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Edwardsiella tarda in Freshwater Catfish and Their Environment

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Edwardsiella tarda was isolated from 47, 88, and 79% of skin, visceral, and dressed-fish samples, respectively. This species was also isolated from 30% of imported dressed fish, 75% of catfish pond water samples, 64% of catfish pond mud samples, and 100% of frogs, turtles, and crayfish from catfish ponds. The incidence of *Edwardsiella* increased during the summer months, as water temperatures increased. Of several isolation media evaluated, the most effective was selective enrichment in double-strength *Salmonella-Shigella* broth and subsequent plating on single-strength *Salmonella-Shigella* agar. The significance of the incidence of *Edwardsiella* in catfish, catfish disease, and public health could not be substantiated.

The occurrence of *Edwardsiella tarda* in catfish was first recorded by Meyer and Bullock (16). *Edwardsiella* was isolated from lesions on the posterolateral areas of infected catfish. The disease was described as abscesses that rapidly increased in size and developed as large cavities filled with gas that emitted a foul odor when incised. The disease was termed "emphysematous putrefactive disease of catfish," aptly describing the gross appearance of infected fish. The proposed etiological agent of emphysematous putrefactive disease of catfish, *E. tarda*, is a member of the family *Enterobacteriaceae*. The species was first recognized by Trabulski and Ewing (24) in work published in 1962 and was introduced as a new group of *Enterobacteriaceae* in 1964 (7). King and Adler (14) described the biochemical reactions of an isolate from a patient with acute enteric fever and gastroenteritis. They called this group of *Enterobacteriaceae* the Bartholomew group. In 1965, Sakazaki (19) presented work describing new *Enterobacteriaceae* called the Asakusa group. The strains reported by each of these workers had similar biochemical reactions. In 1975, Ewing et al. (8) proposed the name *Edwardsiella* and defined the genus as "composed of motile bacteria that conform to the family *Enterobacteriaceae* and the tribe *Edwardsiella*." The genus is composed of only one species. A. C. McWhorter, W. H. Ewing, and R. Sakazaki (Bacteriol. Proc., p. 89, 1967) developed a provisional scheme for the O and H antigenic characteristics of *Edwardsiella*, but it was incomplete because it covered less than 90% of the serotypes.

Edwardsiella has been implicated in gastroenteritis in humans (10, 13, 14, 20) and in bacteremic infections that include wound abscesses (9, 11, 13) and meningitis (18, 22). It has been isolated from a diseased pig (17) and a diseased ostrich (27) and has been implicated as the causative agent of a disease in pond-reared eels (26). *Edwardsiella* has been isolated from snakes (12, 19), tortoises (12), crocodiles (12), seals (13, 19), frogs (21), aquarium water (3), and swine (2, 17) and from seagull roosting areas (4). There are many unanswered questions as to the significance of *Edwardsiella* as an animal, fish, or human pathogen and to its relative occurrence in nature. This study was initiated when *Edwardsiella* was isolated on several occasions during examination of dressed catfish for *Salmonella* (28). This report provides information on the isolation, identification, and incidence of *Edwardsiella* in freshwater catfish and their environment.

MATERIALS AND METHODS

Sample collection. This study was performed in conjunction with a study on the occurrence of *Salmonella* in catfish (28) in which the sampling protocol has been reported. Samples tested were 92 fresh, domestic catfish carcasses; 61 frozen, imported catfish carcasses; 16 catfish skin samples; 16 catfish visceral samples; 86 pond water samples; 86 pond mud samples; 12 crayfish; 4 turtles; 2 frogs; 5 commercial feed samples; and 46 cattle fecal samples.

Bacteriological procedures. Isolation of *Edwardsiella* was performed in conjunction with the *Salmonella* procedure previously described (28). The *Salmonella* method included preenrichment in 0.5% lactose broth, followed by selective enrichment in tet-

rathionate and selenite-cystine which were steaked on *Salmonella-Shigella* (SS) agar (Difco) with 1% sucrose and 0.65% agar as recommended by Sperber and Deibel (23), brilliant green agar with sulfadiazine, and bismuth sulfite agar. All incubations were at 35°C for 24 h. Double-strength SS broth (DSSS) and gram-negative (GN) broth were added as selective enrichment for *Edwardsiella*. DSSS was prepared with 120 g of SS agar, 5 g of glucose, and 1 liter of distilled water. The mixture was stirred without heat to dissolve the ingredients. The agar was removed by suction filtration through a Whatman no. 1 filter. The broth was boiled for 5 min and dispensed in 10-ml quantities into tubes (18 by 150 mm). The tubes were steamed for 30 min. A 1-ml amount of the lactose broth preenrichment was transferred to each of these broths. After incubation at 35°C for 24 h, they were streaked onto SS agar and either deoxycholate-citrate agar or xylose-lysine-deoxycholate agar. The selective agars were incubated at 35°C for 24 h. Typical colonies were picked to triple sugar iron agar slants and motility-indole-lysine deeps (28) and incubated at 35°C for 24 h. Any isolate exhibiting an alkaline slant and acid butt with H_2S and gas in triple sugar iron agar and with indole and lysine decarboxylase production in motility-indole-lysine deeps was selected for additional testing. Suspect isolates were tested for citrate utilization (Simmons citrate agar); methyl red Voges-Proskauer reactions (methyl red-Voges-Proskauer broth); acid production from mannitol, maltose, and glucose (0.5% in phenol red broth); ornithine dihydrolase activity (motility-indole-ornithine medium); and growth in KCN.

