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Histopathology and Pathogenesis of Experimental Infection with *Edwardsiella tarda* in Channel Catfish

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Abstract.—The histopathology and pathogenesis of *Edwardsiella tarda*-induced edwardsiellosis in juvenile channel catfish *Ictalurus punctatus* were characterized. The lateral body surfaces of fish were scraped before infection by immersion in a bath of *E. tarda* for 30 min. Scattered cutaneous petechiae and ulcerations were seen on the mouth, operculum, isthmus, abdomen, and lateral body after infection. Injured skin developed focal ulcerative necrotizing dermatitis, increased mucus, and irregular areas of skin depigmentation scattered over the entire body. Internal organs were congested, and livers showed patchy discoloration and petechiae. Histologically, the liver, head kidney, trunk kidney, and spleen had severe multifocal necrotizing inflammation. Scraped controls did not show gross or microscopic lesions except for mild dermatitis of injured skin at the scraping site. Bacteria in hepatic lesions were specifically identified by immunohistochemistry that used rabbit anti-*E. tarda* serum. Colony-forming units of *E. tarda* per gram of tissue or milliliter of blood peaked 3 d postinfection (PI) in the liver, trunk kidney, and blood. No bacteria were isolated from infected fish after 6 d PI. Clinical signs and internal gross lesions declined by 8 d PI and were absent thereafter. No histological lesions were observed in the intestine, stomach, heart, gills, or brain. Infected fish were sluggish and had rapid opercular movements and pale gills before death. Histological lesions were similar to those caused by *E. ictaluri* in channel catfish.

Edwardsiellosis, caused by the bacterium *Edwardsiella tarda*, is an acute to chronic disease of fish, primarily in warm water. *E. tarda*, in the family Enterobacteriaceae, is a gram-negative, noncapsulated, facultative anaerobic bacillus with peritrichous flagella (Farmer and McWhorter 1984). Fish species most commonly infected with *E. tarda* are eels *Anguilla* spp. and channel catfish *Ictalurus punctatus*; however, a variety of other species around the world are susceptible (Plumb 1999).

Edwardsiellosis in channel catfish was first observed on farms in Arkansas, Mississippi, Louisiana, and Texas (Meyer and Bullock 1973). Although not as economically important as enteric septicemia of catfish caused by *E. ictaluri*, edwardsiellosis continues to be a problem in cultured channel catfish. Prevalence of the disease in ponds is seldom above 5%; however, when channel catfish are confined in tanks, prevalence can reach 50% (Meyer and Bullock 1973).

Histopathology of edwardsiellosis has been described for tilapias (Ciclidae) and Japanese eel *Anguilla japonica* (Miyazaki and Egusa 1976a, 1976b; Miyazaki and Kaige 1985) but not for channel catfish. The objective of this research was to describe the histopathology, pathogenesis, and lesions in juvenile channel catfish infected by waterborne exposure to *E. tarda* preceded by skin abrasion.

Methods

Bacteria.—*E. tarda* (strain AL-92-255) was used in two experimental infection trials with yearling channel catfish (experiments 1 and 2). Before each experiment, four consecutive serial passages of the bacteria in channel catfish were executed by intraperitoneal injection of about 2×10^6 colony forming units (CFU) (established by pour plate count) and reisolation. After the fourth passage,

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the bacteria were suspended in a saline solution (0.9% NaCl) and glycerin (25%) and stored at −80°C for experiment 1. The frozen material was thawed and cultured in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, Michigan) before fish exposure. In experiment 2 the bacteria were used immediately after the fourth passage in fish. Pure cultures were verified according to the method of Farmer and McWhorter (1984).

Experimental fish.—Ten days before each experiment, 160 channel catfish (average weight, 37 g) were divided into 16 equal groups in 45-L aerated static aquaria filled with well water. The water had a hardness ($CaCO_3$) of 50 mg/L, temperature of 25 ± 1°C, dissolved oxygen above 6 mg/L, nitrite below 1 mg/L, and pH between 6.5 and 7.0. Ammonia, nitrite, and pH were measured on alternate days by the use of a water- testing kit (Hach Co., Loveland, Colorado). About 50% of the water was replaced with fresh water daily, and sodium chloride (100 mg/L) was added to prevent methemoglobinemia. The fish were fed a maintenance ration every other day until 3 d before infection and in experiment 1 were not fed thereafter, but in experiment 2 fish were fed every other day starting 5 d postinfection (PI). The study was approved by the Auburn University Institutional Animal Care and Use Committee.

Experimental infection.—Waterborne exposure to *E. tarda* after skin scraping with a scalpel was the chosen method of infecting the fish because it is a more natural route of exposure. An approximate 1.0 × 0.5-cm skin area was scraped on both sides dorsal to the lateral line between the dorsal and adipose fins to remove mucus and epithelium. Before each experiment, preliminary studies were performed to estimate the concentration of bacteria that would kill 50% (LD50) of the scraped fish in 96 h after a 30-min exposure to serial 10-fold dilutions of the bacterium. Bacterial concentrations were determined by the optical density of the inoculum that had been calibrated with pour plates. In experiment 1, the LD50 was approximately 6.0×10^6 CFU/mL, whereas in experiment 2, the LD50 was approximately 4.3×10^7 CFU/mL.

