

Susceptibility of nonictalurid fishes to experimental infection with *Edwardsiella ictaluri*

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ABSTRACT: Studies were conducted to determine the potential pathogenicity of *Edwardsiella ictaluri* to economically important nonictalurid fishes in California, USA. White sturgeon *Acipenser transmontanus*, striped bass *Morone saxatilis*, and chinook salmon *Oncorhynchus tshawytscha* were immersion-challenged in parallel with channel catfish *Ictalurus punctatus*. During a 14 d period, chinook salmon and channel catfish succumbed to infections with *E. ictaluri* but the other species did not. An immersion exposure to 4.0 and 7.9×10^8 cfu ml⁻¹ of *E. ictaluri* for 30 s resulted in a 92 and 48 % mortality among chinook salmon and rainbow trout *O. mykiss*, respectively. A Gram-negative septicemia occurred in infected fishes, and pure cultures of *E. ictaluri* were recovered from dead and surviving fish. There was a moderate to severe necrosis of the liver and kidney in both salmonids and channel catfish. Intracellular bacteria occurred within mononuclear inflammatory cells and hepatocytes. These results suggest that *E. ictaluri* is a potential pathogen of salmonid fishes.

INTRODUCTION

Edwardsiella ictaluri is the causative agent of enteric septicemia of channel catfish *Ictalurus punctatus* (Hawke 1979, Hawke et al. 1981). The disease caused by this Gram-negative bacterial pathogen is considered to be one of the most economically important in commercial catfish farms in the southeastern USA (Francis-Floyd et al. 1987). The number of documented disease outbreaks due to *E. ictaluri* represented more than 50 % of all fish mortalities in Mississippi in 1985 and 1986 (Francis-Floyd et al. 1987). Chen & Kumlin (1989) first reported the occurrence of an epizootic in channel catfish in California during the summer of 1987.

Edwardsiella ictaluri is reportedly specific to ictalurids (Waltman et al. 1985). However, isolation of the bacterium from nonictalurid species has been reported in natural disease outbreaks in green knifefish *Eigemannia virescens* (Kent & Lyons 1982), danio *Danio devario* (Blazer et al. 1985, Waltman et al. 1985), and walking catfish *Clarias batrachus* (Kasornchandra et al. 1987). In addition, an *E. ictaluri*-like organism was recently isolated from cultured sea bass *Dicentrarchus labrax* in Spain (Blanch & Jofre 1989). Experimental exposures to *E. ictaluri* by intraperitoneal injection

showed that tilapia *Sarotherodon aureus* were also susceptible to the bacterium, but that golden shiners *Notemigonus crysoleucas*, bighead carp *Aristichthys nobilis*, and largemouth bass *Micropterus salmoides* were resistant (Plumb & Sanchez 1983).

The following report describes the results of studies to determine the susceptibility of chinook salmon *Oncorhynchus tshawytscha*, rainbow trout *O. mykiss*, white sturgeon *Acipenser transmontanus*, and striped bass *Morone saxatilis* to experimental immersion exposures to *Edwardsiella ictaluri*.

MATERIALS AND METHODS

Bacterial strain. The *Edwardsiella ictaluri* strain used was isolated from diseased channel catfish from a private culture facility in Reno, Nevada, in October 1987. The fish were submitted to the Fish Disease Laboratory at the University of California, Davis, as a routine clinical case. Evidence of septicemia was observed in all fish examined, and pure cultures of *E. ictaluri* were isolated. The bacterium was passed twice in channel catfish and then lyophilized for subsequent experiments. Prior to each challenge, bacteria were grown on Brain-Heart Infusion (BHI) agar (Difco, MI, USA) for 24 h. Isolated colonies were inoculated into BHI broth and then incubated for 48 h at 26 °C on a

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rotary shaker. Plate counts were made to determine the approximate number of viable bacteria for each challenge.

