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Efficacy of Aquamycin 100® (22.045% Erythromycin Thiocyanate) to Control Mortality in Chinook Salmon Fingerlings Diagnosed with Bacterial Kidney Disease

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Bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum (R. sal.)*, adversely affects cultured and wild salmonids worldwide (Noga 2000). External clinical signs are not always evident but can include pale gills, exophthalmia, abdominal extension, skin blisters, shallow ulcers, and hemorrhages (Noga 2000). Internally, creamy-white granulomatous lesions are often present in the kidney (Evelyn 1993). *Renibacterium salmoninarum* is a small, Gram-positive, non-acid-fast, non-spore-forming, non-motile, fastidious diplobacillus that grows best at 15 - 18°C and not at all at 25°C (Evelyn 1993).

Chemotherapeutic control of BKD has been relatively ineffective because of the intracellular nature of the pathogen (Fryer and Sanders 1981). Erythromycin has been used with limited success when administered in feed for 28 d to young fish (Munson et al. 2010). Aquamycin 100® (22.045% erythromycin thiocyanate; ET; Bimeda USA, Inc., Oakbrook Terrace, Illinois USA) is the product most commonly used in the U.S. to treat BKD; however, it can only be used under a U.S. Food and Drug Administration (FDA) Investigational New Animal Drug exemption. Treatment efficacy has been inconsistent, and some investigators have noted signs of toxicity. Nevertheless, efforts to obtain FDA approval continue. To that end, we cooperated in a trial to evaluate the efficacy of Aquamycin 100® administered in feed to control mortality in freshwater-reared Chinook salmon (CHS) *Oncorhynchus tshawytscha* diagnosed with BKD.

Methods

The trial was conducted 24 June - 9 August 2010, at the Idaho Department of Fish and Game, Eagle Fish Health Laboratory (EFHL), Eagle, Idaho USA. Test fish were CHS fingerlings (mean weight, 4.6 g; mean length, 7.2 cm). Aquamycin 100®-medicated feed was administered at an intended target dosage of 100 mg ET per kg fish per d for 28 consecutive days. The treatment objective was to demonstrate a significant difference in mean percent cumulative mortality between treated and control groups.

Before the trial began, mortality in the reference fish population had become elevated, and some fish exhibited clinical signs of BKD. The presence of R. sal. in moribund and dead fish was confirmed via polymerase chain reaction and Direct Fluorescent Antibody Test (DFAT) methods. These results confirmed BKD was the primary cause of mortality and triggered initiation of the trial. Treatment conditions and impartially captured reference population fish were randomly allocated to eight 129-L test tanks (four Aquamycin 100®-treated tanks and four nontreated control tanks; 250 fish per tank). Tanks were supplied with first-pass spring water at flow rates suitable for rearing healthy CHS.

The 47-d trial comprised a 5-d acclimation period, 28-d treatment period, and 14-d posttreatment period. During the treatment period, Aquamycin 100®-medicated feed was administered to fish in treated tanks, and nonmedicated feed was administered to fish in control tanks. Nonmedicated feed was administered to fish in all tanks during the pre- and posttreatment periods. Automatic feeders were used to dispense feed at 2.0% mean fish body weight (BW) per d; however,

feed should have been dispensed at 1.5% BW per d to administer the intended target dose. Consequently, the nominal ET dose administered to fish in treated tanks was 132 mg per kg fish per d or 1.32 times greater than target. During the trial, feed amounts were not adjusted for mortality or growth.

Mortality, general fish behavior, fish-feeding behavior, water temperature, and dissolved oxygen concentration data were collected daily. Feeding behavior was scored on a 5-point ordinal scale. Briefly, a score of "0" meant no feed was consumed, and a score of "4" meant that approximately 100% of feed was consumed and that fish were feeding aggressively. Water hardness and alkalinity were measured once, and pH was measured twice.

During the treatment period, five moribund or recently dead fish were collected from each tank for fish health evaluations. During the posttreatment period, five moribund or recently dead fish were collected from each control tank for fish health evaluations. Thirty eight days after the study ended, five apparently healthy fish were collected from each treated tank for fish health evaluations. Fish health evaluations included (1) visual examination of external and internal organs, (2) examination of skin-scrapes by light microscopy for secondary bacteria and parasites, and (3) testing for *R. sal*. via DFAT. Erythromycin concentrations in medicated and nonmedicated feeds were not analytically verified.

A SAS PROC GLIMMIX-based model (logit link) was used to compare ($P < 0.05$, two-sided) mean percent cumulative mortality between treatment groups.

Results and Discussion

At the end of the trial, mean cumulative mortality in treated tanks (9%; range, 5 - 11% per tank) was significantly ($P = 0.0004$) different from mean cumulative mortality in control tanks (25%; range, 21 - 28% per tank; Figure 1). The DFAT results from fish sampled during the treatment and posttreatment periods confirmed *R. sal*. was associated with mortality. In contrast, all treated fish sampled at 38 d poststudy were DFAT-negative for *R. sal.* and showed no clinical signs of BKD.

General fish behavior was characterized as normal in all tanks throughout the trial. During the treatment period, fish in treated tanks appeared to eat less feed than fish in control tanks (50 - 75% vs. 75 - 100% of feed offered). During the posttreatment period estimated amount of feed eaten was similar in both groups (50 - 75% of feed offered).

Mean ± SD water temperature and dissolved oxygen concentration were 13.7 ± 0.03°C and 6.7 ± 0.5 mg per L, respectively. Water hardness and alkalinity (as $CaCO_3$) were 82 and 9 mg per L, respectively. Mean pH was 7.3. All of these water quality parameters were considered suitable for rearing CHS at EFHL.

In this trial, treatment efficacy might have been enhanced because the nominal ET dose administered (132 mg per kg fish per d) was 1.32 times the target dose (100 mg per kg fish per d). However, because fish in treated tanks were estimated to have consumed only about 50 - 75% of feed offered per d, the actual ET dose received by treated fish was likely close to the target dose (e.g., $132 \times 0.75 = 99$ mg per kg fish per d). In conclusion, results suggest Aquamycin 100® administered in feed can be used to control mortality in CHS and likely in other freshwater-reared salmonids diagnosed with BKD.

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Figure 1. Mean ± SD percent cumulative mortality of Chinook salmon fingerlings diagnosed with bacterial kidney disease.