In both experiments the 16 aquaria (10 fish each) were divided into 8 infected aquaria and 8 uninfected controls; 4 control aquaria contained scraped fish, and 4 contained uninjured fish. A pure culture of confirmed *E. tarda* was used to inoculate a flask containing 1,000 mL of BHI broth and was incubated at room temperature for 14 h with moderate stirring. Fish to be infected were removed from their home aquaria, their skin was scraped (fish were not anesthetized), and the fish were placed in 10 L of aerated water containing *E. tarda* for 30 min and then returned to their home aquaria. Uninfected, injured, and noninjured controls were similarly handled but not exposed to *E. tarda.*

Sampling for histopathology.—Fish used for histological analysis were sampled every day for 5 d in experiment 1 and on alternate days for 12 d in experiment 2. At each sampling, three infected fish and one from each scraped and nonscraped control group were selected. Moribund fish were sampled first; otherwise fish were collected at random. The fish sampled for histopathology were euthanatized by immersion in 300 mg methanesulfonate 3-aminobenzoic ethyl ester/L and immediately dissected, and organs were fixed in 10% zinc-buffered formalin (Z-Fix; Anatech, Ltd., Battle Creek, Michigan) for 12–16 h. After fixation, the tissues were stored in 50% isopropanol. When tissues were processed, they were dehydrated in isopropanol, cleared in Histosol (National Diagnostics, Inc., Somerville, New Jersey), embedded in Paraplast (Oxford Labware, St. Louis, Missouri), sectioned (5 m thick), and stained with hematoxylin and eosin (H&E). Lesions were graded for severity and scored as mild, moderate, or severe histopathology.

Sampling for gross pathology and bacterial analysis.—In experiment 1, five additional moribund (or selected-at-random) fish from the infected treatment and one from each control treatment were collected daily to study the external and internal lesions and to make CFU counts of *E. tarda* in tissues. Livers and trunk kidneys were aseptically removed, weighed, and homogenized in 4 mL of sterile saline (0.9% NaCl solution). Tenfold serial dilutions were made in saline, and 40 L of each dilution was spread on duplicate petri plates containing *Edwardsiella* isolation medium (EIM) (Shotts and Waltman 1990). Blood was drawn from the caudal vein or artery into a 1-mL tuberculin syringe with a 26-gauge needle. The volume of blood drawn was recorded, added to 4 mL of sterile saline, mixed thoroughly, serially diluted, and inoculated onto EIM plates in a manner similar to that used for homogenized tissue. Culture plates were incubated for 24–36 h at 30°C, and the CFU per gram of tissue or milliliter of blood were calculated. The identity of colonies was confirmed by their appearance on EIM agar and by biochemical tests (Farmer and McWhorter 1984).

Because of the wide distribution of the means, the daily value of CFU per gram or milliliter for each sample was logarithmically transformed and

statistically analyzed by a general linear model for an unbalanced analysis of variance. The significant differences (95% confidence level) among means within each tissue were determined by the least significant difference (SAS Institute, Cary, North Carolina). In experiment 2, three infected fish and two control fish were sampled every other day for gross pathology examination and for bacterial isolation and identification.

Rabbit anti-E. tarda serum.—To identify *E. tarda* by immunostaining in tissue sections, polyclonal antiserum specific for *E. tarda* (isolate AL-92-255) was produced by a modification of the technique of Myer (1995). Bacteria were cultured on BHI agar, incubated at 30°C for 16 h, and checked for purity. Bacteria were suspended in 10 mL of sterile saline in a vial with glass beads and shaken for 2 min. After cell clumps settled, the suspension was inactivated with 0.14 mL of formalin (37%) for 1.5 h. The bacteria were washed three times in saline by centrifugation for 15 min at 1,700 × gravity and diluted to a concentration approximating a number 2 McFarland's nephelometer standard. Bacterin sterility was tested in fluid thioglycolate medium for 48 h at 30°C, and then the bacterin was emulsified with an equal volume of Titer Max adjuvant (Vaxel, Inc., Norcross, Georgia). A pathogen-free New Zealand white rabbit was bled to collect preimmune serum, and 100 μL of the bacterin–adjuvant mixture was injected subcutaneously into both shoulders and hind quadriceps. Three weeks later, a booster bacterin in Titer Max was injected intramuscularly into both hind quadriceps (40 μL per site). The rabbit was bled 2 weeks after the first booster vaccination, at which time the antibody titer was estimated to be 1:10,000 by an indirect enzyme-linked immunosorbent assay (ELISA) in microtiter plates that used sonicated formalin-killed *E. tarda* as the antigen (Hornbeck 1991). The rabbit was then given a second 0.2-mL booster of bacterin, without adjuvant, subcutaneously on both thighs and shoulders. One week later the rabbit was bled, at which time the anti-*E. tarda* titer was 1:20,000 by ELISA. Serum was stored in 300-μL aliquots at −20°C. Specificity of the serum antibody was checked by ELISA that used formalin-killed cells of *Escherichia coli*, *Aeromonas hydrophila*, and *E. ictaluri*.

Immunohistochemistry.—The rabbit anti-*E. tarda* serum and a modified avidin–biotin conjugate immunoperoxidase- staining technique were used to identify *E. tarda* in tissue sections (Bourne 1983; Erickson et al. 1993). After sections were mounted on glass slides, paraffin was removed, and the tissue was hydrated, endogenous peroxidase activity was blocked by flooding the slides with 0.3% hydrogen peroxide for 30 min at 37°C. Sections were trypsinized for 10 min (Robinson 1982), and nonspecific binding was blocked by immersion for 1 h at 37°C in goat serum (Vector Laboratories, Burlingame, California) diluted 1:20 in phosphate- buffered saline (PBS) at pH 7.2 with 0.1% crystalline bovine serum albumin added (PBS-BSA). Rabbit anti-*E. tarda* serum diluted 1:750 in PBS-BSA was applied for 1 h at 37°C, sections were washed, and biotinylated goat anti-rabbit immunoglobulin G (Vectastain ABC Elite; Vector) diluted 1:1,000 in PBS was applied for 1 h. The avidin–biotin peroxidase complex diluted in carbonated buffer (pH 9.6) was then applied for 1 h. Color was developed with 3–3′-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, Missouri) solubilized in 0.1 M tris buffer (pH 7.2) with 0.02% hydrogen peroxide and 0.04% nickel chloride. Color development was monitored visually, and the reaction was stopped with a distilled water wash. The immunohistochemistry procedure was applied to liver and skin sections from lesions of infected fish on the third and fourth day PI along with the negative and positive controls. The positive controls consisted of *E. tarda*-infected tissue; negative controls were catfish tissue free of *E. tarda* and infected tissues with one of the following omitted: the primary antibody (preimmune serum was substituted), the secondary antibody, or 3–3′-diaminobenzidine tetrahydro chloride.

