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Aquatic Animal Drug Approval Partnership

DRUG RESEARCH INFORMATION BULLETIN

Efficacy of Chloramine-T to Control Mortality in Largemouth Bass Naturally Infected with External Columnaris

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Columnaris disease (causative agent, *Flavobacterium columnare*) is an acute-to-chronic bacterial infection with a worldwide distribution capable of infecting most freshwater fish (Noga 2000). The disease most commonly occurs as an external infection; however, it can also occur as a systemic infection with no visible external signs (Plumb 1999). *Flavobacterium columnare* is more pathogenic at temperatures > 15°C, and both mortality and acuteness of disease increase with temperature (Noga 2000). Typically, a columnaris outbreak requires intervention (e.g., improving fish culture conditions or using chemotherapeutants) to reduce the external bacterial load on fish. Several chemotherapeutants have historically been used to control mortality caused by external columnaris, and chloramine-T (CLT) is regarded as one of the most effective. To support U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM) approval of CLT for use in aquaculture, data are required that demonstrate its effectiveness.

In this bulletin, we summarize the results of two trials conducted to demonstrate the effectiveness of CLT to control mortality in largemouth bass (LMB) *Micropterus salmoides* fingerlings naturally infected with external columnaris.

Methods

Two independent trials were conducted in 2007 at the Florida Bass Conservation Center's Richloam Fish Hatchery (RFH) in Webster, FL. Test fish were LMB fingerlings (Trial 1, mean length, 6.9 cm; Trial 2, mean length, 10.8 cm).

After moribund fish from a naturally infected reference population were presumptively diagnosed with external columnaris, completely randomized design procedures were used to assign fish and treatment conditions (treated vs. nontreated control) to test tanks. Test tanks were stocked with fish impartially collected from the reference population. Each treatment condition was replicated six times in Trial 1 (n = 12 test tanks; n = 314 fish/tank) and four times in Trial 2 (n = 8 test tanks; n = 800 fish/tank). Each trial comprised a 1-d acclimation period, 3-d treatment period, and 14-d posttreatment observation period. During the treatment period, CLT was administered to treated tanks at a target concentration of 20 mg/L in a static bath for 60 min per day on three consecutive days, and control tanks received a sham treatment of pure hatchery water under static-bath conditions. Mortality, fish behavior, feeding behavior, water temperature, and dissolved oxygen concentration data were collected daily throughout the trial. Water samples were collected for CLT dose verification from each test tank 30 - 45 min into each 60-min treatment. Analytical dose verification was done with a HACH DR/890 colorimeter (HACH Co., Loveland, CO).

For each trial, SAS PROC GLIMMIX (logit link) was used to (a) compare mean cumulative mortality in control tanks to that in treated tanks on each day of the treatment and posttreatment periods and (b) generate mean daily odds ratios. Mean daily odds ratios > 1 indicated that odds of mortality in control tanks was greater than odds of mortality in treated tanks. Treatment levels were judged statistically significant if $P < 0.05$.

Results

At the end of Trial 1 (trial day 17; Figure 1), mean cumulative mortality in treated tanks (27%; range, 21–34% per tank) was significantly less ($P = 0.010$) than mean cumulative mortality in control tanks (35%; range, 33–42% per tank). Also, a

significant difference was detected in mean cumulative mortality between treated and control tanks on all trial days after the first CLT treatment had been administered. Mean daily odds ratios were relatively constant throughout the trial (range, 1.4 – 1.6), indicating that odds of mortality in control tanks was always greater than that in treated tanks.

In Trial 2 (Figure 2), mean cumulative mortality in treated tanks was significantly less ($P < 0.05$) than mean cumulative mortality in control tanks from the last treatment day (trial day 3) through posttreatment day 9 (trial day 12). However, on posttreatment day 8 (trial day 11), mortality had begun to increase in one of the four treated tanks. The increasing mortality in this tank produced a steady rise in mean cumulative mortality in the treated group through the end of the trial. Consequently, at the end of Trial 2, mean cumulative mortality in treated tanks (27%; range, 21–34% per tank) was not significantly different ($P = 0.484$) from that in control tanks (35%; range, 33–42% per tank). Although the outcome of Trial 2 was not significant, mean daily odds ratios that varied between 1.3 and 2.9 indicated that odds of mortality in control tanks was always greater than odds of mortality in treated tanks.

Throughout Trial 1, all fish appeared to behave normally and feed aggressively. In Trial 2, fish behavior appeared normal in one test tank and hyperactive in seven test tanks during the treatment period. Concurrently, feeding behavior appeared semi-aggressive in all tanks on treatment day one and aggressive in all tanks on treatment days two and three. During the posttreatment period, all fish in all test tanks appeared to behave normally and feed aggressively.

