

INVASIVE INFECTIONS DUE TO A FISH PATHOGEN, *STREPTOCOCCUS INIAE*

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**ABSTRACT**

**Background** *Streptococcus iniae* is a pathogen in fish, capable of causing invasive disease and outbreaks in aquaculture farms. During the winter of 1995–1996 in the greater Toronto area there was a cluster of four cases of invasive *S. iniae* infection in people who had recently handled fresh, whole fish from such farms.

**Methods** We conducted a prospective and retrospective community-based surveillance for cases of *S. iniae* infection in humans. To obtain a large sample of isolates, we studied cultures obtained from the surface of fish from aquaculture farms. Additional isolates were obtained from the brains of infected tilapia (*oreochromis* species). All the isolates were characterized by pulsed-field gel electrophoresis (PFGE).

**Results** During one year, our surveillance identified a total of nine patients with invasive *S. iniae* infection (cellulitis of the hand in eight and endocarditis in one). All the patients had handled live or freshly killed fish, and eight had percutaneous injuries. Six of the nine fish were tilapia, which are commonly used in Asian cooking. Thirteen additional *S. iniae* isolates (2 from humans and 11 from infected tilapia) were obtained from normally sterile sites. The isolates from the nine patients were indistinguishable by PFGE and were highly related to the other clinical isolates. There was substantial genetic diversity among the 42 surveillance isolates from the surface of fish, but in 10 isolates the PFGE patterns were identical to those from the patients with *S. iniae* infection.

**Conclusions** *S. iniae* can produce invasive infection after skin injuries during the handling of fresh fish grown by aquaculture. We identified a clone of *S. iniae* that causes invasive disease in both humans and fish. (N Engl J Med 1997;337:589-94.)

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**S**TREPTOCOCCUS *iniae* was first reported in 1976 to cause subcutaneous abscesses in Amazon freshwater dolphins (*Inia geoffrensis*) at aquariums in San Francisco and New York.<sup>1,2</sup> Since the early 1980s, epizootic meningoencephalitis caused by streptococci has been recognized as an important cause of morbidity and mortality in cultured fishponds.<sup>3-8</sup> Outbreaks in Japan, Taiwan, Israel, and the United States have affected tilapia (*oreochromis* species), yellowtail (*Seriola quinqueradiata*), rainbow trout, and coho salmon.<sup>3-10</sup> Several bacteria, includ-

ing *S. iniae*, *S. agalactiae*,<sup>6,11</sup> and *Lactococcus garvieae*,<sup>12,13</sup> have been shown to cause meningoencephalitis in fish grown by aquaculture. *S. iniae* may colonize the surface of fish or cause invasive disease associated with 30 to 50 percent mortality in affected fishponds.<sup>6</sup> Infected tilapia become lethargic, swim erratically, have dorsal rigidity, and die within several days. Pathological studies show extensive infection in the central nervous system.<sup>7</sup>

During the winter of 1995–1996, four persons in the greater Toronto area had bacteremic illnesses due to *S. iniae* infection. Three had cellulitis, and the fourth had sepsis with endocarditis, meningitis, and arthritis. All the patients were of Asian descent and reported having recently prepared whole, fresh fish for cooking. In three cases the fish was identified as tilapia (also known as St. Peter's fish or Hawaiian sunfish) (Fig. 1). We conducted an investigation of the clinical features and epidemiology of this illness.

**METHODS****Patients**

After the first four patients (Patients 1 to 4) were identified at a community hospital in the greater Toronto area (population, 4.2 million) between December 1995 and February 1996,<sup>14,15</sup> retrospective and prospective surveillance was carried out to identify additional patients. Twelve hospitals in greater Toronto were invited to participate. Infection-control practitioners were asked to review their medical records according to the codes defined in the *International Classification of Diseases, 9th Revision* (ICD-9) for all patients hospitalized with cellulitis in the upper limb from October 1, 1995, through March 31, 1996, when there was no predisposing cause for the cellulitis, such as an intravenous line in place, a burn, a chronic skin disease, or lymphedema. Patients were excluded from the study if their blood cultures revealed an etiologic agent other than *S. uberis*, *S. iniae*, or some other, unidentified streptococcal species. Once identified, the patients were interviewed with a standardized questionnaire to obtain clinical and epidemiologic data. Beginning on April 1, 1996, the emergency departments at the hospitals were asked to identify prospectively patients who presented with acute upper-limb cellulitis.

