<tr>
<td>(10^9)</td>
<td>40*</td>
</tr>
<tr>
<td>(10^{10})</td>
<td>0</td>
</tr>
<tr>
<td>(10^{11})</td>
<td>3</td>
</tr>
<tr>
<td>(10^{12})</td>
<td>7*</td>
</tr>
</tbody>
</table>

### Table 2

Percentages of fry, fingerling, and juvenile channel catfish exhibiting clinical signs after immersion or injection challenges with *Streptococcus ictaluri*.

<table>
<thead>
<tr>
<th>Clinical sign</th>
<th>Fry</th>
<th>Fingerlings</th>
<th>Juveniles</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
<td><strong>Number treated</strong></td>
<td><strong>Cumulative percent mortality</strong></td>
<td><strong>Confined to bottom of tank</strong></td>
</tr>
<tr>
<td>All control groups</td>
<td>60</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Immersion</strong></td>
<td>150</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>Fry</td>
<td>50</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>Fingerlings</td>
<td>50</td>
<td>0</td>
<td>44</td>
</tr>
<tr>
<td><strong>Injection</strong></td>
<td>50</td>
<td>14</td>
<td>52</td>
</tr>
<tr>
<td>Fingerlings</td>
<td>50</td>
<td>6</td>
<td>81</td>
</tr>
<tr>
<td>Juveniles</td>
<td>50</td>
<td>20</td>
<td>31</td>
</tr>
</tbody>
</table>

PASNIK ET AL.
in experimentally challenged fish, (2) behavioral changes, and (3) no lesions at the jaw or in the spine. Based on the isolation of *S. ictaluri* from four distinct anatomic sites in sampled dead fish, the bacteria must have spread systemically. This points to an *S. ictaluri* septicemia as the cause of mortalities. Other than the ulcerations at the base of the fins, the clinical signs were nonspecific (Table 2). Few of the fish exhibited erratic swimming behavior, and no fish displayed C-shaped posturing, signs often associated with *Streptococcus*-induced disease (Evans et al. 2007). Despite the fact that *S. ictaluri* could be cultured from the brain, clinical signs of neurologic disease were rarely noted.

Despite the fact that *S. ictaluri* can cause mortalities, this bacteria does not appear to be substantially pathogenic for channel catfish. This corresponds to other studies that have indicated that channel catfish are not highly susceptible to streptococcal disease (Robinson and Meyer 1966; Perera et al. 1997; Shoemaker et al. 2001; Camus et al. 2008). None of our immersion-challenged fingerlings died during the study. Limited mortalities were observed, even with a challenge isolate that had been passed through channel catfish five times before the study challenge. Groups that had mortalities required relatively high challenge doses to cause significant mortalities: fry immersion of $10^8$ cfu, fingerling injections of $10^7$ cfu, and juvenile injections of $10^8$ cfu. However, with two of these high doses there were considerable mortalities: immersion-challenged fry had 40 CPM at $10^9$ cfu and injection-challenged fingerlings had 50 CPM at $10^8$ cfu.

Though the incidence of streptococcal disease in channel catfish is limited, several authors have described such disease in naturally infected (Plumb et al. 1974; Camus et al. 2008) and experimentally infected channel catfish (Plumb et al. 1974; Teska and Shotts 1994; Chang and Plumb 1996; Evans et al. 2007). Others have indicated that channel catfish are not susceptible to certain species of *Streptococcus* (Robinson and Meyer 1966; Perera et al. 1997). However, streptococcal pathogenesis in channel catfish might be linked to injury or stressors (Chang and Plumb 1996; Evans et al. 2007). Increased stressors may be the reason why natural *S. ictaluri* infections have been observed in broodstock; Camus et al. (2008) hypothesized that spawning activity might be essential for disease development. Environmental stressors, such as low dissolved oxygen, that can increase streptococcal disease susceptibility (Evans et al. 2004) may also increase mortalities in *S. ictaluri*-infected fry, fingerling, and juvenile channel catfish. However, more research is needed on *S. ictaluri* disease transmission and pathogenicity before this can be definitively understood. Channel catfish were only susceptible to high concentrations of *S. ictaluri*, and older channel catfish were less susceptible, possibly explaining why little mortality has been attributed to *S. ictaluri* infection in catfish aquaculture.

Stoffregen et al. (1996), Roach et al. (2006), and Russo et al. (2006) used BIOLOG to presumptively identify *S. iniae* isolates cultured from fish. We used BIOLOG to provide a carbon utilization profile for the ATCC *S. ictaluri* and the *S. ictaluri* isolated from dead channel catfish. Only 14 reactions were positive and 81 were negative, indicating low versatility in carbon substrate utilization. The automated system utilized the MicroLog and AAHRL-user databases and did not identify the isolate, much like Shewmaker (2007) found for the Rapid ID32 Strep identification system, which misidentified *S. ictaluri* as *Gemella hemolysans*. Given that *S. ictaluri* is a novel species, its biochemical profile is not in the species database of automated systems. However, BIOLOG-positive reactions of isolates taken from dead and moribund fish matched positive reactions of the original ATCC BAA-1300 isolate, and BIOLOG indicated that the isolates were most closely related to *S. agalactiae*, one of the most important fish streptococcal diseases along with *S. iniae* (Evans et al. 2006).

When comparing the ATCC fish *S. ictaluri* profile with an ATCC fish *S. agalactiae* profile, the following was observed: (1) all positive *S. ictaluri* results were also positive for *S. agalactiae*, except dextrin and pyruvic acid, and (2) the remaining *S. agalactiae* profile contained additional positive results for N-acetyl-D-mannosamine, D-galactose, maltose, β-methyl-D-glucoside, salicin, sucrose, D- trehalose, adenosine-5'-monophosphate, thymidine-5'-monophosphate, uridine-5'-monophosphate, fructose-6-phosphate, and glucose-6-phosphate (Evans et al. unpublished; MicroLog Database, BIOLOG). When comparing the ATCC fish *S. ictaluri* profile with an ATCC marine mammal *S. iniae* profile, the following was observed: (1) all positive *S. ictaluri* results were also positive for *S. iniae*, and (2) the remaining *S. iniae* profile contained additional positive results for arbutin, D-psicose, salicin, sucrose, D-mannitol, adenosine-5'-monophosphate, thymidine-5'-monophosphate, and uridine-5'-monophosphate (Evans et al. unpublished; MicroLog Database). Currently, the *S. ictaluri* isolate cannot be definitively identified by any one system or specific test. Multiple methods are needed to identify the isolate and discriminate this streptococcal isolate from similar or closely related streptococcal isolates. Because the *S. ictaluri* BIOLOG profile in our study does not correspond to any existing BIOLOG database profile and because the ATCC *S. ictaluri* isolate and postchallenge *S. ictaluri* isolates provided the same
BIOLOG profile, this system has potential to presumptively identify *S. ictaluri*.

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**References**