Overall, mean daily CLT concentrations of 19.5 mg/L and 20.5 mg/L were administered to treated tanks in Trials 1 and 2, respectively. Chloramine-T was not detected in control tanks. Mean water temperature and mean dissolved oxygen concentration in Trial 1 (25.2°C; 15.8 mg/L) and Trial 2 (24.8°C; 15.2 mg/L) were suitable for rearing healthy LMB. At RFH, obligatory O₂ supplementation results in high dissolved oxygen levels at this facility.

Discussion

Results from Trial 1 clearly demonstrated the efficacy of CLT administered at 20 mg/L for 60 min daily in a static bath on three consecutive days to control mortality in LMB naturally infected with external columnaris. In Trial 2, however, significant differences in mortality between treated and control tanks were only detected on trial days 3–12. Although not confirmed by additional fish health evaluations, we suspect that a columnaris reinfection occurred near posttreatment day 8. Such a reinfection was evidenced by a steady decline in mean daily odds ratios during the last 7 d of the trial and by the nonsignificant outcome of the trial.

Both trials were summarized in final study reports (FSRs) and have been submitted to FDA/CVM in support of an initial approval for the use of CLT to control mortality in a variety of freshwater finfish due to external columnaris disease associated with *Flavobacterium columnare*. As of February, 2008, FDA/CVM acceptance of these FSRs is pending.

Figure 1. Trial 1: Mean ($\pm 1SD$) percent cumulative mortality of LMB in treated and control tanks and mean daily odds ratios (odds of mortality in control tanks:odds of mortality in treated tanks), Richloam Fish Hatchery, Webster, FL. Dashed vertical line represents the last day of treatment.

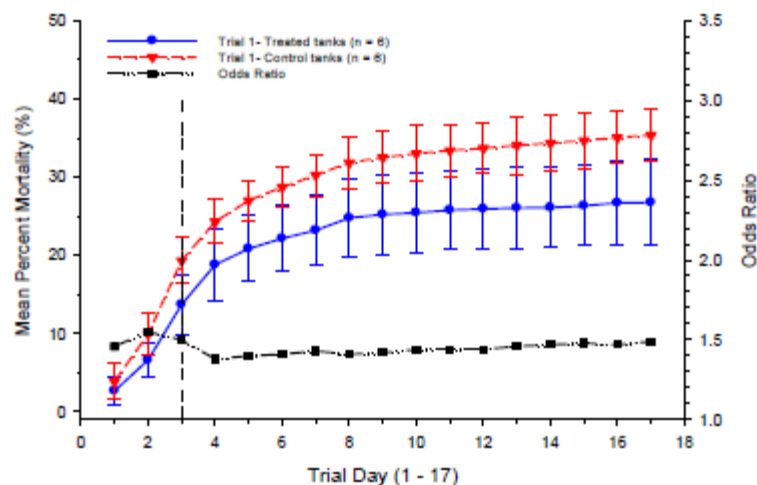
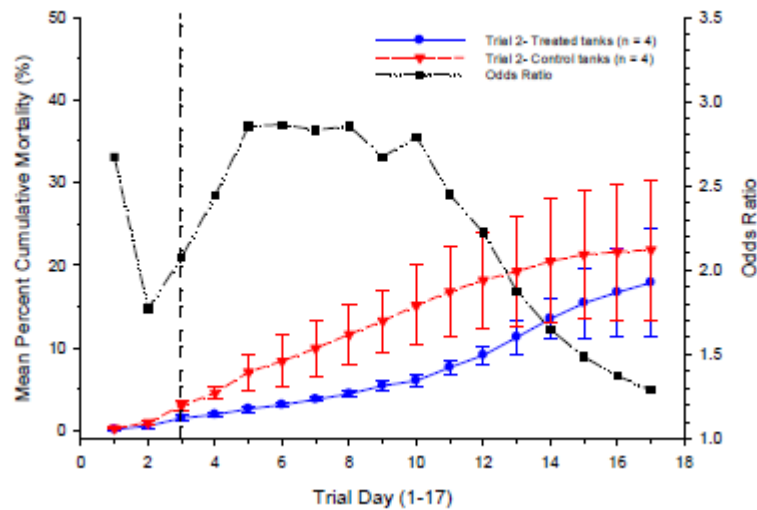


Figure 2. Trial 2: Mean ($\pm 1SD$) percent cumulative mortality of LMB in treated and control tanks and mean daily odds ratios (odds of mortality in control tanks:odds of mortality in treated tanks), Richloam Fish Hatchery, Webster, FL. Dashed vertical line represents the last day of treatment.



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