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**Figure 1.** A Tilapia (*Oreochromis* Species), Also Known as St. Peter's Fish or Hawaiian Sunfish.

In Cantonese the name is pronounced "laap yu," and in Mandarin "lee yu." In Asian cuisine, tilapia are typically bought live, steamed, and served simply, so that their mild flavor can be enjoyed. When they are bought frozen or as fillets, they are usually poached, grilled, baked, microwaved, or fried.

Patients in whom *S. iniae* was isolated from any sterile body site were considered to have confirmed cases of invasive disease. Patients with diagnosed upper-limb cellulitis who had handled fresh, whole fish within the 72 hours before the onset of signs and symptoms were considered to have suspected cases.

#### Additional Patients and Isolates of *S. iniae*

We reviewed the records of the Centers for Disease Control and Prevention (CDC), Atlanta; the Public Health Laboratory of Ontario, Toronto; and the National Centre for Streptococcus, Edmonton, Alberta, to determine whether *S. iniae* had been identified previously. To determine whether workers whose jobs included processing whole fish had had cellulitis, we reviewed injury claims made to the Workers' Compensation Board of Ontario over the preceding five years.

All live tilapia imported to greater Toronto originate in fishponds in the United States. A sample of such fish was taken to identify the extent to which the surface of the fish was colonized with *S. iniae*. In May and June 1996, officials of the Canadian Department of Fisheries and Oceans identified five shipments of tilapia that entered Canada from five of the seven U.S. farms supplying Toronto. At least three live tilapia were randomly selected from each shipment, and a culture was taken from the surface of each fish.

In addition, surface cultures were obtained from fish grown by aquaculture and purchased at retail in greater Toronto and in Vancouver (courtesy of Dr. N. Press and E.A. Bryce, Vancouver Hospital Health Science Centre). Clinical isolates were also received from tilapia that had acquired meningoencephalitis during epizootics in 1993 in Texas and Virginia (CDC and courtesy of Dr. P. Frelter, Texas A&M University). Strains of *S. iniae* from the American Type Culture Collection (ATCC, Rockville, Md.; types 29177 and 29178) were used as controls.

#### Epidemiologic Investigation

We attempted to identify the source of the live tilapia responsible for the infections in humans by tracing the origin of the tilapia sold by retailers to the first four patients. We studied the purchase orders from these retailers and their wholesale suppliers that corresponded to a six-week period preceding the purchase of

the fish, because live tilapia may be stored that long before being sold at market. We used importation records from the Inspection Branch of the Department of Fisheries and Oceans to confirm the origin of the live tilapia.

#### Microbiologic Analysis

Isolates were identified as *S. iniae* by standard microbiologic methods.<sup>1,2,16</sup> The characteristics used to identify streptococcal species as *S. iniae* were that they had a  $\beta$ -hemolytic reaction on trypticase soy agar with 5 percent sheep's blood; that they were not groupable with Lancefield groups A through V antiserum; that they were susceptible to vancomycin, not gas-producing, nonmotile, and positive for pyrrolidonyl arylamidase and leucine aminopeptidase; and that they produced negative results on bile-esculin, Voges-Proskauer, and hippurate tests. Most strains grew at 10°C but not at 45°C, and most did not grow in 6.5 percent sodium chloride. We used a commercial system (Bio-Mérieux Vitek, Hazelwood, Mo.) that identified the isolates as *S. uberis* or reported them as "unidentified," since *S. iniae* is not included in the data base.

