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Field Efficacy of Florfenicol for Control of Mortality in Channel Catfish, *Ictalurus punctatus* (Rafinesque), Caused by Infection With *Edwardsiella ictaluri*

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Abstract

A field study to assess the efficacy of florfenicol (FFC) against enteric septicemia of catfish (ESC) was conducted with pond-reared channel catfish fingerlings held in 0.1-acre earthen ponds. Fish were challenged with *Edwardsiella ictaluri* in a natural pond outbreak or by cohabitation with *E. ictaluri*-infected fish held in netpens. Fourteen ponds were assigned in equal number to two treatment groups, that is, either treated (with 10 mg FFC/kg body weight in medicated feed) or not treated (control) for 10 consecutive d. The threshold for enrollment into the study was 0.3% cumulative mortality attributed to ESC. Treatment was initiated on different dates for each pond because each pond was enrolled when 33 fish/pond were diagnosed with ESC based on clinical signs, lesions, or positive cultures. Mortality was monitored during the 10-d treatment period and during a 14-d posttreatment observation period. At the end of the 14-d posttreatment observation period, all fish were euthanized, and 20 fish from each pond were examined by gross necropsy and evaluated for the presence of *E. ictaluri* by bacterial culture. The odds of a mortality in the control group were 2.20 times the odds of a mortality in the FFC-treated group. Significantly fewer ($P \leq 0.05$) FFC-medicated catfish died in comparison to unmedicated catfish. The minimum inhibitory concentration of FFC for this strain of *E. ictaluri* was 0.25 μg/mL in all fish that were assayed. The mean zone of inhibition (Kirby Bauer) was 36.8 mm from *E. ictaluri* isolates of test fish. There were no FFC treatment-related lesions seen on gross pathology. FFC was efficacious and safe for control of mortality from *E. ictaluri* infection in catfish.

According to the 2003 National Animal Health Monitoring System (NAHMS) report, bacterial disease is the leading cause of loss in fingerling operations in the USA (USDA 2003). Enteric septicemia of catfish (ESC) was the most frequently reported fish bacterial disease in the southeastern USA from 1986 to 1996, causing mass mortality of catfish and millions of dollars of loss (MacMillan 1985; Thune 1991; Plumb 1999). Outbreaks of ESC usually occur in the spring and fall months when water temperatures are 22–28 C, conditions that are optimal for growth of *Edwardsiella ictaluri*, the bacterial cause of ESC (Hawke et al. 1981; Baldwin and Newton 1993).

Treatment for bacterial diseases in fish intended as food is limited to oxytetracycline and a sulfadimethoxine/ormetoprim combination, two commercial antibiotic preparations approved by the US Food and Drug Administration (FDA). While the sulfadimethoxine/ormetoprim combination is approved for use against *E. ictaluri* in catfish, oxytetracycline is only approved for use against *Aeromonas* and *Pseudomonas*. However, there are reports of bacterial resistance to these antibiotics (Johnson 1991; Plumb et al. 1995). In addition, palatability problems have been reported with sulfadimethoxine/ormetoprim (Poe and Wilson 1989). Compounding these problems, oxytetracycline is primarily manufactured as a sinking pellet, making it difficult for farmers to gauge feeding activity, and the sinking feed represents a diet change for sick fish typically fed floating diets.

For these reasons, producers have been reluctant to use these antibiotics when their fish are

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diagnosed with ESC. The approval of a new and efficacious antibiotic would give producers another viable option in treating bacterial disease in fish.

Florfenicol (FFC) ([*R*-(*R**,*S**)]-2,2-dichloro-*N*-[1-(fluoromethyl)-2-hydroxy-2-[4-(methylsulfonyl)phenyl]ethyl-acetamide) is a fluorinated derivative of thiamphenicol, a chloramphenicol analogue (Nagabhusahan et al. 1992). Thiamphenicol is used therapeutically for humans, but FFC was developed exclusively for animal use and is approved for cattle, swine, and poultry in many countries. Most bacteria that are resistant to chloramphenicol and thiamphenicol are sensitive to FFC (Cannon et al. 1990).