Fish. In susceptibility studies, channel catfish and 3 nonictalurid species were tested in parallel immersion challenges. Groups of 50 channel catfish (mean weight 2.0 g), 20 white sturgeon (30.0 g), and 20 striped bass (6.6 g) were acclimated to 25°C for 5 d prior to challenge. Groups of 20 chinook salmon (36.6 g) were similarly acclimated to 20°C. Fish were held in 132 l tanks receiving flow-through well-water and were maintained at the above temperatures during the experiments. Four groups of chinook salmon (50 fish each, 39 g) and 4 groups of rainbow trout (25 fish each, 45 g) were also acclimated as above and maintained at 20°C for LD₅₀ experiments.

Challenges. In susceptibility studies, groups of 50 channel catfish and groups of 20 fish of the other species were exposed for 30 s to a 48 h culture (full strength broth) of the bacterium in separate 5 l containers and then returned to their tanks. Dead fish were collected once daily and kidney tissues from these were inoculated onto BHI agar. At 14 d post-exposure, 15 channel catfish and all survivors in the other challenged groups were examined (by culture) for the presence of *Edwardsiella ictaluri* in the kidney.

In LD₅₀ studies, replicate groups of 50 chinook salmon and replicates of 25 rainbow trout were challenged in the same manner but exposed to full strength, 10⁻¹ and 10⁻² dilutions of a 48 h broth culture of *Edwardsiella ictaluri* containing 4.0 × 10⁸, 4.0 × 10⁷ and 4.0 × 10⁶ cfu ml⁻¹, respectively, for chinook salmon and 7.9 × 10⁸, 7.9 × 10⁷ and 7.9 × 10⁶ cfu ml⁻¹, respectively, for rainbow trout. Identical control groups of each species in both studies were exposed only to uninoculated BHI broth. Dead fish in the chinook salmon LD₅₀ studies were collected once daily. The kidneys of these dead fish and of those surviving to 14 d post-exposure were cultured aseptically on BHI agar. Isolates recovered from the kidney samples were grown in pure culture and subsequently tested for their biochemical properties and by agglutination with hyperimmune rabbit anti-*E. ictaluri* serum. In LD₅₀ rainbow-trout trials, dead fish were collected twice daily and their kidneys, along with those from fish surviving to 14 d, were similarly cultured on BHI agar. Kidney tissues from 4 rainbow trout were also examined for their *E. ictaluri* concentrations. Individual kidneys from these 4 fish were weighed, homogenized in 0.85% NaCl (1:10 w:v) with a sterile glass homogenizer, and diluted for bacterial plate counts. Freshly dead fish were preserved for later histopathological examinations. Values for LD₅₀ were calculated using the procedure of Reed and Muench (1938).

Antibody titers in fish serum. Caudal blood (0.5 ml) was collected from each surviving fish in nonheparinized microhematocrit tubes (Fisher, PA, USA) at 14 d post-exposure and then centrifuged at 2000 × g for 5 min. An equal amount of serum from each of 10 fish was pooled and then serially diluted in 1:2 steps with 0.85% NaCl in standard V-bottom microtiter plates (Costar, MA, USA). Formalin-killed *Edwardsiella ictaluri* (optical density = 0.9) was used as antigen and the agglutination titer was determined after 24 h (Saeed & Plumb 1987).

Histopathology. For histopathological examination, whole channel catfish and striped bass were placed in Bouin's fixative and salmonid tissues (including heart, kidney, liver, gills, intestinal tract, and spleen) were placed in Davidson's fixative. Following fixation for 24 h, specimens were transferred to 70% ethanol prior to routine processing (Humason 1979). Tissues were embedded in paraffin, and 6 μm sections were stained with Harris' haematoxylin and eosin (H&E) or Warthin Starry reagents.

RESULTS AND DISCUSSION

The bacterium isolated from dead and surviving fish was a Gram-negative straight rod that measured ca 1 × 2 μm; it was oxidase-, indole-, and citrate-negative, and produced an alkaline slant and an acid butt on Triple Sugar Iron agar (Difco). On BHI agar, colonies were round, white, and ca 1 mm diam after 3 d incubation at 26°C. Isolates were confirmed as *Edwardsiella ictaluri* based on these differential characteristics (Hawke et al. 1981) and on the basis of a positive agglutination reaction with hyperimmune rabbit serum prepared against *E. ictaluri* (Saeed & Plumb 1987).