Surface swabs obtained from fresh, whole fish were inoculated onto colistin-nalidixic acid blood agar (Unipath, Basingstoke, United Kingdom) and incubated at 35°C in 5 percent carbon dioxide for 18 to 24 hours. In vitro susceptibility testing was carried out by broth microdilution according to the methods of the National Committee for Clinical Laboratory Standards.<sup>17</sup>

#### Molecular Typing

Pulsed-field gel electrophoresis (PFGE) was performed on all isolates of *S. iniae* obtained from humans and fish. PFGE was performed with the CHEF DRII apparatus (Bio-Rad, Mississauga, Ont., Canada) and restriction endonucleases *Sma*I and *Apa*I (Boehringer Mannheim, Mannheim, Germany), with use of a modified version of the method of Murray et al.<sup>18</sup> The modifications included the supplementation of the Enzyme Commission lysis buffer with 20  $\mu$ g of mutanolysin per milliliter (Sigma Chemical, Mississauga), a reduction in lysis time from overnight to 2 to 5 hours, and the use of the following for electrophoresis: pulse times of 5 to 60 seconds, a temperature of 12°C, and 175 V for 20 hours. Standard interpretive criteria were used to assess the PFGE patterns.<sup>19</sup>

**TABLE 1.** DEMOGRAPHIC CHARACTERISTICS OF PATIENTS WITH CULTURE-CONFIRMED CASES OF INVASIVE *S. INIAE* INFECTION.

PATIENT No.	AGE (YR)/SEX	DATE OF CULTURE	UNDERLYING ILLNESS	TYPE OF INFECTION		TYPE OF FISH	TREATMENT	
				PRIMARY	SECONDARY		ANTIBIOTIC USED	DURATION (DAYS)
1	74/F	12/19/95	None	Cellulitis of hand	None	Unknown	Penicillin	10
2	64/F	12/16/95	None	Cellulitis of hand	None	Tilapia	Penicillin	10
3	40/F	12/22/95	Rheumatic heart disease	Cellulitis of hand	None	Tilapia	Penicillin, cloxacillin	10
4	77/M	2/1/96	Rheumatic heart disease, chronic renal failure, diabetes, osteoarthritis	Unknown	Endocarditis, meningitis, arthritis	Tilapia	Erythromycin, cefuroxime	35
5	80/M	4/17/96	Diabetes, osteoarthritis	Cellulitis of hand	None	Unknown	Ampicillin	10
6	69/F	2/29/96	None	Cellulitis of hand	None	Unknown	Cloxacillin	10
7	70/F	8/20/96	Diabetes	Cellulitis of hand	Cellulitis of leg	Tilapia	Ampicillin, cephalixin	10
8	71/M	8/29/96	None	Cellulitis of hand	None	Tilapia	Penicillin	10
9	58/F	12/6/96	None	Cellulitis of hand	None	Tilapia	Penicillin	10
10	79/M	8/94	Unknown	Unknown	Arthritis of knee	Unknown	Cloxacillin	Unknown
11	Unknown	1991	Unknown	Cellulitis	None	Unknown	Unknown	Unknown

RESULTS

**Clinical Findings and Characteristics**

Eleven of the 12 hospitals agreed to review their clinical records for cases of cellulitis, and 10 of them completed the review. Thirteen emergency departments from 3 tertiary care and 10 community hospitals participated in the prospective case finding.

From December 1995 through December 1996, nine patients with bacteremic *S. iniae* infections were identified (Table 1). Their median age was 69 years (mean, 67.0; range, 40 to 80), and the female:male ratio was 2:1. All the patients with confirmed infections were of Asian descent: eight Chinese and one Korean. All the patients reported preparing whole, raw fish, and eight patients recalled injuring their hands by puncturing the skin with the dorsal fin, a fish bone, or a knife used in the cleaning and scaling. None had prior breaks in the skin. Six patients were able to identify the fish they were preparing as tilapia; three were not certain of the species. No fish remained for possible culture. For all the clinical isolates tested, the minimal inhibitory concentrations of penicillin, cefazolin, ceftriaxone, erythromycin, clindamycin, and trimethoprim-sulfamethoxazole were 0.25 µg per milliliter or less; that of ciprofloxacin was 0.5 µg per milliliter; and that of gentamicin was 16 µg per milliliter.

