DRIB No. 18
October 2010

U.S. Fish & Wildlife Service

Aquatic Animal Drug Approval Partnership

DRUG RESEARCH INFORMATION BULLETIN

Efficacy of AQUAFLOR® (50% Florfenicol) to Control Mortality in Chinook Salmon Diagnosed with Bacterial Kidney Disease

Jim Bowker*, Doug Munson[1], and Miranda Dotson

U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program
4050 Bridger Canyon Road, Bozeman, Montana, 59715, USA

[1]Idaho Department of Fish and Game, Eagle Fish Health Lab
1800 Eagle Trout Road, Eagle, Idaho 83616, USA

Bacterial kidney disease, caused by Renibacterium salmoninarum (Sanders and Fryer 1980), is a serious disease of cultured and feral salmonids (Earp et al. 1953; Smith 1964). Bacterial kidney disease (BKD) can result in significant mortality, and is wide-spread throughout North America, Chile, Europe, and Japan. Fish with BKD may or may not show external clinical signs (e.g., pale gills, exophthalmia, abdominal extension, 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). *Renibacterium 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, and 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 lack of efficacy by non-antibiotic therapeutants, 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 recommended for treating young hatchery fish (Wolf and Dunbar 1959). Currently, AQUAMYCIN® 100 (erythryomycin thiocyanate; Bimeda, a Division of Cross Vetpharm Group, Ltd., Lehigh, Iowa USA) is the product most commonly used for treatment of BKD in the U.S., albeit solely under an Investigational New Animal Drug (INAD) exemption. However, 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.

AQUAFLOR® (50% florfenicol; Intervet/Schering-Plough Animal Health Corp., Roseland, New Jersey USA) is 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. Legally, this product must be used under veterinary prescription and administered at a dosage of 10 mg florfenicol/kg fish/d for 10 consecutive days. In the interest of expanding the current label for AQUAFLOR®, additional potential uses in aquaculture are being tested experimentally. As such, this bulletin summarizes the results of a field trial conducted to evaluate the efficacy of AQUAFLOR® to control mortality in freshwater-reared Chinook salmon Oncorhynchus tshawytscha (CHS) diagnosed with BKD.

Methods

The trial was conducted May 22 – June 15, 2010 at the Idaho Department of Fish and Game, Eagle Fish Health Lab (EFHL), Eagle, Idaho, USA. Test fish were CHS fingerlings (mean weight, 2.91 g; mean length, 6.3 cm). AQUAFLOR®-medicated feed was administered at a target dosage of 15 mg florfenicol/kg fish/d for 10 consecutive days.

Before the trial began, kidney tissue of dead or moribund fish sampled from the reference population were confirmed via polymerase chain reaction to harbor *R. salmoninarum*. In turn, it was thus deduced that noted mortalities were caused by BKD. Fish from the reference population were then impartially collected by dipnetting, counted, and randomly allocated among eight 98-L test tanks (4 treated and 4 control; 206 fish/tank). Treatment conditions (AQUAFLOR®-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 trial comprised 1-d acclimation, 10-d treatment, and 14-d posttreatment periods. During the treatment period, AQUAFLOR®-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 study, feed was administered at 4.0% of mean fish body weight/d, and amounts were not adjusted for growth.

Mortality, general fish behavior, feeding behavior, water temperature, and dissolved oxygen concentration data were collected 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 twice during the trial. During both the treatment and post-treatment periods, five dead or moribund fish were collected from each tank for fish health evaluation. Fish health examinations were comprised 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 direct fluorescent antibody test (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 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 trial, mean cumulative mortality in treated tanks (14.8%; range, 11.7 – 18.9% per tank) was significantly different ($P = 0.0202$) than mean cumulative mortality in control tanks (24.3%; range, 20.9 – 32.0% per tank; Figure 1). Results from DFAT confirmed that *R. salmoninarum* was associated with mortality during the study.

During the treatment period, fish consumed between 50 and 75% of the feed offered and hence, the mean feeding behavior score in both treated and control tanks was 2.8. During the posttreatment period, fish consumed nearly 100% of the feed offered, and the mean feeding behavior score was 4.0 in treated tanks and 3.9 in control tanks. Overall (treatment and posttreatment period combined), the mean feeding behavior in both treated and control tanks was 3.3. Throughout the trial, general fish behavior was characterized as normal.

Mean water temperatures and dissolved oxygen concentration during the trial were 13.6°C (range, 13.3 – 13.7°C) and 6.1 mg/L (range, 4.5 – 7.5 mg/L) respectively. Mean water hardness (89 mg/L $CaCO_3$), alkalinity (7 mg/L $CaCO_3$), and pH (range, 7.1 – 7.3) were considered normal and within ranges suitable for rearing CHS at EFHL. The analytically verified florfenicol dose administered to fish was 16.0 mg florfenicol/kg fish/d. Based on the estimated amount of feed consumed by fish during the treatment period, it was likely that the actual dose administered to fish in treated tanks was between 8 and 12 mg florfenicol/kg fish body weight/d. No florfenicol was detected in control feed.

Based on results from this trial, we concluded that AQUAFLOR®-medicated feed was efficacious in controlling mortality when administered at target dosage of 15 mg florfenicol/kg fish/d for 10 consecutive days to CHS fingerlings diagnosed with BKD. The results from this trial have been summarized in a final study report and submitted to FDA's Center for Veterinary Medicine in support of expanding the approval of AQUAFLOR® in U.S. aquaculture to include use to control mortality in all freshwater-reared, Chinook salmon due to BKD associated with *R. salmoninarum*.

Acknowledgments

We thank Gregg Anderson, Eagle Fish Health Lab, and Molly Bowman, FWS AADAP, for their help conducting or coordinating this study. Tom Bell, Dan Carty, and Dave Erdahl, FWS AADAP, critically reviewed this bulletin.

References

Earp, B. J., C. H. Ellis, and E. J. Ordal. 1953. Kidney disease in young salmon. State of Washington Department of Fisheries. Special Report Series, Number 1.

Fryer, J. L., and J. E. Sanders. 1981. Bacterial kidney disease of salm-onid fish. Annual Review of Microbiology 35:273-298.

Inglis, V., R. J. Roberts, and N. R. Bromage. 1993. Bacterial diseases of fish. Halstead Press: an imprint of John Wiley & Sons, Inc. New York.312 pp.

Sanders, J. E., and J. L. Fryer. 1980. Renibacterium salmoninarum gen. nov., sp. nov., the causative agent of bacterial kidney disease in salmonid fishes. International Journal of Systemic Bacteriology 30:496-502.

Smith, I. W. 1964. The occurrence and pathology of Dee disease. Freshwater and Salmon Fishery Research 34:1-12.

Wolf, K., and C. E. Dunbar. 1959. Test of 34 therapeutic agents for control of kidney disease in trout. Transactions of the American Fisheries Society 88:117-134.

***Corresponding author: jim_bowker@fws.gov**

Figure 1. Mean (± SD) percent cumulative mortality (treated tanks vs. control tanks) of Chinook salmon fingerlings diagnosed with bacterial kidney disease. Treatment period equals trial days 1 – 10.