FFC is efficacious against a number of fish pathogens, including *Aeromonas salmonicida* and *Vibrio salmonicida* (Fukui et al. 1987; Inglis and Richards 1991; Nordmo et al. 1998; Samuelson et al. 1998; Bruun et al. 2000; Schmidt et al. 2000), and its efficacy against *E. ictaluri in vitro* has recently been demonstrated (McGinnis et al. 2003). It is currently approved for aquaculture to control susceptible bacterial diseases in Japan (yellowtail *Seriola quinqueradiata*, red sea bream *Pagellus bogaraveo*, coho salmon *Oncorhynchus kisutch*, horse mackerel *Trachurus* spp., rainbow trout *Oncorhynchus mykiss*, sweetfish *Plecoglossus altivelis*, tilapia *Oreochromis* spp, Japanese eel *Anguilla japonica*), South Korea (yellowtail, eel), Norway (salmon), Canada (salmon), and the UK (salmon). FFC was shown to be effective *in vivo* against *E. ictaluri* in a preliminary laboratory study wherein infection was experimentally induced in channel catfish fingerlings (Gaunt et al. 2003). In support of a new animal drug application approval for FFC to control mortality in channel catfish caused by ESC, a dose titration, dose confirmation, and field study were undertaken. Research protocols followed were drafted according to specific US FDA guidelines. The determination of the optimum dose rate was studied in a tank-dose titration followed by a dose confirmation study, and was found to be 10 mg/kg body weight (bw) one time a day for 10 d (Gaunt et al. 2004). Herein, we report the results of the field efficacy study conducted during May and June 2001 in which this dose rate was administered to channel catfish fingerlings held in experimental 0.1-acre ponds.

Materials and Methods

Experimental Design

Fourteen 0.1-acre ponds were randomly assigned in equal numbers to each of two treatment groups: (1) challenged with *E. ictaluri* and fed unmedicated feed (control) and (2) challenged with *E. ictaluri* and fed 10 mg FFC/kg bw in medicated feed. Approximately 11,000 fingerlings were allotted to each pond in an undetermined ratio of males and females; during acclimation, they were fed to satiation on a commercial diet once per day. Challenge with *E. ictaluri* occurred either from a natural outbreak (two ponds) or from natural outbreaks in each of the 12 remaining ponds combined with experimentally infected fish placed in netpens and water from the initial natural outbreak. The criterion for enrollment of ponds into the study was 0.3% (33) fish from each pond manifesting either clinical signs or lesions of ESC, or positive culture for *E. ictaluri*. Initiation of treatment began at the feeding immediately after ponds met the inclusion criterion. Because enrollment of ponds was staggered and dependent upon when the inclusion criterion was met, acclimation to the experimental conditions ranged from 40 d (one pond) to 59 d (five ponds).

Fish

Approximately154,000 farm-reared, 1-year-old channel catfish fingerlings with no known history of exposure to *E. ictaluri* were obtained from L&S Fish Farm, Leland, Mississippi, USA. Fingerling fish were collected with a seine, and three samples of fingerlings were collected, counted, and weighed to determine the mean fingerling weight (count range: 345–383 head/sample). These fish were released back into the pond from which they had been collected, and fish for the study were collected from the same seine and weighed to yield a weight equivalent to ~11,000 fish. Groups of 11,000 fingerlings in an undetermined ratio of males and females were placed into 14 separate

oxygenated wells on live haul trucks and were transported to Delta Western Research Center, Indianola, Mississippi, USA, where they were stocked in previously prepared 0.1-acre ponds at a rate of approximately 11,000 fish per pond.

Fish Environment, Handling, and Feeding

Earthen ponds at Delta Western Research Center supplied with well water were used in the study. Prior to stocking, all test ponds underwent routine conditioning with potassium permanganate (to kill existing fauna), sodium chloride (to prevent nitrite toxicosis), and cottonseed meal (to promote phytoplankton growth), in accordance with the research farm's standard operating practices.