Eight of the nine patients had cellulitis of the hand. They all had similar clinical presentations, with fever and lymphangitis originating from the site of injury. The cellulitis developed within 16 to 24 hours after the injuries. No patient had evidence of skin necrosis or bulla formation. The leukocyte

counts were elevated (range, 12,900 to 33,400 cells per cubic millimeter), with neutrophil predominance and leftward shifts. Patient 4, who did not have cellulitis, met the Duke criteria for infective endocarditis<sup>20</sup> of the mitral valve. He also had clinical and laboratory evidence of meningitis and arthritis in his right knee, but cerebrospinal and synovial fluid cultures performed 12 hours after the start of treatment with appropriate antibiotics were negative. All the patients were admitted to the hospital and given parenteral antibiotics; they responded to treatment within two to four days (Table 1).

Twelve patients with suspected cases of *S. iniae* infection were identified. Their median age was 46 years (mean, 50.0; range, 36 to 68), and the female:male ratio was 1:1. Eleven of the patients with suspected infections were of Asian origin; one was white. All reported having injured themselves while handling whole or partially prepared fresh fish. Nine reported the fish as being tilapia, and one as bass; the remaining two did not know the type of fish they had been preparing. One patient with a suspected infection purchased a tilapia from the same retail store, and on the same day, as a patient with a confirmed infection (Patient 7). Microbiologic cultures were negative, except in the one white patient, whose tissue culture was positive for *Aeromonas hydrophila*. That patient did not know the type of fish he had been handling when he was injured.

**Additional Patients and Isolates of *S. iniae***

The review of Workers' Compensation Board claims failed to identify any suspected cases of invasive *S. iniae* infection. The review of the data base at the

CDC microbiology laboratory revealed two additional cases in which *S. iniae* had been isolated (Patients 10 and 11) (Table 1). Patient 10 was employed as a cook in Ottawa, and had *S. iniae* isolated from synovial fluid from his knee. Patient 11 had *S. iniae* bacteremia; he was from Texas, but no other demographic information was known. Additional isolates of *S. iniae* included 11 isolates from tilapia brains obtained during epizootics, 11 from cultures of live fish obtained at retail stores, and 27 from tilapia obtained from fish suppliers (Table 2).

### Molecular Typing

The PFGE patterns of the isolates from Patients 1 through 9 were identical and were termed pattern A (Fig. 2). Patients 10 and 11 had pattern A', which differed from pattern A by one band. The strains isolated from the tilapia brains had either pattern A (1 isolate) or pattern A' (10 isolates). Pattern A was also found in two cultures of fish from two retail stores in the greater Toronto area, all four isolates from tilapia sampled in Vancouver, and four of the isolates of tilapia from two of the seven fish suppliers sampled. The remaining strains, including the ATCC type strains, yielded a total of 19 different unrelated patterns (Table 2).

**TABLE 2.** PFGE PATTERNS DETECTED IN ISOLATES OF *S. INIAE* OBTAINED FROM HUMANS AND FISH.

SOURCE OF ISOLATE	No. STUDIED	PFGE PATTERN*		
		PATTERN A	PATTERN A'	NEITHER†
		no. of isolates		
Patients 1 through 9	9	9	—	—
Patients 10 and 11	2	—	2	—
Greater Toronto retail stores‡	11	2	—	9
Greater Toronto suppliers§	27	4	—	23
Vancouver retail stores¶	4	4	—	—
Tilapia brains				
From Texas	10	—	10	—
From Virginia	1	1	—	—
ATCC type strains**				
29177	1	—	—	1
29178	1	—	—	1

\*Pattern A' differed by a single band from pattern A when PFGE was performed with restriction enzymes *Sma*I and *Apa*I.

†Nineteen different, unrelated patterns were included in this category.

‡The fresh fish sampled included 25 tilapia, 4 perch, and 7 bass, and *S. iniae* isolates were recovered from 8 tilapia, 2 striped bass, and 1 green bass.

§Thirty-three live tilapia were tested from convenience samples provided by Fisheries and Oceans Canada.

¶Four tilapia from Vancouver retail stores were sampled.

||Samples were obtained during epizootics among fish in 1993.

\*\*ATCC denotes American Type Culture Collection.