The *Edwardsiella* most-probable-number tests for pond water were conducted as previously reported for *Salmonella* (28). Volumes of water tested ranged from 100 through 0.01 ml, and selective enrichment was in DSSS and GN broth.

Statistical methods. Analysis of variance and the new Duncan multiple range test were used to identify significant differences among broths and samples. The modified new Duncan multiple range test (15) for unequal samples was used to detect differences among agars.

RESULTS AND DISCUSSION

Based on the media used in the work of Iveson (12) with tiger snakes in Australia and the use by Sakazaki et al. (20) of GN for *Edwardsiella* isolation, bile salts, rather than chemical agents, were the best selective agent for the isolation of *Edwardsiella*. The DSSS medium developed in this study was made from SS agar because it had the highest bile salt content of the available media.

SS agar and deoxycholate-citrate agar were selected for testing the recovery of *Edwardsiella* from the experimental enrichment broths because of initial isolations in this study on SS agar and the report of Sakazaki et al. on the use of deoxycholate-citrate agar (20). In addition, brilliant green, bismuth sulfite, and xylose-lysine-deoxycholate agars were used. Pure cultures of *Edwardsiella* did not grow on brilliant green agar. Colonies on bismuth-sulfite agar were black and about 1 mm in diameter. Colonies on SS agar varied from 1 to 3 mm in diameter and were characterized by small, black centers to predominantly black colonies. *Edwardsiella* was visually differentiated from *Salmonella* colonies on SS agar because of the more rapid production of H_2S and the raised shape of the colony, whereas *Salmonella* colonies were typically flatter and opaque with small, black centers. On xylose-lysine-deoxycholate agar *Edwardsiella* produced clear colonies with black centers surrounded by reddened medium due to the decarboxylation of lysine.

Different formulations of SS broth were prepared and inoculated from lactose preenrichments of catfish samples. Single-strength SS broths with and without 0.5% glucose were autoclaved. This resulted in very few suspect *Edwardsiella* colonies, with the majority of the other isolates being either lactose or sucrose positive. When this same formulation was used, but boiled and steamed for 30 min, approximately one-half of the isolates were suspect *Edwardsiella*. DSSS broths with and without 0.5% glucose were boiled and steamed for 30 min. The use of DSSS without 0.5% glucose resulted in the majority of the colonies on the SS agar plates being suspect *Edwardsiella*. When DSSS with 5% glucose was used, almost all isolates were suspect *Edwardsiella*. All suspect colonies tested from the various broths proved to be *Edwardsiella*. Based on the results of this initial screening, DSSS was used as a selective enrichment for *Edwardsiella*. GN broth was also used as a selective enrichment for *Edwardsiella* and served as a control medium. These two selective enrichments were streaked on SS and deoxycholate-citrate agars during the early part of the study. Later, xylose-lysine-deoxycholate agar was substituted for deoxycholate-citrate because of the H_2S indicator in xylose-lysine-deoxycholate agar.

The Texas Department of Health Resources Laboratory verified the identification of one typical and three atypical (one nonmotile, one indole negative, one maltose negative) isolates as *E. tarda*. The results given in Table 1 were obtained from 74 isolates taken from catfish and their environment. The results of these tests are in close agreement with those published by Ewing et al. (6).

Edwardsiella was present on 47% of the 16 skin samples; 88% of the 16 visceral samples; 79% of the 92 domestic fish; 30% of the 61 imported fish; 75% of the 86 pond water samples; 64% of the 86 pond mud samples; 100% of the 2 frogs, 4 turtles, and 12 crayfish; and 2% of the 46

TABLE 1. *Biochemical results of 74 Edwardsiella isolates from catfish and their environment*

Test	Reaction	% of isolates
Indole	+	98.9
Methyl red	+	100
Voges-Proskauer	−	100
Simmons citrate	−	100
Lysine decarboxylase	+	100
Ornithine dihydrolase	+	100
Motility	+	94.5
KCN	−	100
Glucose		
Acid	+	100
Gas	+	100
Mannitol	−	100
Maltose	+	98.9

cattle fecal samples and was absent in the 5 commercial feed samples.