Water temperatures during the treatment (started May 14, 2001) and posttreatment observation (ended June 25, 2001) periods ranged from 20 to 31 C. Supplemental aeration was provided when dissolved oxygen levels could not be determined (because of inclement weather) or were approaching <4 ppm. The total ammonia nitrogen, pH, nitrite, and chloride were measured twice weekly. The mean total alkalinity (as $CaCO_3$) and total hardness (as $CaCO_3$) measured once prior to study initiation ranged from 205–291 ppm and 273–307 ppm, respectively. Water supplying the ponds was analyzed for the presence of lead, copper, organochlorines, and organophosphates by Mississippi State Chemical Laboratory (Mississippi State, MS, USA), and no harmful levels were found in the water that would interfere with the husbandry of the channel catfish. Water quality was within acceptable limits for the maintenance of channel catfish fingerlings throughout the study (Tucker and Robinson 1990).

Fish were hand fed once daily, feeding activity was observed, and their feed consumption was recorded. Fish were fed to satiation during acclimation and during the treatment and posttreatment periods were fed to either to satiation or to 2.5% of their bw, whichever was less. During acclimation, fingerlings were fed commercial floating 35% protein fingerling feed (Delta Western, Indianola, MS, USA), with the exception of the day of stocking and 1 d after stocking, when feed was not provided.

At termination (after the 14-d observation period), the ponds were seined and scrapped using a 3/8″ seining net. The overall weight of fish from the ponds was obtained by weighing the fish on a hydroscale. Fish were placed in tanks at the test facility where samples of 20 fish were counted and weighed from each pond to obtain an average weight per fish. From the overall weight and the weight of the samples of 20 fish, a final number of fish recovered was estimated for each pond. Twenty fish from each pond were delivered to Delta Research and Extension Center for necropsy and microbial sampling, with the exception that only 19 fish were inadvertently sampled from two ponds. All remaining fish were euthanized.

Preparation of Feed

Florfenicol Aquaculture Premix (50% Type A Medicated Article) was provided by Schering-Plough Animal Health Corp., Union, New Jersey, USA. A batch of the feed (Delta Western Fry II, 2.5-mm pellets) was medicated at the in-life facility to deliver 10 mg FFC/kg bw. Batches of unmedicated control diet were manufactured in a similar fashion to the medicated feed, except no drug was added. The floating feed was produced on the same equipment for both unmedicated and medicated diets. To minimize the chances of FFC contamination, a batch of unmedicated feed was prepared by commercial methods, followed by preparation of the medicated feed. The medicated feed was prepared to achieve a target concentration of 400 ppm FFC, which would allow delivery of 10 mg FFC/kg bw at a 2.5% bw feeding rate (400 ppm = 10 mg FFC/kg bw ÷ 2.5% bw). The concentration of FFC in feed, as measured by an HPLC–UV method (Hayes, submitted), was 372.34 ppm (93.1% of nominal). No contamination from oxytetracycline, Romet®, or organochlorine pesticides was detected in the feed (Woodson-Tenent Laboratories and SGS Agricultural Services, Memphis, TN, USA).

Preparation of Edwardsiella ictaluri *Inoculum and Challenge of Fish*

The *E. ictaluri* isolate (S01-602-14) was obtained from a natural outbreak in one of the

experimental ponds. The isolate was confirmed as *E. ictaluri* by biochemical characteristics (Hawke et al. 1981) using a biochemical test kit according to the manufacturer's instructions (BBL Crystal, Cockeysville, MD, USA). One milliliter of the *E. ictaluri* isolate was used to inoculate each of six 1-L brain heart infusion broth, and the cultures were incubated at 25 C overnight with rotary aeration. One control culture was incubated overnight at 25 C and one at 37 C to evaluate contamination and purity. The 1-L cultures were combined to yield two 3-L volumes to attain uniformity for plate counts and dosing. Bacterial concentrations determined by serial dilutions and plate counts were calculated to be $\sim 24 \times 10^7$ and 15×10^7 cfu/mL, respectively.

