EFFICACY OF AQUAFLOR™ PREMIX AGAINST FURUNCULOSIS IN ATLANTIC SALMON SMOLTS IN BRITISH COLUMBIA

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Abstract

An efficacy and field safety trial of a 50% florfenicol premix (AQF) was conducted under commercial aquaculture conditions during a furunculosis epizootic. The *Aeromonas salmonicida* affecting the Atlantic salmon smolts (*Salmo salar*) demonstrated neither *in vitro* nor *in vivo* susceptibility to various antibiotics. The AQF premix was incorporated into an extruded feed, and was administered to 12 pens at 0.5% body weight / day delivering a daily dose of 10 mg florfenicol / kg fish for 10 days. Eight pens served as negative controls. The AQF pens demonstrated an immediate, substantial and sustained drop in mortality rates when compared to the negative control pens. Seven days following the cessation of the treatment, the mean weekly mortality rates in the AQF pens dropped to 0.05% while the control pen mortalities remained at 1.45%. Appetites of the fish remained vigorous throughout the trial period.

Introduction

Aeromonas salmonicida infections are common in Atlantic salmon (*Salmo salar*) populations under various physiological and environmental conditions (1). The infections can progress to a clinical disease status called furunculosis. The economic losses associated with furunculosis outbreaks in Atlantic salmon are often substantial and highly significant (2, 3). Despite improvements in salmonid husbandry practices, occasions arise where antimicrobial therapy is essential in the containment of outbreaks and to minimize fish mortalities.

It is generally accepted that *Aeromonas salmonicida* can become non-responsive to certain chemotherapeutants (4). To date, the limited availability of effective and safe antibiotic compounds in Canada has contributed to the global, competitive disadvantage facing the Canadian aquaculture industry in terms of fish survival, production performance, and ultimately in the cost of production of farmed salmon internationally (5, 6). The Canadian finfish aquaculture industry requires alternative antimicrobials to help control inevitable bacterial infections and epizootics.

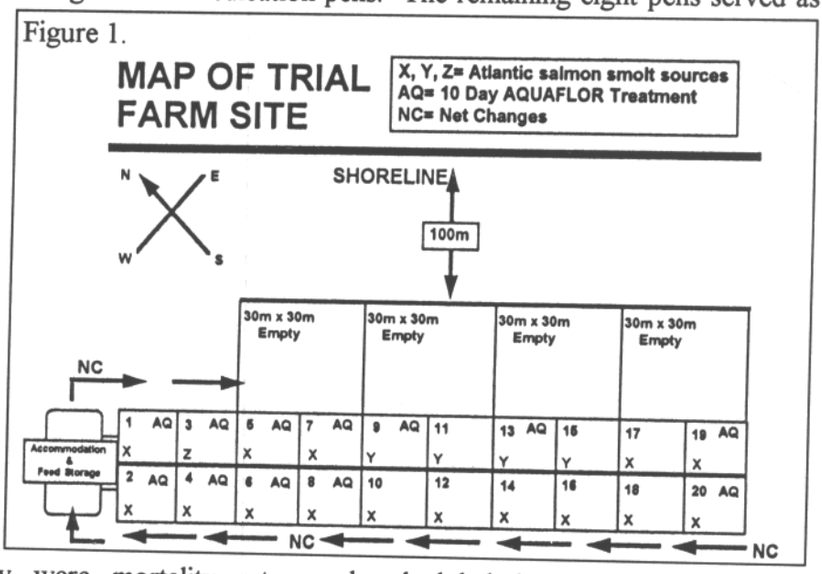
The active component of the 50% Aquaflor™ premix is florfenicol; a fluorinated analogue of thiamphenicol and chloramphenicol with significantly unique chemical properties (7). Florfenicol is a broad spectrum antibiotic with a bacteriostatic mechanism of action. Many experiments have been conducted using florfenicol to assess the therapeutic efficacy and suitability of use within fish (8, 9). The present study was undertaken to assess the field efficacy of the Aquaflor premix during a severe furunculosis outbreak under commercial sea farming conditions.