In susceptibility studies, mortalities did not occur in control groups of any of the 4 species tested or among white sturgeon exposed to the bacterium. In contrast, there was a 32% (16/50) mortality in channel catfish, 75% mortality (15/20) in chinook salmon, and 5% mortality (1/20) in striped bass over the 14 d test period following challenge (Table 1). External clinical signs of a Gram-negative septicemia occurred in the catfish and chinook salmon that died during the experiment. There was cutaneous petechiation and ecchymosis around the mouth, on the ventral portions of the body, and at the base of the fins. Bilateral exophthalmos and pale gills were occasionally observed. Pure cultures of *Edwardsiella ictaluri* were recovered from the dead fish and survivors in channel catfish (5/16, 3/15, respectively), chinook salmon (7/15, 2/5, respectively), and from the single striped bass mortality (Table 1). White sturgeon were considered resistant to infection with *E. ictaluri* because the bacterium was not reisolated

Table 1. *Edwardsiella ictaluri*. Mortality and antibody response of different fish species following immersion challenges with the pathogen. Each group of fish was exposed separately for 30 s to a 48 h broth culture (1.0×10^8 cfu ml⁻¹) and then placed in 132 l tanks receiving 25 °C well-water (except for chinook salmon which were maintained at 20 °C). A corresponding group of controls for each species was treated identically but were exposed to BHI broth with no bacteria. None of the control fish of each species died during the duration of the experiment and none developed detectable agglutination titers. No.: No. mortalities/no. fish exposed; Dead: Fish positive for *E. ictaluri*/no. examined

Fish	Mean wt (g)	Mortality		Reisolation		Mean agglutination titer ^a
		No.	%	Dead	Surviving	
Channel catfish	2.0	16/50	32	5/16	3/15	64
Chinook salmon	36.6	15/20	75	7/15	2/5	63
Striped bass	6.6	1/20	5	1/1	0/19	32
White sturgeon	30.0	0/20	0	–	0/20	16

^a Blood (0.5 ml) was withdrawn from the caudal vessel in microhematocrit tubes from each surviving fish at 14 d post exposure. Resulting sera were pooled in each group (10 fish/group) and serially diluted: 1:2 in 0.85 % NaCl. Formalin-killed *E. ictaluri* (optical density = 0.9) was used as antigen

Table 2. *Edwardsiella ictaluri*. Mortality and antibody response of chinook salmon and rainbow trout following immersion challenges with selected concentrations of bacterium. Chinook salmon (50 fish, mean weight 39.0 g) and rainbow trout (25 fish, 45.0 g) were used and groups of fish were exposed to full strength, 10^{-1} or 10^{-2} dilutions of a 48 h broth cultures of *E. ictaluri*. An identical control group for both species was exposed to uninoculated BHI broth. Water temperature was maintained at 20 °C. Dead: no. fish positive for *E. ictaluri*/no. examined

Bacterial dose (cfu ml ⁻¹)	Mortality		Reisolation		Mean agglutination titer ^a
	No.	%	Dead	Survivor	
Chinook salmon					
4.0 × 10 ⁸	46	92	6/46	0/4	128
4.0 × 10 ⁷	9	18	2/9	0/41	128
4.0 × 10 ⁶	2	4	2/2	0/48	64
Control	0	0	–	0/50	0
Rainbow trout					
7.9 × 10 ⁸	12	48	12/12	1/13	128
7.9 × 10 ⁷	0	0	–	0/25	32
7.9 × 10 ⁶	0	0	–	0/25	8
Control	0	0	–	0/25	0

^a Blood (0.5 ml) was withdrawn from the caudal vessel in microhematocrit tubes from each surviving fish at 14 d post exposure. The resulting sera were pooled in each group (10 fish/group) and serially diluted 1:2 in 0.85 % NaCl. Formalin-killed *E. ictaluri* (optical density = 0.9) was used as antigen

from the kidneys and there was no mortality or evidence of clinical disease following challenge.