Approximately 1400 stocker size fish were loaded from vats in the Thad Cochran National Warmwater Aquaculture Center into two ~460-L tanks on a live hauler and were transported to Delta Western Research Center in aerated water. The dissolved oxygen and the temperature of the water in the live hauler were 5 ppm and 25.5 C, respectively. Three liters of *E. ictaluri* culture was added to each side tank on the live hauler followed by a 2-h incubation. The final concentration of bacteria in the live hauler tanks was 1.54×10^6 cfu/mL on Side 1 and 9.74×10^5 cfu/mL on Side 2.

In the 12 experimental ponds that did not meet the inclusion criterion quickly through a natural outbreak of infection, a cylindrical netpen constructed of 1/4″ mesh was placed in ponds and fixed to the pond bottom by two metal fence posts. After seed fish had been challenged with *E. ictaluri* for 2 h, 100 seed fish were hand counted from the live hauler and placed into each netpen. Seed fish established infection in ponds by cohabitation by allowing bacterial shedding into the water while preventing inadvertent sampling of seed fish. Netpens were enclosed on the bottom and had removable covers on the top to prevent bird predation. In addition, approximately 53 L of water from the live hauler were added to each seeded pond. Infection was confirmed in at least 10 seed fish from each netpen, with the exception of four netpens from which infection was confirmed in fewer fish.

Assessment of Efficacy

Ponds were visually observed at least two times daily, throughout the 10-d treatment and 14-d posttreatment observation period. No fish were cultured for bacteria from ponds that received medicated feed during the treatment period in accordance with the study protocol. A maximum of 10 moribund/dead fish were collected for microbiological assessment from ponds that received unmedicated feed during the treatment period. During the posttreatment observation period, a maximum of 10 moribund/dead fish from each medicated and each unmedicated pond were examined by gross necropsy and sampled for bacterial culture. Upon completion of the posttreatment observation period, 20 fish from each pond were arbitrarily sampled for necropsy and microbiological analysis.

Postmortem Examination of Fish

Moribund and dead fish were removed from the ponds. Moribund fish were euthanized with an overdose of MS-222 (Tricaine-S, Western Chemicals Inc., Ferndale, WA, USA). Dead fish were transported on ice to the laboratory. Postmortem examination of the fish included an inspection of the skin, fins, mouth, eyes, gill, and coelomic viscera.

Microbial Methods

Select fish were sampled in accordance with the study protocol to obtain bacterial isolates at the time of mortality or end-of -study euthanasia. Brain and posterior kidney were cultured for bacterial pathogens on Mueller–Hinton agar containing 5% sheep's blood. Plates were then incubated at 25 C for 2 d. Cultures that yielded small, white, punctate, weakly β-hemolytic colonies characteristic of *E. ictaluri* were considered positive. *E. ictaluri* was identified biochemically through tests kits supplied by Difco (Becton Dickinson and Co., Cockeysville, MD, USA) and BBL Crystal. The tests included negative results for oxidase, (Difco, Becton Dickinson and Co.) and positive results for arginine, *p-n-p-N*-acetyl glucosaminide, lysine, and

p-n-p phosphate. Test results for mannose, tetrazolium, urea, prolinenitroanilide, and galactose were variable (BBL Crystal). Quality control strains for the BBL Crystal identification system were *Klebsiella pneumoniae* ATCC 33495 and *Escherichia coli* ATCC 25922.

The minimum inhibitory concentration (MIC) was determined for the inoculum bacteria and 39 other arbitrarily selected bacteria cultured during the study. Briefly, colonies were removed from the Mueller–Hinton blood plates and suspended in a broth media to a density of 0.5 McFarland barium sulfate turbidity standard. One microliter of each bacterial isolate was evenly inoculated on the surface of blood agar plates prepared with concentrations of FFC ranging from 0.06 to 16 μg/mL. Plates were incubated at 25 C for 2 d and observed to determine which concentration completely inhibited growth of *E. ictaluri* (NCCLS 1999).

The disk diffusion zone (formerly Kirby Bauer zone of inhibition) was determined for 388 of 410 isolates. Briefly, *E. ictaluri* was suspended in inoculum fluid, and the density was standardized by dilution with sterile saline to a 0.5 McFarland barium sulfate turbidity standard. The bacteria were uniformly streaked on a Mueller–Hinton blood agar plate and were overlaid with a control (blank paper) disc (BBL Crystal) and a disc impregnated with 30 μg of FFC (Difco Laboratories, Detroit, MI, USA). The plates were incubated at 25 C until the diameter of the zone of inhibition was clearly defined to facilitate accurate measurement.