Methods and Materials

Aquaflor™ (Schering-Plough Animal Health, Union N.J., USA) was incorporated into a 2.5 mm extruded commercial fish diet manufactured by Moore-Clark Co. (Canada) Inc. (Vancouver B.C.) creating a complete medicated fish feed of the same constituency that was fed to the fish during the pre-medication period. Under an Emergency Drug Release protocol (EDR No. 935183), the medicated ration was designed to be fed at 0.5% biomass consumption daily to deliver a target dose of 10 mg florfenicol / kg fish / day for 10 days. Multiple one kg feed samples were collected both before and after the extrusion process, and were analyzed for drug concentration by Schering-Plough Animal Health (Pointe Claire, Que.) by means of high performance liquid chromatography.

A single-year-class, commercial, salmon farming unit raising 320,000 S1 Atlantic salmon smolts served as the study farm in a remote location of the British Columbia coastline. The smolts originated from three separate hatchery sources and weighed 115 to 275g approximately 60 days after sea water entry. The farm site consisted of 20 pens, of which 12 were selected and designated as medication pens. The remaining eight pens served as negative control pens (Fig. 1).

Figure 1.



The trial was divided into three time blocks: a pre-medication period of five weeks, a 10 day medication period from July 31 to August 09, 1993, and a post-medication period of four weeks. The pivotal parameters for response to therapy were mortality rates and scheduled bacteriological analyses throughout the three trial time periods. Individual pen mortality rates, post-mortem examinations, and kidney cultures of 10 carcasses from each trial group were conducted and collected on days 0, 2, 5, 7, 10, 13, and 21 in relation to the medication period.

Tryptic Soy Agar (TSA) with and without 5% sheep's blood (Prepared Media Laboratories microbiologicals - PML, Tualatin, Oregon, USA.) served as the primary culture media. The inoculated agar plates were immediately transported from the study site to the Moore-Clark Technical Service laboratory in Campbell River, B.C. by float plane for analysis. The isolates were incubated at 20 C for 36 to 48 hours prior to assessing *in vitro* drug susceptibilities. Mueller-Hinton agar (PML, Oregon, USA) and antimicrobial susceptibility discs (Oxoid OT 30mcg, Oxoid SXT 25mcg, Difco SOR 25 mcg, Oxoid E 15mcg) were employed. Secondary inoculates were forwarded to the B.C Ministry of Agriculture, Food and Fish laboratory in Abbotsford, B.C. to verify bacterial identification, and to perform the *in vitro* AQF susceptibility test with Difco FFC 30 mcg discs. The parameters monitored daily at the study site included water quality, environmental conditions, feeding volumes and appetite.

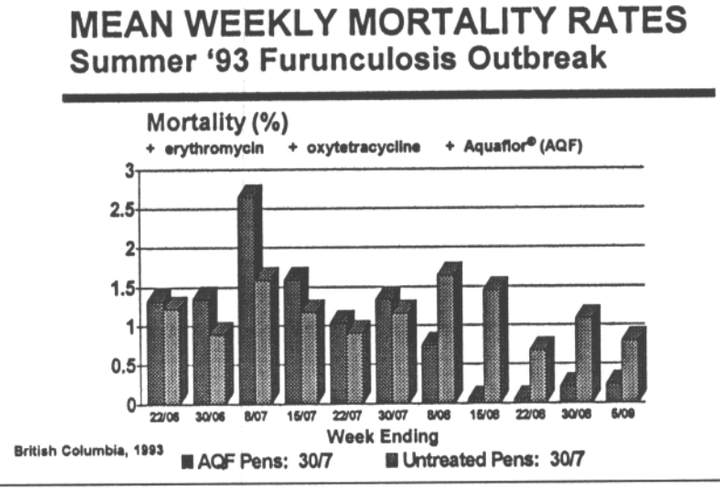
Results and Discussion

Sea water temperatures increased from 14 to 16 C during the AQF medication period. Other water quality parameters expressed adequate dissolved oxygen levels, no identifiable pathogenic plankton, and good visibility. The appetite of the fish remained vigorous during the trial period in each group of smolts.

