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Efficacy of Terramycin® 200 for Fish (Oxytetracycline dihydrate) to Control Mortality in Tiger Musky Due to Columnaris

Niccole Wandelear*[1], James D. Bowker[1], Geofrey Eckerlin[2]

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

[2]*New York Department of Environmental Conservation, Rome Fish Health Laboratory*
8314 Fish Hatchery Road, Rome, New York, 13440, USA

Columnaris (causative agent, *Flavobacterium columnare*) is an acute-to-chronic external or systemic bacterial disease affecting freshwater-reared finfish worldwide (Bullock et al. 1986). Infections begin when *F. columnare* invades epithelial tissues, most commonly affecting the gills and buccal, opercular, dorsal, and caudal surfaces (Post 1987). Lesions form as the infection progresses, first as light-colored patches with slight hemorrhaging at the periphery and later as large circular lesions to which yellowish mucoid material commonly adheres. These lesions are often observed at the base of the dorsal fin, the caudal fin, or sites of abrasion. The bacteria are not usually found systemically until a relatively large amount of external epithelial damage has taken place. In virtually all instances, columnaris outbreaks require intervention to prevent significant losses. If left untreated, every member of the population may become infected (Post 1987).

In the U.S., only two "in feed" products are approved by the U.S. Food and Drug Administration (FDA) for use to control mortality in cultured fish diagnosed with systemic columnaris. Terramycin® 200 for Fish (TM200; 44% oxytetracycline dihydrate; OTC; Phibro Animal Health, Corp., Teaneck, NJ) is approved for use on all freshwater-reared *Oncorhynchus mykiss*. AQUAFLOR® (50% florfenicol; Merck Animal Health, Roseland, NJ) is approved for use on all freshwater-reared finfish.

Oxytetracycline is a broad-spectrum antibiotic with bacteriostatic properties that is active against a wide variety of gram-positive and gram-negative bacteria (Hochstein et al. 1953). As a chemotherapeutant, it can be administered via enteral or parenteral routes with good tissue distribution (Stoffregen et al. 1996). Since its isolation and development in 1950, OTC has become one of the most commonly used antibiotics in aquaculture (Xu and Rogers 1994; Rigos et al. 2004).

In an effort to expand the current TM200 label, we coordinated a field trial to evaluate the effectiveness of TM200 to control mortality in freshwater-reared Tiger Muskellunge Esox lucius × muskellunge E. masquinongy (TMU) diagnosed with systemic columnaris.

Methods

The trial was conducted June 16-July 10, 2017 at the New York Department of Environmental Conservation's South Otselic Fish Culture Station (South Otselic, NY). Test fish were TMU fingerlings (mean length ± 1 SD, 10.3 cm ± 1.2 cm). TM200-medicated feed was administered at a target dosage of 82 mg OTC/kg fish/d (3.75 g OTC/100 lbs fish/d) for 10 consecutive days.

Before the trial began, ten moribund fish sampled from a reference population of approximately 6,590 fish were diagnosed with columnaris disease based on confirmation of *F. columnare* via Polymerase Chain Reaction (PCR) performed by staff at the U.S. Fish and Wildlife Service Bozeman Fish Health Center (BFHC; Bozeman, Montana). Sixteen hundred fish were subsequently netted impartially from the reference population, weighed, and randomly allocated among eight 82-L

rectangular, aluminum test tanks (4 treated and 4 control; mean, 200 fish/tank). Treatment conditions (TM200-treated vs. nontreated control) were randomly assigned to test tanks using completely randomized procedures. Tanks were supplied with first-pass water at flow and turnover rates suitable for rearing TMU.

The 25-d trial comprised 1-d acclimation, 10-d treatment, and 14-d posttreatment periods. During the treatment period, TM200-medicated feed or nonmedicated feed (Skretting Extruded Sinking Salmon 1.6 mm fish feed) was administered to the appropriate tanks. During the posttreatment period, nonmedicated feed was administered to all tanks. Feed was administered to tanks at 1.9% mean body weight/tank/d via an automated belt feeder set to distribute feed every 5 minutes; feed amounts were not adjusted for growth.

Mortality, general fish behavior, water temperature, and dissolved oxygen concentration data were collected daily. During treatment and posttreatment periods external and internal gross necropsies were performed on selected moribund fish. In addi-tion, skin scrapes and gill tissue from each fish necropsied were examined microscopically to presumptively identify cause of mortality. Bacterial isolates from skin and liver were cultured on Hsu-Shotts media and sent to the BFHC for pathogen confirmation via PCR. Oxytetracycline concentrations in medicated and nonmedicated feed samples were analytically verified with high-pressure liquid chromatography by Phibro Animal Health Corporation (State College, Pennsylvania).

Results and Discussion

At the end the trial, mean cumulative mortality in treated tanks (4.1%; range, 0.5 – 11.8%) was significantly different (P = 0.0024) than that in control tanks (59.1%; range, 44.5 – 69.9%; Figure 1). Bacterial isolates recovered from moribund fish before the start of the trial and during and after the treatment period positively confirmed *F. columnare* as the cause of mortality. Necropsy results were also consistent with systemic columnaris and indicated no concomitant pathogens present during the trial.

During the treatment and posttreatment periods, general fish behavior was characterized as normal in treated tanks on virtually every day and in every tank. Conversely, fish behavior was characterized as lethargic in the control tanks during 60% of the observation instances during the treatment period and 25% of the observation instances during the posttreatment period.

The mean analytically verified OTC dose administered to fish was 68.2 mg/kg fish/d, which was 78.3% of the target dose. No OTC was detected in control feed.

Water temperature and dissolved oxygen concentration means during the trial were 16.7°C (range, 13.8 – 19.6°C) and 6.7 mg/L (range, 4.3 – 8.7 mg/L) respectively. Water hardness (120 mg/L $CaCO_3$), alkalinity (80 mg/L $CaCO_3$), and pH (6.9) were normal and within ranges suitable for rearing TMU at the testing facility.

In conclusion, TM200-medicated feed administered at a target dose of 82 mg OTC/kg fish/d for 10 consecutive days resulted in a clinical reduction in mortality in freshwater-reared TMU fingerlings diagnosed with systemic columnaris. Results from this study were submitted to the FDA Center for Veterinary Med-icine demonstrating the efficacy of OTC at the target dose to sup-port expanding the use of TM200 in U.S. aquaculture to include treatment of systemic columnaris in all freshwater-reared finfish.

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

Figure 1. Mean (± SD) percent cumulative mortality (treated tanks vs. control tanks) of Tiger Musky fingerlings diagnosed with systemic columnaris disease. Treatment period equals trial days 1 – 10.