



Efficacy of AQUAFLO[®] (50% Florfenicol) to Control Mortality in Chinook Salmon Diagnosed with Bacterial Kidney Disease (2014)

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Bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum* (Sanders and Fryer 1980), is a serious disease of cultured and feral salmonids (Earp et al. 1953; Smith 1964). This disease can result in significant mortality, and is widespread throughout North America, Chile, Europe, and Japan. Fish with BKD may or may not show external clinical signs (e.g., pale gills, exophthalmia, abdominal distension, skin blisters, shallow ulcers, or hemorrhages). Internally, infected fish are most frequently observed with creamy-white granulomatous lesions in the kidney (Inglis et al. 1993). *R. salmoninarum* is a small (0.5 × 1.0 μm), Gram-positive, non-acid-fast, non-spore-forming, non-motile, fastidious diplobacillus that grows best at 15 – 18 °C, but not at all at 25 °C (Inglis et al. 1993).

Chemotherapeutic control of BKD has proven to be relatively ineffective due to the intracellular nature of the pathogen (Fryer and Sanders 1981). Due to the ineffectiveness of external antimicrobial agents, fisheries professionals began to evaluate the effectiveness of antibiotic treatment. Erythromycin has been identified as the antibiotic of choice for combating BKD, and a 21-d oral regimen is recommended for treating young hatchery fish (Wolf and Dunbar 1959). AQUAMYCIN[®] 100 (erythromycin thiocyanate; Bimeda, a Division of Cross Vetpharm Group, Ltd., Lehigh, Iowa USA) has been the product most commonly used for treatment of BKD in the U.S., albeit solely under an Investigational New Animal Drug (INAD) exemption. Despite routine use under the INAD exemption, its progress towards FDA-approval has been slow, treatment efficacy has been somewhat inconsistent, and some investigators have noted signs of toxicity. As a result, there has been a desire to evaluate the effectiveness of alternative antibiotics, preferably one that is already approved by FDA for use in salmonids.

AQUAFLO[®] (50% florfenicol; Merck Health Corp., Roseland, New Jersey) is approved for the control of mortality in all freshwater-reared salmonids due to furunculosis, coldwater disease, and columnaris disease. Florfenicol is a broad-spectrum antibiotic with bacteriostatic and bactericidal properties and is active against a variety of Gram-positive and Gram-negative

bacteria. This product is approved as a veterinary feed directive drug and can be legally administered at a dosage of 10-15 mg florfenicol/kg fish/d for 10 consecutive days. In the interest of expanding the current label for AQUAFLO[®], additional potential uses in aquaculture are being tested experimentally, including studies to evaluate its effectiveness to control mortality caused by BKD. Two field effectiveness studies have shown that AQUAFLO[®] administered at a dosage of 15 mg florfenicol/kg fish/d for 10 d is effective in reducing mortality in freshwater-reared Chinook Salmon *Oncorhynchus tshawytscha* (CHS) diagnosed with BKD (Bowker et al. 2010; Bowker et al. 2011). Although Final Study Reports summarizing results from these two studies were submitted to and accepted by the U.S. Food and Drug Administration's Center for Veterinary Medicine Aquaculture Team, a third successful study is required to complete the effectiveness technical section for this claim. As such, this bulletin summarizes the results of a third field study that was conducted to evaluate the efficacy of AQUAFLO[®] to control mortality in freshwater-reared CHS diagnosed with BKD.

Methods

The study was conducted June 25 – July 19, 2014 at the Idaho Department of Fish and Game Eagle Fish Health Lab (EFHL), Eagle, Idaho. Test fish were CHS fingerlings (mean weight, 2.8 g; mean length, 6.2 cm). AQUAFLO[®]-medicated feed was administered at a target dosage of 15 mg florfenicol/kg fish/d for 10 consecutive days.

Before the study began, *R. salmoninarum* was cultured from kidney tissue of 10 dead or moribund fish sampled from the reference population before the start of the study and the pathogen was confirmed via Direct Fluorescent Antibody Test (DFAT). Based on clinical signs, an initial presumptive diagnosis, and the resulting DFAT confirmation, it was determined that BKD was causing the mortality. Fish from the reference population were then collected by dipnetting, individually counted, and randomly allocated among eight test tanks (113-L water volume; 4 treated and 4 controls;

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250 fish/tank [range 243–261 fish/tank]). Treatment conditions (AQUAFLO[®]-medicated feed treated vs. nontreated control) were allocated among tanks using a completely randomized design. Tanks were supplied with first-pass water at flow rates suitable for rearing healthy CHS.

The 25-d study comprised of 1-d acclimation, 10-d treatment, and 14-d posttreatment periods. During the treatment period, AQUAFLO[®]-medicated feed was administered to treated tanks and nonmedicated feed was administered to control tanks. During the posttreatment period, nonmedicated feed was administered to all tanks. Feed was administered at 2.0% of mean fish body weight/d and amounts were not adjusted for mortality or growth during the treatment period.