The twelve AQF pens and the eight negative control pens were retrospectively assessed over the pre-AQF period. Throughout the months of May, June, and July 1993, the entire smolt population experienced weekly losses in excess of 1.0% due to furunculosis. On four scheduled collection dates, the bacterial isolates from each trial group were confirmed to be *Aeromonas salmonicida*. With the exception of erythromycin and AQF, all isolates expressed no *in vitro* zones of inhibition to three common registered antibiotics. AQF susceptibility discs consistently elicited 40 to 55 mm zones of inhibition. Potentiated sulfonamides, oxytetracycline, and erythromycin administered orally had little or no *in vivo* affect.

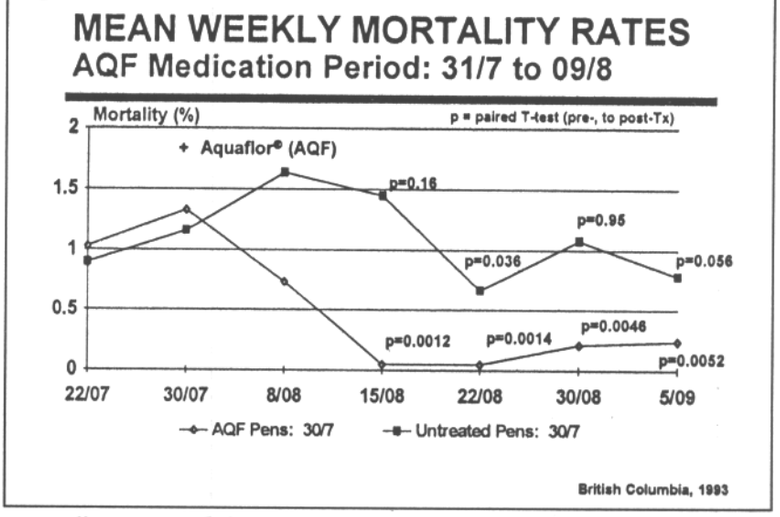
As depicted in figure 2, a 10 day erythromycin phosphate treatment was administered to each of the 20 pens at 100 mg / kg daily from June 12 to 21. The response was minimal and short-lived despite a

Figure 2.



moderate *in vitro* susceptibility. Mortality rates remained above 1% weekly. Oral oxytetracycline was prescribed at 150 mg / kg daily from July 6 to 20 but again, the weekly mortality rates did not fall below 1.0%. The 10 day AQF medication period was initiated on July 31, and the response in the 12 AQF pens was demonstrated by an immediate, substantial, and sustained drop in mortality rates when compared to the negative control pens (Fig. 3).

Figure 3.



On day 0, the statistical difference between the trial groups was not significant (p=0.68). By day 7 of therapy, the mean weekly mortality rate of the AQF group fell from 1.33% to 0.74%, while the control pens increased from 1.16% to 1.64%. Statistically, the weekly mortality rates of the two groups had already become significant (p=0.007). On day 17, the mean weekly mortality rate had fallen to 0.05%. P-values of 0.0000 indicated that the difference between the fish groups was due completely to treatment effect. Subsequently, the mortality remained below 0.25% for four weeks following the medication period (p=0.009 to 0.015).

Under the conditions of this study, it was established that the 50% Aquaflor premix is efficacious in the control of furunculosis in commercial Atlantic salmon farming units, with no behavioural adverse affects expressed by the recipient fish population. The limited availability of registered chemotherapeutants within the Canadian aquaculture industry leaves Aquaflor as a viable candidate to help improve the health status and performance of our cultured salmon.

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