The LD₅₀ of *Edwardsiella ictaluri* for chinook salmon in our study was 3.4×10^7 cfu ml⁻¹. An LD₅₀ value for rainbow trout could not be calculated because mortalities occurred only at the highest dose tested (Table 2). However, a concentration of 7.9×10^8 cfu ml⁻¹ of *E. ictaluri* was sufficient to cause a 48 % mortality in rainbow trout. Gross signs of hemorrhagic septicemia were less severe in moribund or dead rainbow trout than in affected chinook salmon. These external signs were less consistent and generally restricted to the lateral line or pectoral fins in rainbow trout, although *E. ictaluri* concentrations in the kidney ranged from 4.0 to 7.1×10^7 cfu g⁻¹.

In both susceptibility and chinook salmon LD₅₀ studies, *Edwardsiella ictaluri* was reisolated from less

than 50 % of the fish that died during the experiment. We assume that the cause of death was in all cases *E. ictaluri* but that the presence of faster growing bacteria in the tissues masked the colonies of *E. ictaluri* (Rogers 1981). To test this hypothesis, in the rainbow trout LD₅₀ trials, dead fish were collected twice, rather than once, daily. This resulted in the reisolation of the bacterium from all of the dead fish in this study. This suggests that *E. ictaluri* does not survive well in dead tissue or that secondary invaders out compete the pathogen once the fish dies, making subsequent attempts at recovering the pathogen difficult.

Agglutinating antibody titers were higher in *Edwardsiella ictaluri*-exposed channel catfish, chinook salmon, and striped bass than in exposed white sturgeon (Table 1). The control fish of each species did not