Statistical Methods

The cumulative mortality data at the end of observation period (Day 24) was analyzed by logistic regression using a general linear mixed model (SAS, %GLIMMIX Macro, SAS Institute, Cary, NC, USA). Analyses of cumulative mortalities were performed based on both the total number of fish allocated (estimated by biomass) and the total number of fish recovered at the study end (assumed allotted equaled mortalities plus live fish recovered). The differences between least squares means were compared by a one-sided test. Differences were declared significant at the 5% level.

Because the cumulative mortality data only capture a fish's living status (dead or alive) at the end of the study, the information obtained about when the fish died during the study period was not used in the analyses of cumulative mortality data. A survival analysis was performed by the Kaplan–Meier method using SAS Proc Lifetest, which enabled estimation of the survival rate over the whole study period and therefore to better understand the treatment effect over time.

Both analyses (cumulative mortality and survival analysis) were performed based on both the total number of fish allocated (estimated by biomass) and the total number of fish recovered at the study end (assumed allotted equaled mortalities plus live fish recovered). Because the results were similar, only the results based on the total number of fish recovered at the study end will be discussed in the Results.

Results

The first deaths attributable to ESC in the study were confirmed in Pond 7 approximately 35 d after stocking, and this pond was enrolled 5 d afterward. Although some deaths from natural outbreaks of ESC were noted in all ponds, only two ponds (one control and one treated) met the inclusion criterion quickly enough from the natural outbreak. Outbreaks in the 12 remaining ponds were enhanced with the addition of exposed caged fish so that ESC could affect the experimental ponds during the small temperature window required by *E. ictaluri*. These 12 ponds were enrolled 7–19 d after the first one was enrolled.

Daily mortality for all treatment groups are summarized in Table 1 and plotted in Fig. 1. The FFC-treated group showed a decrease in mortality after Day 4, while the control group did not show a decrease in mortality until after Day 7.

The cumulative mortality at the end of the study period was defined as total known mortality during and after treatment. The total mortality accounted for was 2300 fish across the seven control ponds (mean = 329 fish/pond) and 1478 fish across the seven FFC-treated ponds (mean = 211 fish/pond). The cumulative

TABLE 1. *Summary of daily mortality of channel catfish, Ictalurus punctatus, that were challenged with* Edwardsiella ictaluri *followed by a 10-d treatment with unmedicated or FFC-medicated feed.*

	Treatment group	
Day	Control	FFC (10 mg/kg)
2	183	191
3	290	216
4	201	241
5	230	190
6	250	188
7	262	165
8	197	94
9	191	39
10	134	33
11	117	25
12	78	19
13	57	8
14	25	6
15	14	3
16	7	3
17	14	4
18	11	8
19	21	3
20	3	4
21	9	17
22	3	8
23	3	13
24	0	0

FFC = florfenicol.

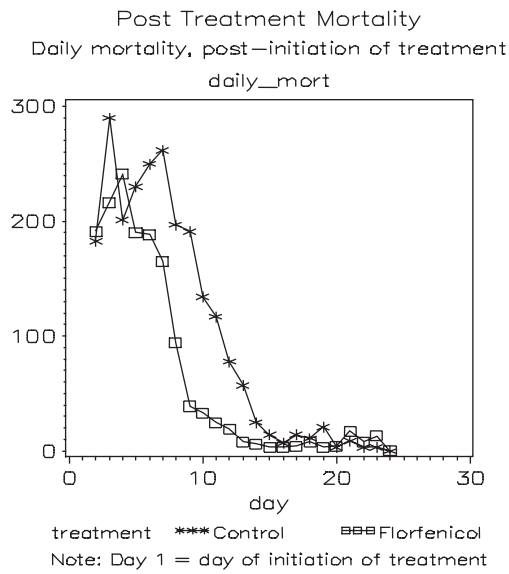

FIGURE 1. *Plot of daily mortality of* Edwardsiella ictaluri*-exposed channel catfish treated with 0 or 10 mg/kg florfenicol.*

mortality at the end of the study period was analyzed using logistic regression with a binomial error and logit link.