In March when the water temperature was 15°C, each of 30 ponds in central Texas was tested for the presence of *Edwardsiella* in 500 ml of water and in a 25-g mud sample. *Edwardsiella* was present in water samples from 14 ponds and in mud samples from 15 ponds. Of these positive ponds, only eight were positive in both water and mud samples. Further testing was done monthly on five ponds that initially had *Edwardsiella*-positive water samples and five ponds that had negative water samples. A most-probable-number test was performed to determine if the number of *Edwardsiella* increased during the warmer months. Table 2 gives the *Edwardsiella* most probable numbers found in the ponds from April through August. The increase in *Edwardsiella* can probably be explained by (i) the increase in water temperature, (ii) an increase in the organic content of the pond due to heavier feeding rates, and (iii) an observed increase in the numbers of amphibians in the ponds.

The data generated for the isolation techniques, media, and sample groups were evaluated to determine the efficiency of each method. The results are presented in Table 3. For all samples tested the experimental selective enrichment broth, DSSS, had a significantly higher recovery rate than the other three media. The performance of each combination of broth and agar was evaluated to determine the effect of the agar in successful isolation of *Edwardsiella*. The DSSS combinations were significantly different from the GN broth combinations. Each GN-agar combination was significantly different from the other, indicating the significant role played by the agars in overall effectiveness.

Subdividing the samples to evaluate the recovery effectiveness of each broth for each sample type could be used to illustrate the relative concentrations of *Edwardsiella* present. This

TABLE 2. *Edwardsiella most probable numbers per 100 ml for 10 catfish ponds*

Pond	*Edwardsiella* presence				
	March[a]	Most probable no./100 ml in:			
		April	May	June	August
1	−	110	110	>1,100[b]	>11,000[b]
2	−	0.73	21[b]	>1,100[b]	750
3	−	>110	460[b]	1,100[b]	2,400[b]
4	−	110	46	>110[b]	2,400[b]
5	−	9.3	1,100[b]	>1,100[b]	750
6	+	>110	240[b]	1,100[b]	93
7	+	110	1,100[b]	43	43
8	+	>110	23	9.1	43[b]
9	+	>110	240[b]	>1,100[b]	2,400[b]
10	+	>110	150[b]	1,100[b]	2,400[b]

[a] +, *Edwardsiella* present; −, *Edwardsiella* absent.
[b] Increase from previous sample.

TABLE 3. *Comparison of DSSS, GN, tetrathionate, and selenite-cystine broths for efficacy of Edwardsiella isolation*

Samples	Selective agar	Isolation rate[a] from broth			
		Selenite-cystine	Tetrathionate	GN	DSSS
All		0.12	0.16	0.74	0.96
	Xylose-lysine-deoxycholate			0.30	0.69
	Deoxycholate-citrate			0.43	0.71
	Salmonella-Shigella			0.50	0.87
Viscera		0.13	0.53	0.87	1.00
Skin		0.25	0.63	0.75	1.00
Dressed fish		0.08	0.2	0.61	0.92
Water		0.11	0.04	0.62	0.96
Mud		0.30	0.30	0.63	0.96

[a] No significant difference ($P = 0.05$) for underscored results.

also shows that DSSS is effective at lower population levels of *Edwardsiella*. There was no significant difference between isolation rates for DSSS and GN broth for viscera samples or between DSSS and GN and tetrathionate broths for skin samples. However, DSSS was significantly better in all other sample divisions. No *Salmonella* was isolated with DSSS from known *Salmonella*-positive samples, whereas *Edwardsiella* and *Salmonella* were both isolated from tetrathionate broth in five samples. The data presented show that DSSS can be used for the specific isolation of *Edwardsiella* from environmental samples with a high degree of success.

The reported worldwide isolations of *Edwardsiella* indicate that water-borne animals may be carriers. These reported isolations have primarily been incidental to *Salmonella* recovery (1, 3, 4, 12, 21, 25). The results of this study suggest that the recovery rates found by these workers are probably far below the actual levels of *Edwardsiella* present. This adds additional uncertainty to the role of *Edwardsiella* as a primary human pathogen capable of causing gastroenteritis. Studies by Sakazaki et al. (20), and Bhat and Meyers (5) did not conclusively correlate gastrointestinal illness to *Edwardsiella*, particularly since it was isolated in cases along with the enteric pathogens *Salmonella* and *Shigella*. In a study by the Food and Drug Administration (1) on retail catfish, the low incidence of *Salmonella* food poisoning from catfish was related to the lack of *Salmonella* isolations from catfish. However, the high incidence of *Edwardsiella* found in dressed catfish cannot support this same theory if it is indeed an intestinal pathogen. Although catfish are thoroughly cooked before consumption, the possibility of cross-contamination exists.

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