Results

Clinical Signs and Gross Lesions

Clinical signs and gross lesions were similar during the first 5 d PI in *E. tarda*-infected fish in experiments 1 and 2 (Table 1). On day 1 PI the scraped site in all fish was reddened and swollen and progressed into ulcerative dermatitis over the next 24 h. The lesion in infected fish then became a hemorrhagic ulcer surrounded by erythemic borders with petechiae. Skin of these fish had scattered areas of cutaneous depigmentation and increased mucus production. Cutaneous petechial hemorrhages and ulcers were also present on the mouth, isthmus, operculum, abdomen, and lateral body during 1–6 d PI (Figure 1). Infected fish became lethargic and developed rapid opercular movements and pale gills before death, which occurred from 3 to 6 d PI.

Principal gross internal lesions included con-

TABLE 1.—Gross lesions in juvenile channel catfish (number positive/number examined) experimentally infected with *Edwardsiella tarda.*

	Experiment 1			Experiment 2		
Days post-infection	Cutaneous lesions[a]	Mottled liver	Hyperemic spleen	Cutaneous lesions[a]	Mottled liver	Hyperemic spleen
1	0/5	0/5	2/5			
2	1/5[b]	0/5	4/5	1/3	0/3	3/3
3	4/5	3/5	3/5			
4	2/5	4/5	4/5	3/3	3/3	3/3
5	1/5	5/5	5/5			
6				3/3	3/3	3/3
8				1/3	1/3	0/3
10				0/3	0/3	0/3
12				0/3	0/3	0/3

[a] Cutaneous lesions do not include those at the scraping site.
[b] Erythema only.

gestion, particularly of the spleen, and pale-tan foci or petechial hemorrhages on the liver. Clinical signs and external and internal lesions were less pronounced on day 8 PI in experiment 2 and were not seen thereafter except in the scraped skin area. On 10 and 12 d PI, ulcers that developed at the scraped site were healing by scarring. None of the noninfected controls (scraped and nonscraped) died or showed clinical signs or gross lesions except for the scraping sites that had mild dermatitis for 3–4 d.

Histopathology

Skin of the scraped site of infected fish at 1 d PI had two to three cell layers of epidermis and intact basement membrane. The hypodermis had

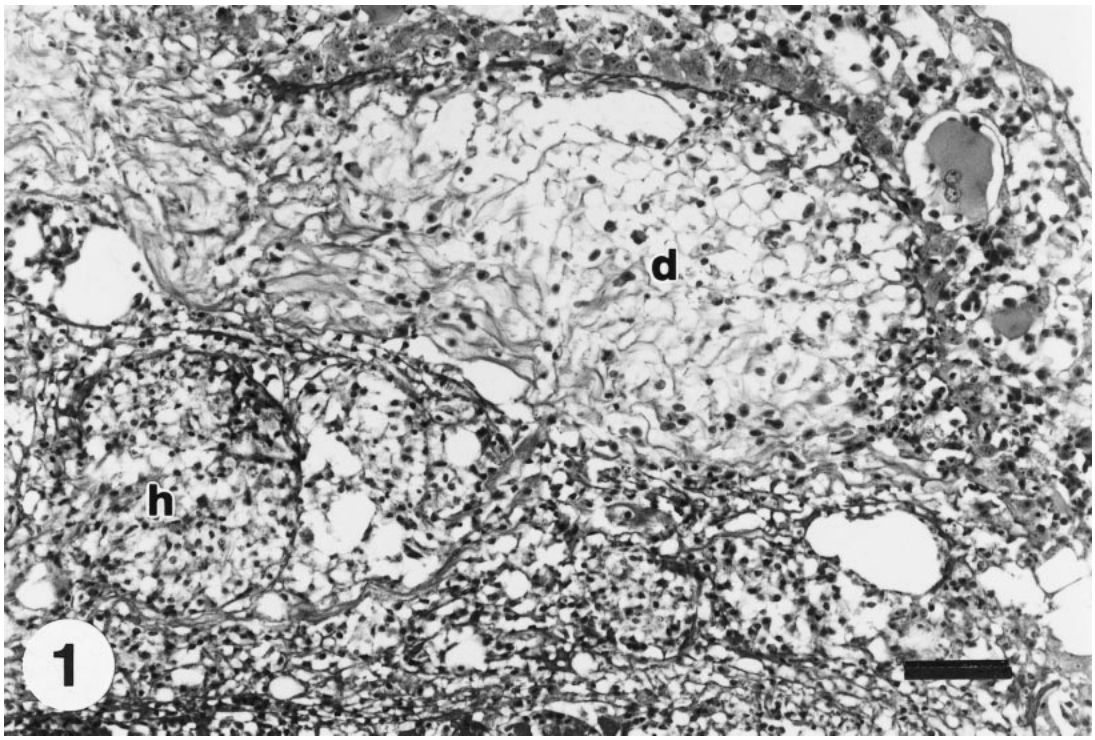

FIGURE 1.—The dermis (d) and the hypodermis (h) of *E. tarda*- infected channel catfish showing severe necrosis with predominantly macrophage infiltration 3 d postinfection; hematoxylin and eosin staining is shown. Bar = 50 μm.