Statistical analysis of the cumulative percent mortality using the number of fish recovered by biomass is shown in Table 2. The FFC group had statistically significant lower cumulative mortality rate than the control group ($P = 0.0397$, one-sided test). The parameter estimate of 0.7906 equates to an odds ratio of 2.20, that is, the odds of a mortality in the control group is 2.20 times the odds of a mortality in the FFC-treated group.

For the purposes of analysis, a fish's survival time was defined as the number of days that a fish lived after the pond was admitted in the study. If a fish died on Day 9, then its survival time was 9, or if a fish lived until the end of the study period (Day 24), then the fish's survival time was considered a censored observation of 24. The survival analysis would not only take into account a fish's living status at the study end (alive or dead) but would also use the information of how long a fish lived. Results from the survival analysis, based on fish recovered, are shown in Table 3. The two treatment groups were significantly different ($P < 0.0001$), with the FFC group having a higher survival rate than the control group. Fig. 2 shows the estimated survival rate of the two treatment groups over time. The survival rate of the FFC group was consistently higher than the control group throughout the entire study period.

Edwardsiella ictaluri was isolated from fish in each netpen and from each pond. Throughout the study, *E. ictaluri* was cultured from 44.8% (182 of 406) of the fish sampled from the FFC-treated group and from 46.7% (228 of 478) of the fish sampled from the control group. The MIC50 and MIC90 for all 40 *E. ictaluri* isolates that were tested against FFC, including the strains of *E. ictaluri* from ponds that met enrollment criterion from a natural outbreak, were 0.25 μg/mL. The 388 of 410 *E. ictaluri* isolates had disk diffusion susceptibility zones to FFC that ranged from 32 mm to 50 mm, with a mean of 36.8 mm.

Postmortem examination of the fish revealed external lesions including inflammation through

TABLE 2. *Logistic regression (using GLIMMIX) of cumulative mortality based on fish recovered.*

The mixed procedure						
Parameter estimates						
Effect	Treatment	Standard estimate	Error	df	*t* value	Pr > |*t*|
Intercept		−3.8124	0.2925	12	−13.03	<0.0001
Treatment	Control	0.7906	0.4125	12	1.92	0.0794
Treatment	Florfenicol	0	—	—	—	
Type 3 tests of fixed effects						
		Numerator	Denominator			
	Effect	df	df	*F* value	*P* value (one side)	
	Treatment	1	12	3.67	0.0397	

the sutra fontanel of the skull (commonly known as ''hole in the head''), hemorrhages on the skin and fins, and exophthalmia, all of which are compatible with ESC (Baldwin and Newton 1993). Internal lesions included gastrointestinal and hepatic hemorrhages, splenic congestion, ascites, and renomegaly.

An average of 4 d prior to enrollment (range of 1–8 d), feeding activity decreased in both medicated and control ponds. During treatment, feed consumption was significantly different between the medicated and the control groups only on Day 10, when the FFC group consumed a mean of 1.70 kg feed/pond and the control group a mean of 1.46 kg feed/pond ($P < 0.0001$). During treatment, the mean feed consumption in medicated ponds was 1.22 kg/pond per d. The nominal dose rate for fish that received FFC-medicated feed was 10 mg FFC/kg bw for 10 d, assuming that all the medicated ration was consumed. The actual mean daily dose rate for the FFC-treated ponds was calculated to be 5.92 mg/kg bw. This indicates a mean daily feeding rate of 1.48%, which was less than the nominal feeding rate of 2.5%. Feed consumption in FFC-treated ponds generally increased to pretreatment levels or higher during the posttreatment period.