Mortality, general fish behavior, feeding behavior, water temperature, and dissolved oxygen concentration data were recorded daily. Appetite behavior data were determined based on the relative amount of feed consumed; values were 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 the feed was consumed, fish were feeding aggressively, and that some fish broke the surface of the water during feeding. Hardness, alkalinity, and pH of source water were measured once at the beginning of the study. Five dead or moribund fish were collected from each tank during both the treatment and posttreatment periods for fish health evaluation, which consisted of (1) external and internal gross necropsy, (2) preparation and examination of wet skin scrape mounts by light microscopy for bacteria and parasites, and (3) imprinting kidney tissue on 12-well 5 mm hydrophobic, autoclavable glass slides to test for the presence of *R. salmoninarum* by DFAT. In addition, kidney and spleen were streaked on appropriate media and cultured for Gram Negative bacteria. Florfenicol concentrations in medicated and nonmedicated feed samples were analytically verified by Eurofins Scientific Inc., Portage, Michigan.

The SAS PROC GLIMMIX procedure was used to statistically compare mean cumulative mortality in control tanks to that in treated tanks on each day of the treatment and posttreatment periods. Treatment differences were judged significant if $P < 0.05$.

Results and Discussion

At the end of the study, mean cumulative mortality in treated tanks (27.4%; range, 21.7 – 41.6% per tank) was lower than that in control tanks (36.3%; range, 18.3 – 49.8% per tank (Figure 1), but differences were not significant ($P = 0.3173$). The presence of *R. salmoninarum* was confirmed in all 40 fish sampled during the treatment period and 34 of the 40 fish (19 treated fish and 15 control fish) sampled during the posttreatment period. In

addition, *Aeromonas hydrophila* was isolated from kidney tissue of 13 of the fish sampled during the treatment period and *A. hydrophila* and/or *Pseudomonas fluorescens* was isolated from kidney tissue of 26 of the fish sampled during the posttreatment period. No *F. psychrophilum* was isolated from kidney tissue of any fish sampled during the treatment or posttreatment periods.

During the treatment period, fish in treated tanks consumed feed more frequently at the 3 (~75% of feed offered) and 4 (~100% of feed offered) levels while fish in control tanks consumed feed more frequently at the 0 (~0% of feed offered), 1, (~25% of feed offered), and 2 (~50% of feed offered) levels. During the posttreatment period, feed consumption was relatively similar between fish in treated tanks and fish in control tanks, and fish in both groups consumed ~50 – 100% of the feed offered. Throughout the study, general fish behavior was characterized as normal.

Mean water temperatures and dissolved oxygen concentration during the study were 13.9°C (range, 13.7–14.6°C) and 6.9 mg/L (range, 6.2–7.7 mg/L) respectively. Water hardness (92 mg/L CaCO₃), alkalinity (78 mg/L CaCO₃), and pH (7.1) were adequate for rearing healthy salmonids. The analytically verified florfenicol dose administered to fish was 14.5 mg florfenicol/kg fish/d.

Results from this study indicate that AQUAFLO[®]-medicated feed was not efficacious in controlling mortality in CHS fingerlings diagnosed with BKD when administered at the target dosage because the difference in mortality was not significant. However, the numerical difference in relative mortality in the present study (8%) was very similar to that observed in two previously conducted studies, wherein differences of 9% (Bowker et al. 2010) and 10% (Bowker et al. 2011) were reported. The relative percent survival (RPS; 100*(treated-control)/control) in this study (12.8%) was also very similar to the two previously conducted studies, where an RPS of 12.5% (Bowker et al. 2010) and 14.4% (Bowker et al 2011) were reported. Mortality in the present study was higher and more variable than that reported in the other two studies mentioned above, and this may have influenced our ability to resolve statistical significance.

The results from the present study have been summarized in a Final Study Report and submitted to FDA’s Center for Veterinary Medicine for review. It is our hope that FDA will consider the “body of evidence” of the three studies conducted in support of expanding the approval of AQUAFLO[®] in the U.S., and consider the effectiveness technical section complete for this claim.

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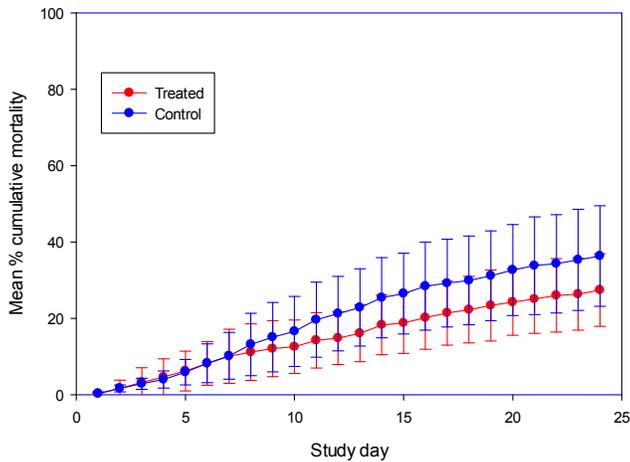


Figure 1. Mean (\pm SD) percent cumulative mortality (treated tanks vs. control tanks) of Chinook salmon fingerlings diagnosed with bacterial kidney disease. The treatment period included study days 1 – 10.

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