### Epidemiologic Investigation

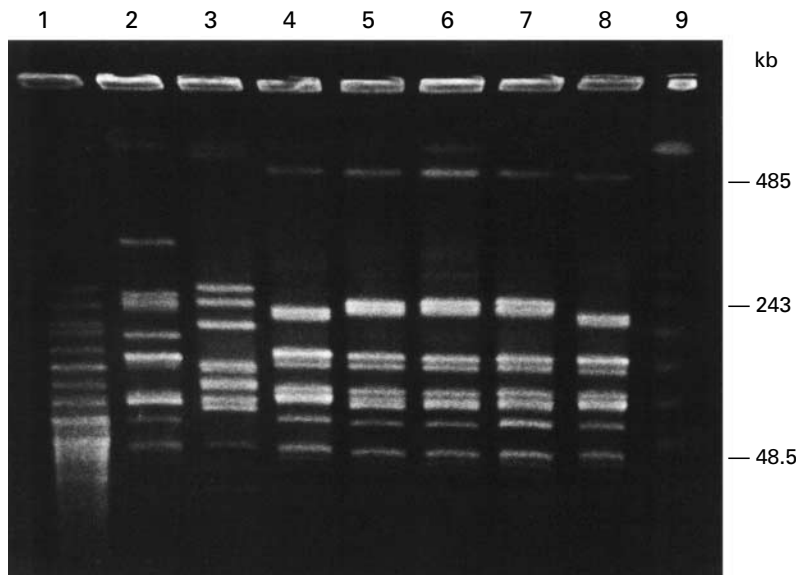
We were unable to identify any one farm as the probable source of the fish associated with the cases of cellulitis. Each of the first four infected patients purchased tilapia from a different retailer in the greater Toronto area. In the six weeks before each purchase, all these retailers had been supplied, through wholesalers, from a total of six fish farms in the United States — two in North Dakota and one each in Tennessee, Arkansas, Delaware, and Illinois. Only one of the suppliers identified in this investigation exported fish during the period of the sampling. *S. iniae* was identified from that supplier's fish, but it did not have the A or the A' PFGE pattern.

### DISCUSSION

Whether these *S. iniae* infections represent the emergence of a new pathogen affecting humans or cases of previously unrecognized disease is unclear. Such infections may not have been recognized in the past as a cause of cellulitis, for several reasons. Cellulitis occurring after local injury or spontaneously is by far most often due to *S. pyogenes* or *Staphylococcus aureus*.<sup>21</sup> Cultures are usually not diagnostic and are therefore not routinely obtained.<sup>22</sup> Hook et al. were able to isolate pathogens from only 26 percent of patients with cellulitis, even though they cultured punch-biopsy specimens, aspirates, and blood.<sup>22</sup> Under certain growth conditions the  $\beta$ -hemolysis of *S. iniae* may not be evident, and it may therefore be misidentified as a viridans streptococcus and considered a contaminant. Even if identification to the species level were performed with current commercial systems of identification, *S. iniae* would probably not be correctly identified, since it is not found in those data bases. Six of the clinical isolates we studied were originally identified as *S. uberis*.

Evidence supporting the possibility that *S. iniae* is a newly emerging pathogen includes the fact that the organism has only recently been identified as a pathogen in fish produced by the aquaculture industry. It has been suggested that streptococcal infections in fish have become increasingly important because of overcrowding in farms and transport.<sup>4,9</sup> We do not believe that *S. iniae* has gone unrecognized in the greater Toronto area because of a failure of identification, since most hospitals routinely refer viridans streptococci isolated from sterile sites to a reference laboratory.

Our surveillance identified 12 patients with suspected infections on the basis of the clinical presentation of cellulitis of the hand and a history of handling fish in the previous 72 hours. Although the case definition may lack specificity, as evidenced by the patient with cellulitis due to *A. hydrophila*, suspected cases may well have been due to *S. iniae* infection. The clinical presentations were remarkably



**Figure 2.** PFGE Analyses of Strains of *S. iniae* after the Digestion of Chromosomal DNA with *Sma*I. Lane 8 shows pattern A, which was seen in all the patients with confirmed infection in the greater Toronto area. Lanes 5 (from a tilapia brain; Texas, 1993), 6 (from Patient 11; Texas, 1991), and 7 (from Patient 10; Ottawa, 1994) have the shift of a single band, known as pattern A'. Lane 4, from a fish obtained at a retail store in Toronto, shows pattern A. The PFGE patterns in lanes 1 (ATCC strain 29177), 2 (ATCC strain 29178), and 3 (*S. iniae* from a fish obtained at a retail store in Toronto) are unrelated. Lane 9 shows a molecular-size ladder, used as a standard.

similar, and most of the patients had been injured while preparing tilapia.