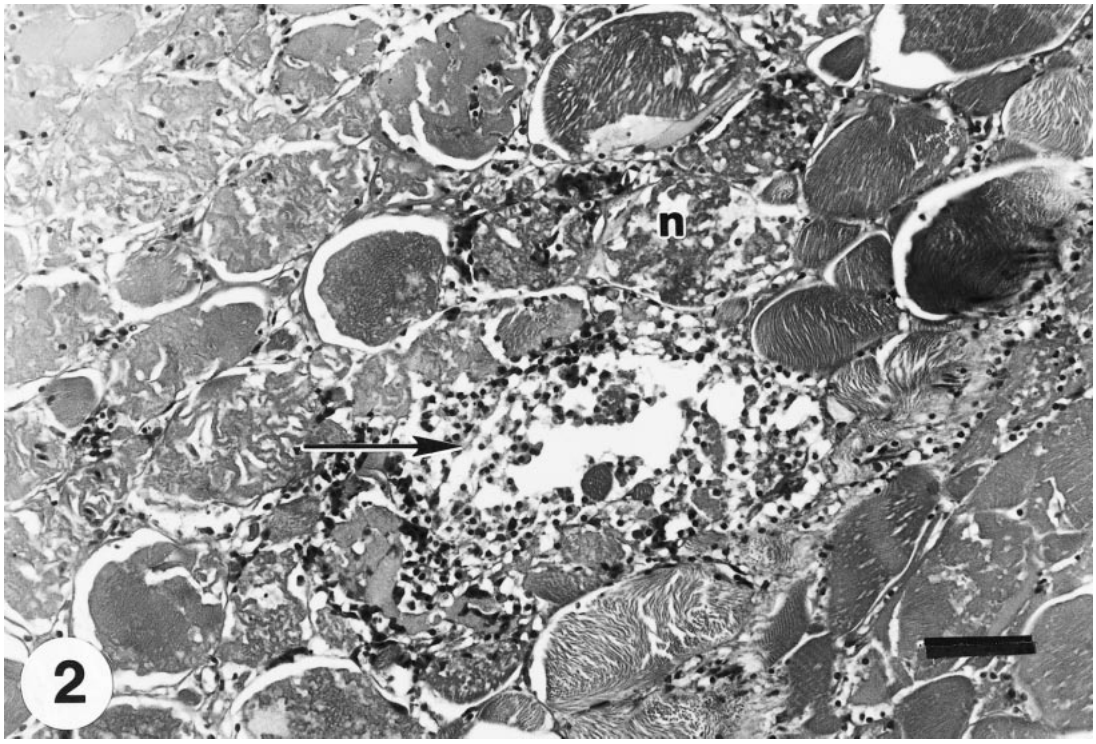

FIGURE 2.—Macrophage infiltration (arrow) in the muscle of *E. tarda*-infected channel catfish 6 d postinfection. Necrotic muscle fibers (n) are also present; hematoxylin and eosin staining is shown. Bar = 75 μm.

congested blood vessels accompanied by infiltration of macrophages, neutrophils, and lymphocytes. On day 2 PI, the scraped site had severe focal ulcerative necrotizing dermatitis with hemorrhage and inflammation of the underlying muscle. The cutaneous lesion 3 d PI was characterized by epidermal spongiosis, hydropic degeneration and nuclear pyknosis, dermal edema, hypodermal vasodilation, hemorrhages, and severe macrophage infiltration (Figure 1). The underlying superficial muscle bundles had necrotic myositis accompanied by macrophages. Ulcerative necrotizing dermatitis continued until 6 d PI, but on 8 and 10 d PI the lesions were covered by epidermis about 11–15 cells thick. The hypodermis and superficial muscle had macrophage infiltration and fibroblasts typical of granulation tissue. On 12 d PI, the dermis and epidermis were more differentiated, and the hypodermal granulation tissue was more organized into scar tissue. Scattered focal necrotic myositis lesions with macrophages were occasionally seen under the injured skin (Figure 2). Skin not associated with the scraped lesion had scattered ulcers (Figure 3). Uninfected scraped control fish had mild dermatitis during the first 4–5 d PI at the scraping site.

Lesions of visceral organs were mainly in liver, head kidney, trunk kidney, and the spleen of infected fish. Multifocal hepatocellular vacuolar degeneration appeared 3 d PI. On 4–6 d PI, livers of infected fish had moderate to severe multifocal necrotizing hepatitis (Figure 4a). The pancreatic tissue surrounding the hepatic portal vein within the liver was not affected. Hepatic lesions included hepatocellular necrosis, eosinophilic cellular debris, and infiltrating macrophages, some of which contained bacteria as indicated by immunohistochemistry (Figure 4b). In some areas the liver lesions extended into the hepatic serosa. On 8 d PI, no histologically examined fish had microscopic hepatic lesions.

Some infected fish had mild focal necrotizing nephritis as early as 1 d PI that became moderate to severe from 2 to 6 d PI. Renal tubules and hemopoietic tissue were degenerated, necrotic, and hemorrhagic with aggregates of predominantly macrophages. Similar to the trunk kidney, the head kidney had mild to severe multifocal necrosis with

