



U.S. Fish & Wildlife Service

Aquatic Animal Drug Approval Partnership

DRUG RESEARCH INFORMATION BULLETIN**Efficacy of AQUAFLO[®] (50% Florfenicol) to Control Mortality in Chinook Salmon Diagnosed with Bacterial Kidney Disease**Jim Bowker*, Dan Carty, Molly P. Bowman, and Doug Munson¹*U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program
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Bacterial kidney disease (BKD) caused by *Renibacterium salmoninarum*, adversely affects cultured and wild salmonids (Earp et al. 1953; Smith 1964, Sanders and Fryer 1980). The disease is widespread in North America, Chile, Europe, and Japan and can cause significant mortality in cultured salmonid populations. External clinical signs of BKD are not always evident but can include pale gills, exophthalmia, abdominal extension, skin blisters, shallow ulcers, and hemorrhages. 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 21 d to young fish (Wolf and Dunbar 1959). AQUAMYCIN[®] 100 (erythromycin thiocyanate; Bimeda, a Division of Cross Vetpharm Group, Ltd., Lehigh, Iowa USA) is the product most commonly used in the U.S. for treatment of BKD; however, it can only be used under a U.S. Food and Drug Administration (FDA) Investigational New Animal Drug exemption. Moreover, progress towards FDA approval of AQUAMYCIN[®] 100 has been slow, treatment efficacy has been inconsistent, and some investigators have noted signs of toxicity. Consequently, the U.S. aquaculture community is interested in exploring other drug treatment options that may be effective for the control of mortality caused by BKD.

AQUAFLO[®] (50% florfenicol; Intervet/Schering-Plough Animal Health Corp., Roseland, New Jersey USA) is FDA-approved for the control of mortality in all freshwater-reared salmonids due to furunculosis (causative agent, *Aeromonas salmonicida*) and coldwater disease (causative agent, *Flavobacterium psychrophilum*). Florfenicol is a broad-spectrum antibiotic with bacteriostatic and bactericidal properties and is active against a variety of Gram-positive and Gram-negative bacteria. Currently, AQUAFLO[®] must be used under veterinary prescription and administered at a dosage of 10 mg florfenicol/kg fish/d for 10 consecutive days. However, additional treatment regimens and aquaculture uses are being researched. As part of that research effort, we designed and coordinated a field trial to evaluate the efficacy of AQUAFLO[®] to control mortality in freshwater-reared Chinook salmon (CHS) *Oncorhynchus tshawytscha* diagnosed with BKD.

Methods

The trial was conducted June 28 – July 22, 2010, at the Idaho Department of Fish and Game, Eagle Fish Health Lab (EFHL), Eagle, Idaho USA. Test fish were CHS fingerlings (mean weight, 4.6 g; mean length, 7.2 cm). AQUAFLO[®]-medicated feed was administered at a target dosage of 15 mg florfenicol/kg fish/d for 10 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, some fish in the reference population exhibited clinical signs of BKD with an associated increase in mortality. The presence of *R. salmoninarum* in moribund and dead fish was confirmed via polymerase chain reaction (PCR)

and Direct Fluorescent Antibody Test (DFAT). Based on these results it was evident that BKD was the primary cause of mortality and triggered the initiation of the trial. Treatment conditions and impartially collected reference population fish were then randomly allocated to eight 129-L test tanks (four AQUAFLO[®]-treated tanks and four nontreated control tanks; 233 fish/tank). Tanks were supplied with first-pass water at flow rates suitable for rearing healthy CHS.

The 25-d trial comprised a 1-d acclimation, a 10-d treatment, and a 14-d posttreatment period. 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. During the trial, automatic feeders were used to administer feed at 2.0% mean fish body weight/tank/d. 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. Source water hardness and alkalinity were measured once during the trial, and source water pH was measured twice.

During the treatment and posttreatment periods, five dead or moribund fish were collected from each tank for fish health evaluations (total, 10 fish collected/tank). 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) detection of *R. salmoninarum* via DFAT. Florfenicol concentrations in medicated and nonmedicated feed samples were analytically verified by Eurofins Scientific Inc., Portage, Michigan USA.

The SAS PROC GLIMMIX procedure was used to compare mean percent cumulative mortality between treatment groups 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 trial, mean cumulative mortality in treated tanks (12.1%; range, 11.4 – 12.7% per tank) was significantly ($P = 0.0003$) different from mean cumulative mortality in control tanks (20.5%; range, 17.3 – 22.9% per tank; Figure 1). The DFAT results from moribund or dead fish sampled during the treatment and posttreatment periods confirmed *R. salmoninarum* was associated with mortality.

General fish behavior was characterized as normal in both treatment groups throughout the trial. However, fish in both treatment groups appeared to feed more aggressively during the treatment period than during the posttreatment period. During the treatment period, observers estimated treated and control fish ate 75 – 100% of feed offered per tank per day. In contrast, during the posttreatment period, observers estimated treated fish ate 50 – 100% of feed offered per tank per day while control fish ate only 25 – 100% of feed offered per tank per day.

Mean water temperature and dissolved oxygen concentration during the trial were 13.7°C (range, 13.6 – 13.8°C) and 6.6 mg/L (range, 4.9 – 9.4 mg/L) respectively. Water hardness (82 mg/L as CaCO₃), alkalinity (9 mg/L as CaCO₃), and pH (range, 7.1 – 7.5) were considered normal and within ranges suitable for rearing CHS at EFHL. The florfenicol dose administered to treated tanks was 15.3 mg florfenicol/kg fish/d (102% of target), which was within FDA-acceptable limits (80 – 110% of target). No florfenicol was detected in control feed.

Based on these results, we concluded that AQUAFLO[®] administered in feed at 15 mg/kg fish/d for 10 d was effective in controlling mortality in a test population of CHS fingerlings diagnosed with BKD. Results have been submitted to FDA and will be used to support expanding the current AQUAFLO[®] label to include control of mortality in all freshwater-reared Chinook salmon due to BKD associated with *R. salmoninarum*.

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Figure 1. Mean (\pm SD) percent cumulative mortality of Chinook salmon fingerlings diagnosed with bacterial kidney disease.