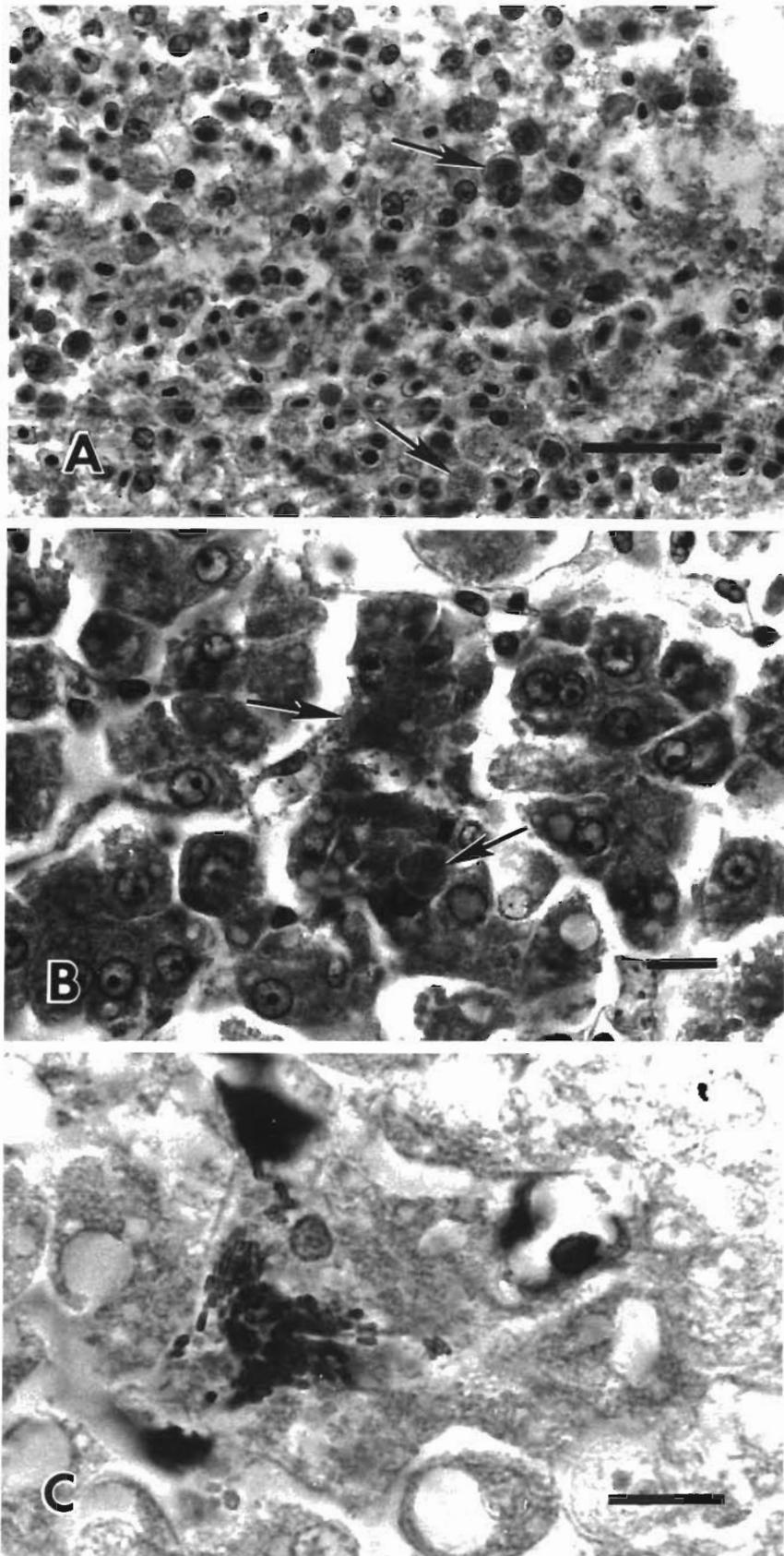


Fig. 1. *Edwardsiella ictaluri*. Chinook salmon *Oncorhynchus tshawytscha* infected by the pathogen. (A) Inflammatory cells (arrows) in the blood sinuses of infected kidney. H & E stain. Bar = 50 μ m. (B) Intracellular bacteria (arrows) within hepatocytes. H & E stain. Bar = 20 μ m. (C) Bacteria in blood sinuses of liver. Warthin and Starry stain. Bar = 20 μ m.

develop any detectable antibody response to the bacterium even at the lowest serum dilution tested.

Histopathological examination of channel catfish revealed a moderate to severe necrosis in the kidney, liver, and spleen at 3 d post-exposure. These lesions were similar to those previously observed in liver, kidney, spleen, and pancreas of channel catfish infected with *Edwardsiella ictaluri* (Areechon & Plumb 1983, Shotts et al. 1986).

At 3 d post-exposure, microscopic lesions in chinook salmon and rainbow trout were most evident in the liver and kidney. There was a moderate to severe hepatic degeneration and necrosis associated with the presence of the bacterium within a few of the hepatocytes and cells lining the hepatic sinusoids. There was also a diffuse, moderate necrosis of the renal interstitial hematopoietic tissue as well as renal tubular degeneration and necrosis. The lumens of the renal tubules often contained a coarse granular debris. There was an apparent thickening of the glomerular membranes, often with hypercellularity of the glomerular tufts. Mononuclear inflammatory cells within the renal interstitial hematopoietic tissue often contained intracellular bacteria. There was also a mild hyperplasia of the reticuloendothelial cells lining the sinuses of the heart but there was no evidence of the intracellular bacteria within these cells.

Histopathological lesions of the single striped bass that died at 3 d post-exposure were similar to those observed in salmonids. There was, however, an increased number of bacteria-laden macrophages in the periorbital region of the eyes. In contrast, all surviving striped bass showed no signs of infection and the pathogen was not isolated at the end of the study.

The results suggest that *Edwardsiella ictaluri* is a potential pathogen of salmonid fishes. The implications of these results should be seriously considered by fisheries managers because certain reservoirs in California are stocked with channel catfish and salmonid fish for sport fishing. Although Plumb & Sanchez (1983) reported that tilapia can be experimentally infected by intraperitoneal injection with *E. ictaluri*, they concluded that only channel catfish are reproducibly susceptible to the bacterium. However, Waltman et al. (1985) considered the possibility that the host range of *E. ictaluri* might be greater than originally documented. The isolations of similar bacteria from various nonictalurid species like walking catfish in Thailand (Kasornchandra et al. 1987), cultured sea bass in Spain (Blanch & Jofre 1989), green knife-fish (Kent & Lyons 1982) and danio (Blazer et al. 1985) make it a potential worldwide pathogen. Our observations extend the experimental host range of *E. ictaluri* to at least 2 salmonid species, chinook salmon and rainbow

trout – 2 species that are among the most important food and recreational fishes in North America and Europe.

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