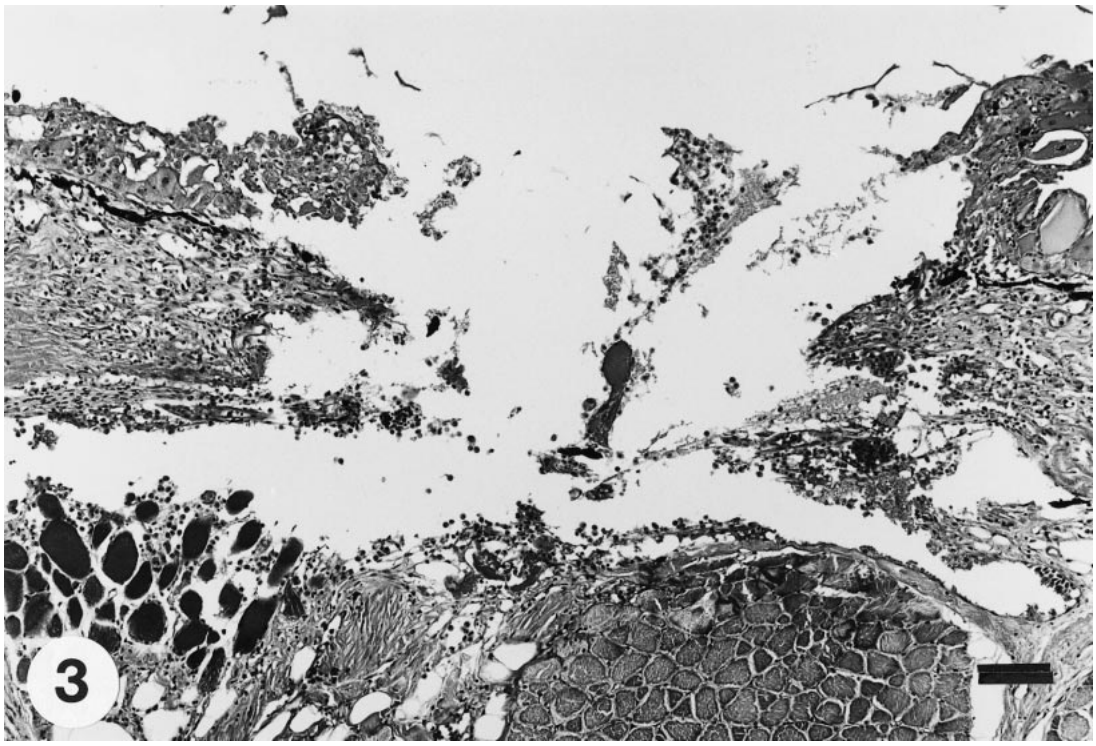

FIGURE 3.—A cutaneous ulcer in *E. tarda*-infected channel catfish with sloughing epidermis and dermis 6 d postinfection; hematoxylin and eosin staining is shown. Bar = 100 μm.

predominantly macrophages as early as 2 d PI. At 3 d and continuing through 6 d PI, lesions of head kidney were severe and either multifocal or coalescing and involved the hemopoietic tissue (Figure 5). No obvious pathology was seen in the head kidney 8 d PI or thereafter.

There was mild to moderate hypocellularity of the splenic white pulp 2 d PI. On 3–6 d PI, fish had severe multifocal necrotizing splenitis (Figure 6) in which the red pulp was either reduced or completely destroyed. After 6 d PI, splenic lesions were absent (Table 2). The control fish showed no histological lesions in visceral organs.

Colony-Forming Units in Tissues

The CFU counts in the trunk kidney, liver, and blood of infected fish failed to change significantly 2 d PI compared with the counts seen 1 d PI ($P > 0.05$) (Figure 7). The number of CFU peaked at 3 d PI ($P < 0.05$), declined significantly at 4 d PI, and continued to do so at 5 d PI. *E. tarda* was isolated from 24 of 25 infected fish assayed in experiment 1 (Table 3). In experiment 2, *E. tarda* was isolated from six of six fish assayed on 2 and 4 d PI, from one of three fish assayed on 6 d PI, and from none of nine fish assayed thereafter. Bacteria were not isolated from control fish.

Serology and Immunohistochemistry

No ELISA cross-reactivity occurred between the rabbit anti-*E. tarda* serum and *Escherichia coli* or *A. hydrophila*; however, *E. ictaluri* cross-reacted at antiserum dilutions of less than 1:40. The rabbit preimmune serum contained no antibodies to *E. tarda*; however, the final ELISA titer in the antiserum was 1:20,000. By the use of the modified avidin—biotin-conjugated immunoperoxidase reagent, *E. tarda* was specifically stained in the liver and skin lesions of infected fish on 3 and 4 d PI but not in the uninfected controls. Numerous inflammatory cells, consistent in size and morphology with tissue macrophages, contained large numbers of positively stained *E. tarda* in the necrotic hepatic lesions (Figure 4b). Large numbers of bacteria were also positively stained at the interface between the dermis and hypodermis at the scraping site, which demonstrates the ability of *E. tarda* to invade injured skin.