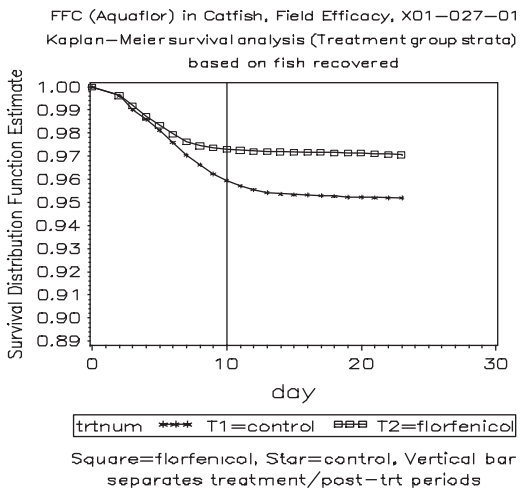

FIGURE 2. *Estimated survival function by treatment (0 or 10 mg florfenicol/kg bw). Kaplan–Meier survival analysis based on number of catfish accounted for (mortalities and harvested numbers*).

Square = florfenicol; star = control. Vertical bar separates treatment/posttreatment periods.

Discussion

In commercial fish farming, disease surveillance is recommended so that intervention can be initiated as soon as clinical signs or lesions indicating the presence of disease are noted. Under field conditions, treatment for ESC is seldom initiated until fish deaths are in plain sight. In an attempt to duplicate field conditions, this research protocol required that 33 fish from each pond manifest ESC before FFC treatment in that specific pond could commence. For this reason, there was a delay ranging from 4 to 18 d from the time the first ESC case in a medicated pond was diagnosed until FFC treatment commenced. Therefore, high mortalities during the early stages of the treatment period (Days 2–7) in the FFC-medicated ponds were not unexpected. Despite this, there was a significant difference in the cumulative mortalities between medicated and control ponds. A netpen study (Meade et al. 1993) testing the efficacy of sarafloxacin in

TABLE 3. *Kaplan–Meier survival analysis (treatment group strata) based on fish recovered with treatment as strata.*

The LIFETEST procedure					
Summary of the number of censored and uncensored values					
Stratum	Treatment number	Total	Failed	Censored	Percent censored
1	T1 = control	47,879	2300	45,579	95.20
2	T2 = florfenicol	50,358	1478	48,880	97.07
Total		98,237	3778	94,459	96.15
Test of equality over strata					
	Test	Chi square	df	Pr > Chi square	
	Log rank	228.0785	1	<0.0001	
	Wilcoxon	223.8872	1	<0.0001	
	−2Log(LR)	235.8182	1	<0.0001	
Univariate chi squares for the log-rank test					
Variable	Test statistic	Standard deviation	Chi square	Pr > Chi square	
Pond	1456.8	419.2	12.0770	0.0005	

which fish were treated immediately following confirmation of *E. ictaluri* infection also showed high mortalities in medicated fish. Although there was not a significant difference in cumulative mortality, a difference in the daily mortality was seen between the treated and the control groups, indicating that there was a change in the rate of mortality caused by intervention with the drug.

However, in another netpen study (Johnson et al. 1992) testing the efficacy of sarafloxacin against *E. ictaluri* in which there was a treatment delay of 3 d from when the first ESC mortality occurred, many fish became infected, anorectic, and subsequently died before receiving adequate levels of drug in the feed. Cumulative survival rates in the sarafloxacin-medicated ponds were significantly different from the unmedicated ponds and ranged from 48 to 73%.

In a previous FFC range finding study (Gaunt et al. 2003) conducted in tanks, treatment with medicated feed was initiated the day after exposure to *E. ictaluri* and prior to clinical signs. Therefore, mortality was minimal in the FFC-medicated fish and ranged only from 0 to 1.25%. In an aquarium dose confirmation study (Gaunt et al. 2004), there was a higher percentage of mortalities in FFC-medicated fish (0.8–2.5%) because treatment was delayed for 2 d after exposure to *E. ictaluri*. A decrease in appetite was observed, and the fish were overcome by infection and succumbed. Clearly, administration of medicated feed at the first sign of disease will decrease mortalities and will also help avoid development of bacterial resistance. Anorexia is one of the first clinical signs of ESC, and it prevents fish from consuming a therapeutic dose of medication. Bacteria may be sensitive to the concentration of an antibiotic when the fish are feeding well but may not be susceptible to the dose achieved in a fish with the loss of appetite.