Although isolates of *S. iniae* obtained from the surfaces of tilapia and other species of fish are genetically diverse, only two distinct, highly related clones (with PFGE patterns A and A') caused invasive disease. There have been similar findings with regard to other bacteria that cause infectious diseases.<sup>23</sup> This suggests that a virulence factor or factors that are not present in all strains may be important for pathogenicity in humans and fish.

The explanation for the finding that tilapia was so frequently associated with disease may be that surface colonization with *S. iniae*, particularly with the invasive clone, is restricted primarily to that species or to tilapia from farms where the invasive clone is endemic. Our surveillance data do suggest that *S. iniae* is a common bacterium on tilapia grown by aquaculture. Our results do not rule out other commercial fish as sources of *S. iniae*. We could not determine from the epidemiologic studies whether the invasive clone of *S. iniae* was restricted to one or more farms. Although the invasive clone that had PFGE pattern A was isolated from fish from two farms, neither was identified in the epidemiologic investigation. Unfortunately, we could not sample fish or water directly from the potentially implicated farms.

All 9 patients with confirmed *S. iniae* infection

and 11 of the 12 who had culture-negative suspected infections were of Asian origin. Asians make up 5.6 percent of the population of greater Toronto, and they were clearly overrepresented in our study.<sup>24</sup> This may be related to the volume of tilapia this population consumes or to the manner in which the fish are processed before cooking. Typically, our patients purchased the fish live from aquariums in retail stores, where they were killed and gutted, but with all their appendages left intact. The fish were kept at 4°C for up to 48 hours, at which point they were cleaned further before cooking. This technique contrasts with the methods of purchasing and preparing fish found in many other ethnic communities, where fish are dead before purchase and kept packed on ice in retail stores. In such communities, fish are usually scaled and cleaned, and the head, tail, and fins are removed, by members of the retail staff. These practices may reduce the potential for the inoculation of pathogens.

Our surveillance for cellulitis associated with injuries during the handling of fish was not population-based, and we do not know the sensitivity of the reports made by the emergency departments during the survey period. Furthermore, current diagnostic tests are not adequate to define the bacterial cause of cellulitis when no cultures are obtained from a sterile site or such cultures are negative. Our data suggest that cellulitis may occur in association with in-

juries received during fish preparation, but they do not allow an estimate of frequency or of the proportion of cases that may be associated with a given pathogen.

Other streptococcal species have been shown to be capable of causing zoonotic infections.<sup>25-34</sup> Outbreaks of *S. suis* septicemia and meningitis have been documented in pigs, especially under adverse environmental conditions.<sup>29,33</sup> Similar disease has been described in humans after contact with live or slaughtered pigs.<sup>30-34</sup> The portal of entry is unknown, but it often appears to be the skin. As we found with *S. iniae*, only certain clones of *S. suis* are commonly associated with disease in humans.<sup>35</sup>

The demonstration of another new pathogen linked to the food industry is not surprising, considering that changes in the production, storage, distribution, and preparation of food, as well as environmental changes, provide increased opportunity for humans to be exposed to new organisms that may be pathogenic.<sup>36,37</sup> *S. iniae* can cause invasive disease in humans, but when proper precautionary measures are taken during the handling of whole, uncooked fish, the infections caused by *S. iniae* should be preventable.

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## APPENDIX

The following persons were also members of the *S. iniae* Study Group: M. Patel and J. Fuller, Department of Microbiology, Mount Sinai Hospital, Toronto; M. Baqi and W. Gold, Department of Medicine, University of Toronto, Toronto; J. Hoeve, Fisheries and Oceans Canada, Toronto; J. Urquhart, Toronto Health Department, Toronto; K. Gorman and C. D'Cunha, City of Scarborough Public Health Department, Scarborough, Ont.; and M. Lovgren, National Centre for Streptococcus, Laboratory Centre for Disease Control, Edmonton, Alta.

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