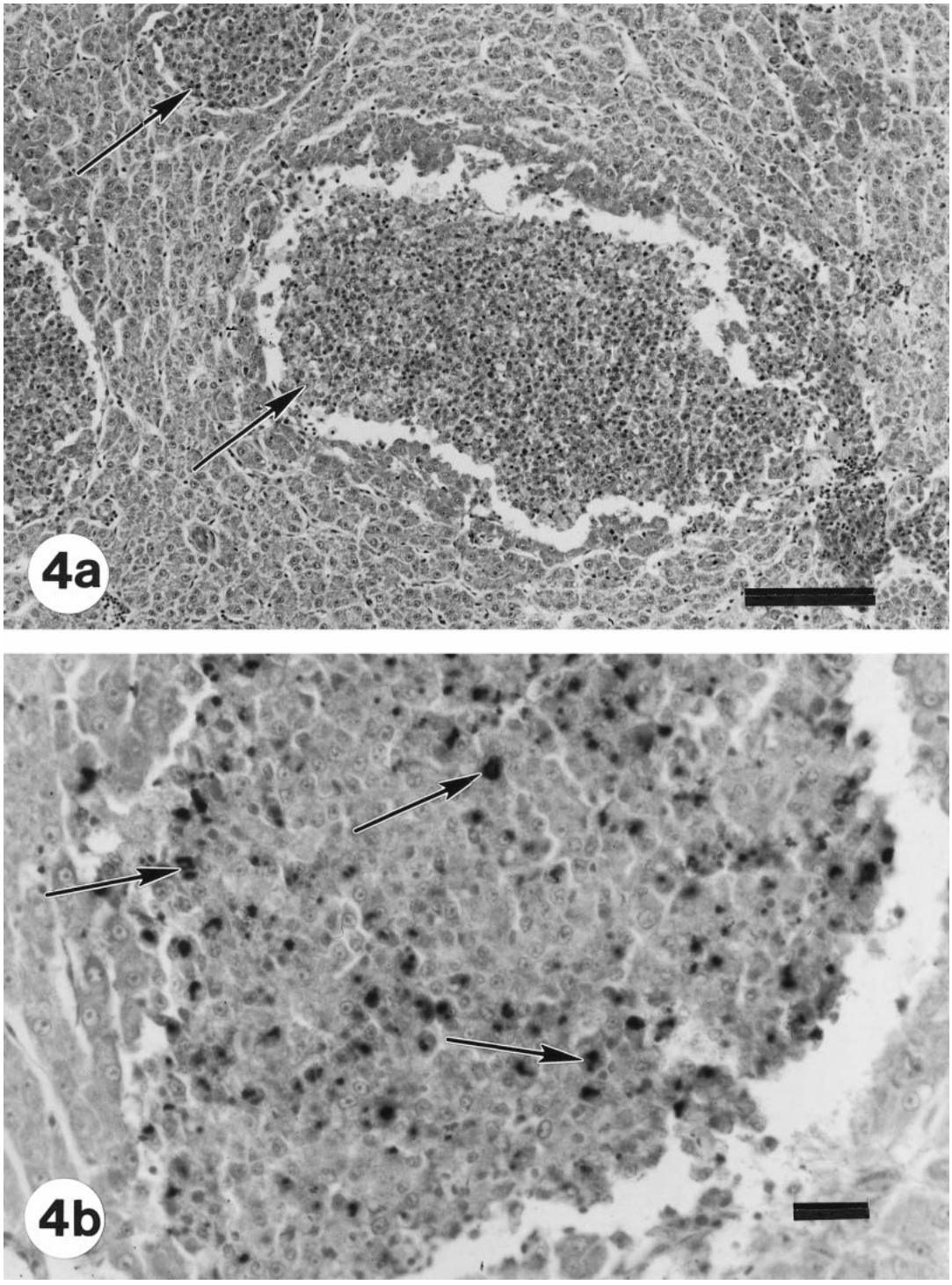

FIGURE 4.—(**a**) Severe multifocal necrotizing hepatitis (arrows) in *E. tarda*-infected channel catfish 4 d postinfection; hematoxylin and eosin staining is shown. Bar = 100 μm. (**b**) A hepatic lesion stained specifically for *E. tarda* by the use of the avidin–biotin immunoperoxidase technique 4 d postinfection. The brown material (arrows) is *E. tarda* bacteria in macrophages. Bar = 25 μm.

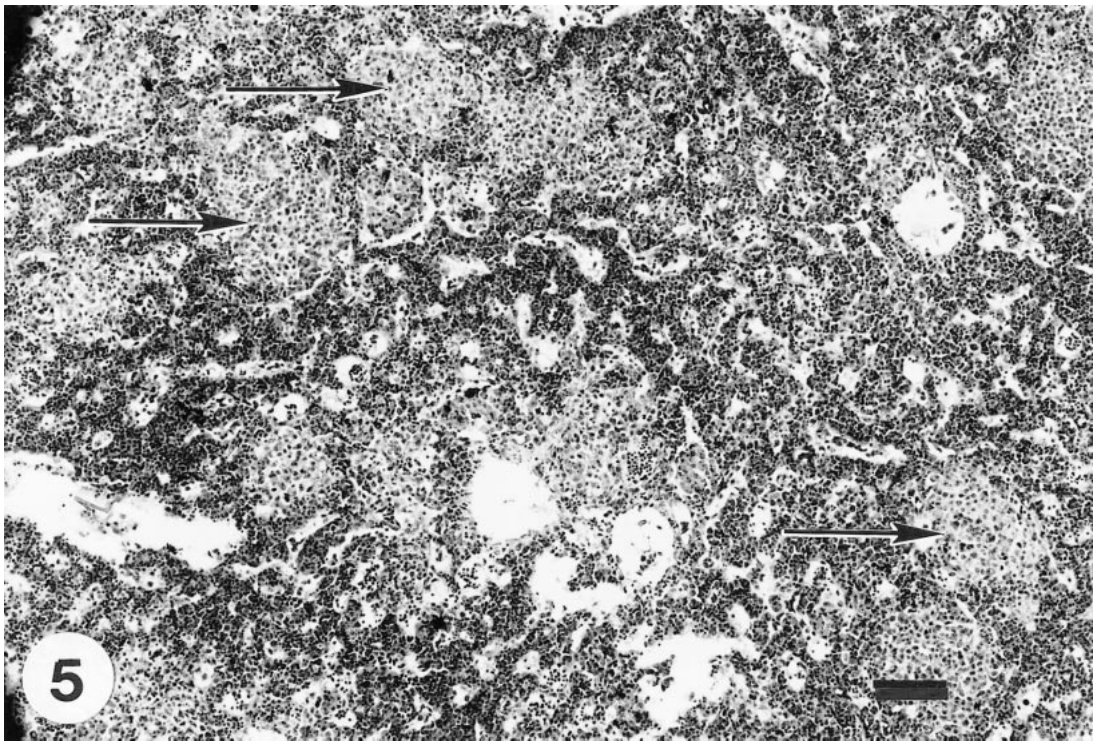

FIGURE 5.—Severe multifocal necrotizing inflammation (arrows) of the head kidney in *E. tarda*-infected channel catfish 4 d postinfection; hematoxylin and eosin staining is shown. Bar = 100 μm.

Discussion

Results of the two experiments complement each other, although the LD50 values of the two studies were about 1 log different. The discrepancy in the LD50 values between the two studies may have been partly due to human error or to the fish in experiment 2 developing a higher innate resistance. Clinical signs and gross and microscopic lesions were similar in both. The petechiae and cutaneous ulcers in experimentally infected fish are also common in naturally *E. tarda*-infected fish (Meyer and Bullock 1973). Focal necrotic myositis was seen in the experimental infection, but gas-filled abscesses like those commonly reported in muscle of naturally infected channel catfish were absent. The rapid course of infection and small size of fish used could be reasons for the absence of gas pockets in muscle. Ulcers seen in our experiments were also similar to those reported in largemouth bass *Micropterus salmoides* infected with *E. tarda* (Francis-Floyd et al. 1993). On the basis of the CFU curves in the organs, it is apparent that infected fish were capable of combating infection.