The nominal dose rate in this study was 10 mg/kg per d, but the actual dose rate was approximately 60% of nominal or 5.92 mg/kg per d. The lower actual dose rate was as a result of the FFC concentration in the medicated feed at 93% of nominal and reduced feed intake resulting from anorexia associated with the progressing ESC infections in the ponds. It is expected that if treatment initiation were dependent upon detecting the first case of ESC rather than upon meeting the specified pond enrollment criterion, the fish would have consumed more medicated feed and a higher survival rate would have been observed among the FFC-treated fish.

A feeding rate of 2.5% bw, which distributed the medication over a large number of feed pellets, likely contributed to a higher survival rate of the FFC-medicated fish. Rates of feeding medicated feed to *E. ictaluri* infected fish are often decreased to 1% because of formulation characteristics of the available antimicrobial medicated feeds and depressed appetite. In a previously reported study (Johnson and Smith

1994), the number of surviving fish was significantly higher when feeding rates of Romet-medicated feed increased from 1 to 3%. Ponds with higher feeding rates had more pellets available to achieve the same medication level, and this allowed medication of more fish, including less aggressive eaters. Inspection of the stomach contents of the fish at necropsy revealed that larger fingerlings had over 15 pellets in their stomachs and smaller fingerlings had over 4 pellets each in their stomachs.

In field studies, it is difficult to reconcile the stocking numbers of fish with the harvested fish numbers because of predation by birds and mammals and cannibalism. Upon termination of this study, the sum of surviving fish plus the observed fish deaths did not equal the stocking rate of approximately 11,000 fish per pond. During this study, pond workers occasionally witnessed losses of experimental fish to bird predators that removed moribund or dead fish from the ponds. In addition, fish were found on the banks between ponds, so the mortality could not be assigned to a specific pond because it was not certain from which pond the fish came. Similarly, in a previous field study documenting the unaccounted losses of salmon in netpens (Moring 1989), losses were attributed to decomposition of carcasses during disease outbreaks and scavenging by birds, mammals, and fishes. These losses were generally not documented until a cage was emptied at the time of harvest. In a field study on catfish production (Tucker et al. 1994), the researchers noted that the total losses calculated from estimates of fish harvested and from the fish allotted were higher than the losses accounted for by retrieving dead fish. The fate of the fish that died but were not recovered was not known. The authors concluded that observed losses will always underestimate the total losses, possibly by a factor of seven or more.

In this study, there was no difference in the cumulative infection rates with *E. ictaluri* of control versus medicated fish. The analysis of infection rates of mortalities (both for inclusion purposes and infection rate analysis) was complicated by the fact that not all fish were suitable for bacterial isolation. Many of the mortalities were autolyzed and were presumed to be dead from ESC, but *E. ictaluri* could not be separated from faster growing bacteria present from postmortem overgrowth. In addition, pond temperatures that rose outside the optimal ESC range (28–31 C) during the 14-d observation period could inhibit growth of *E. ictaluri* (and decrease infection rates).

No specific gross lesions that could be associated with the antibiotic were observed in this field study. This is in agreement with previous range-finding studies in which no gross or histopathological lesions induced by FFC were observed among 640 fish in efficacy and tolerance studies with dose rates ranging from 10 to 40 mg FFC/kg bw and 10 to 100 mg FFC/kg bw, respectively (Gaunt et al. 2003).

The low MIC for FFC indicated potent *in vitro* antimicrobial activity against the *E. ictaluri* isolate tested. This is in agreement with the reported MIC values of FFC for most pathogenic fish bacteria, which range from 0.3 to 1.6 μg/mL (Fukui et al. 1987; Inglis and Richards 1991; Martinsen et al. 1993; Bruun et al. 2000; Schmidt et al. 2000).

In conclusion, FFC administered in feed at a daily dose rate of 10 mg FFC/kg bw of fish for 10 consecutive d was effective in reducing mortalities from *E. ictaluri* in channel catfish fingerlings when fed 4–18 d postinfection.

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