Foci of inflammatory cells in the liver, head kidney and trunk kidney, and spleen appeared to be predominantly macrophages on the basis of their morphology and size; however, further definitive identification in paraffin sections is difficult (Ferguson 1989). By the use of immunohistochemistry staining, large numbers of *E. tarda* bacteria were demonstrated inside phagocytic cells that appear to be macrophages. This large number of bacteria could indicate intracellular replication, but this could not be confirmed. However, the presence of *E. tarda* within phagocytic cells was also demonstrated in eels and tilapias (Miyazaki and Egusa 1976a, 1976b). In the two forms of edwardsiellosis in eels (interstitial nephritis and hepatitis), the initial lesions were considered to be accumulations of bacterial-laden phagocytic cells. Multiplication of *E. tarda* within phagocytic cells was demonstrated in Japanese eel, Japanese flounder *Paralichthys olivaceus*, (also known as olive flounder), and tilapias (Miyazaki and Kaige 1985). Surprisingly no histopathology was detected in tissues of infected fish at 8 d PI or thereafter. This lack of a resolution period of healing is unexplained; however, it may have been due to selection of individual specimens that were not infected.

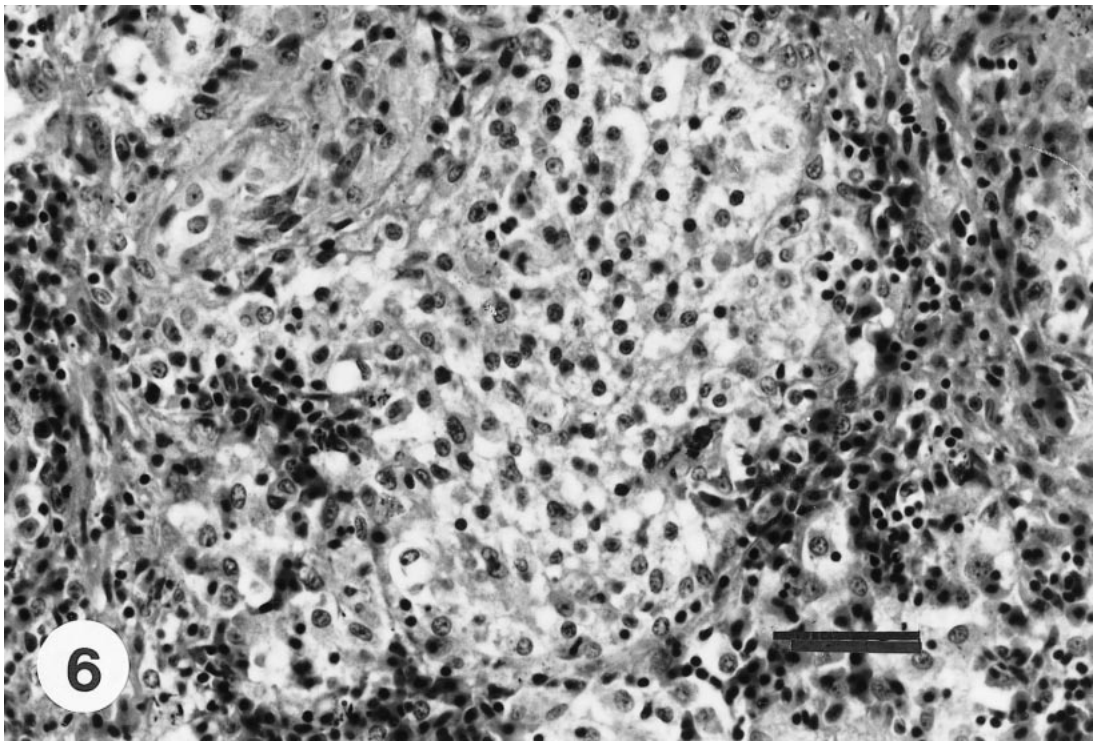

FIGURE 6.—Spleen from an *E. tarda*-infected channel catfish showing necrotic white pulp 4 d postinfection; hematoxylin and eosin staining is shown. Bar = 50 μm.

There are striking similarities between the histologic lesions seen in this study and the lesions described in infections of *E. ictaluri* (Miyazaki and Plumb 1985; Shotts et al. 1986; Newton et al. 1989). Hypocellular hemopoietic tissue, necrotic lesions with accumulation of ostensibly macrophages in the various tissues and possible multiplication of *E. tarda* in macrophages, was

TABLE 2.—Histopathology of juvenile channel catfish (number positive/number examined) after experimental infection with *Edwardsiella tarda.* Number of plus signs indicates severity of lesion: + = Mild, ++ = moderate, +++ = severe.

	Experiment 1				Experiment 2			
Days post-infection	Hepatitis	Nephritis	Head kidney inflammation	Spleenitis	Hepatitis	Nephritis	Head kidney inflammation	Spleenitis
			0/3					
1	0/3	1/3+	1/3+++	0/3				
2	0/3	1/3++		2/3[a]	1/3+	1/3+	1/3+ 1/3++ 1/3+++	3/3[a]
3	2/3[b]	1/3+++	1/3+++	1/3+++				
4	3/3+++	2/3+++	2/3+++	2/3+++	3/3+++	3/3+++	3/3+++	2/3+++
5	2/3+++	2/3+++	2/3+++	2/3+++				
6					3/3+++	1/3+++	3/3+++	3/3+++
8					0/3	0/3	0/3	0/3
10					0/3	0/3	0/3	0/3
12					0/3	0/3	0/3	0/3

[a] Only hypocellular white pulp observed.
[b] Multifocal hepatocellular vacular degeneration.

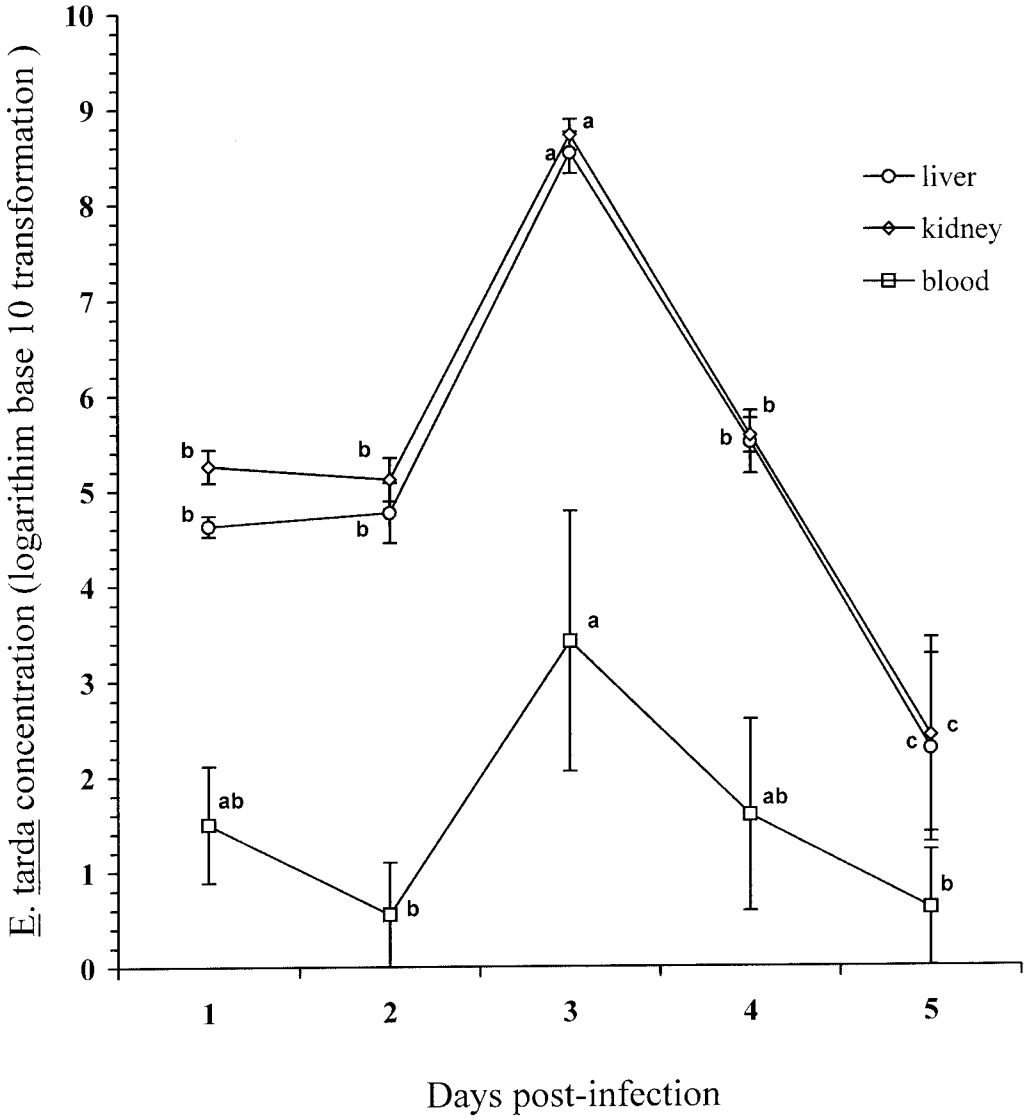

FIGURE 7.—Colony-forming units of *E. tarda* per milliliter of blood or gram of liver and trunk kidney after exposure of channel catfish to the pathogen ($N = 5$). Means labeled with different letters are significantly different ($P < 0.05$) within each tissue.

TABLE 3.—Number of *Edwardsiella tarda*-positive cultures obtained from experimentally infected fish (number positive/number examined).

Experiment	Days postinfection						
	1	2	3	4	5	6	8–12
1	5/5	5/5	5/5	5/5	4/5		
2		3/3		3/3		1/3	0/9

similar to the lesions caused by infection with *E. ictaluri*.

The invasive ability of *E. tarda* is demonstrated in these experiments because immunohistochemical staining demonstrated the bacterium in tissues. The pathogen was also isolated from nearly all fish during the first 5 d PI of both experiments, and histological changes were detected in many organs of nearly all infected fish examined. The invasiveness of *E. tarda* in in vitro-cultured monolayers of HEp-2 cells has been described (Marques

et al. 1984; Janda et al. 1991a, 1991b). Janda et al. (1991b) implicated cell-associated hemolysins (CAH) in the pyknosis and rounding of nuclei and the extensive vacuolization in cell cytoplasm in *E. tarda*- invaded HEp-2 cells. The cytotoxic effect of CAH could perhaps partially explain the necrosis in organs of *E. tarda*-infected fish.

Isolation of *E. tarda* from blood indicates that bacteria had accessed the circulatory system where survival could be due to their ability to acquire available iron (Janda et al. 1991b). Production of CAH and siderophores by *E. tarda* could also enhance the bacteria's survival and replication in blood and within macrophages (Kokubo et al. 1990; Janda et al. 1991a, 1991b). It has been proposed that the CAH of *E. tarda* helps bacteria escape from cytoplasmic vacuoles, thus avoiding lysosomal degradation and placing the bacteria in nutrient-rich cytoplasm (Janda et al. 1991b). The CAH and secreted hemolysins provide the bacteria with iron under iron- deprivation conditions by causing the release of hemoglobin from lysed erythrocytes (Payne 1988).

On the basis of the CFU count and the failure to isolate bacteria from internal organs by 8 d PI (experiment 2), it is apparent that the inflammatory process was associated with decreasing bacterial abundance and their eventual elimination (Table 3). Miyazaki and Kaige (1985) reported that granulomatous lesions in red seabream *Pagrus major* (also known as madi) and tilapias were associated with killing *E. tarda.*

Although it does not conclusively prove the case, this study suggests that physical injury may be a precursor to infections of *E. tarda* in channel catfish. The study also indicates that the organism can become systemic and that fish can combat and clear infections of *E. tarda* when they are held in good environmental conditions. *E. tarda* may not be a major clinical disease problem for cultured channel catfish, but proper fish husbandry and management to prevent skin injury and other stressors are important in avoiding infections.

Acknowledgments

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