

**Evaluation of erythromycin treatment and Renigen vaccination on asymptomatic *Renibacterium salmoninarum* infections in yearling Lahontan Cutthroat trout (CUT-PPW-06-LAH).**

J. Scott Foott and John Bigelow, USFWS  
October 2007

**Background:** The captive, Pilot peak strain Lahontan Cutthroat (PPW), brood stock at Lahontan NFH has a history of asymptomatic infection by the bacterium *Renibacterium salmoninarum* (Rs). While the adult and their progeny have not develop clinical Bacterial Kidney Disease, Rs infection has restricted the movement of these fish into California waters. The current USFWS / AFS Bluebook *Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens 2005* states that direct fluorescent antibody (DFAT) assay of kidney tissue imprints is the inspection method for Rs detection of hatchery populations. This inspection method is accepted by California Department of Fish and Game for testing of salmonid fish populations identified for potential transport into California.

The macrolide antibiotic Erythromycin has been used for the treatment and control of Rs infection in salmonid fish for several decades. This antibiotic is bacteriostatic (inhibits replication but does not necessary kill the bacteria) and is reported to induce toxicity responses in the kidney and liver under certain conditions. Renigen is a commercial “live” vaccine produced by AquaHealth Ltd, Canada that consists of viable *Arthobacter* sp. culture. This bacterium is reported to share antigenic determinants with Rs and after injection induces a brief bacteremia in the fish. An immune response that targets both bacteria is the goal of the vaccination. The objective of this pilot effort was to determine if erythromycin treatment or vaccination would change the Rs detection profile of an asymptomatic cutthroat population (CUT-PPW-06-LAH).

**Methods:** On 17May2007, 60 trout were distributed to four 250 gal tanks in the LNFH quarantine building. These yearling trout had previously been diagnosed with asymptomatic *Renibacterium salmoninarum* (Rs) infections. On June 1, 2007, the following treatments were conducted on trout in 4 tanks:

- 1) Controls – no injections and fish were fed non-medicated diet.
- 2) Renigen - injected intraperitoneally (IP) with the Renigen vaccine prepared as per manufacturer instructions (0.1 mL / fish).
- 3) EIP – a single injection (IP) of Gallimycin 100 (100 mg erythromycin / mL) at dosage of 0.05 mL / fish.
- 4) EF – feed erythromycin (2.25% Skretting MDAqua100- 3mm pellet) at 1% bodyweight / d for 28 days beginning on 11June through 9July.

Immediately prior to any treatment on 01 June, five fish from each tank were captured, euthanized with an overdose of MS222, weighed (0.1g), and the entire kidney sampled for Rs assays (time0 sample). On both 10 July (6 weeks {wk} post treatment) and again on 07 August (10 wk), the entire kidney was removed from 20 fish in each group and frozen until later assayed by DFAT. The kidney sample was homogenized and smears made onto 10 mm diameter well slides. After heat and acetone-methanol fixation, the sample slides and appropriate controls were stained with FITC-conjugated goat anti-Rs (Kirkegaard-Perry), coverslipped with Vectashield mounting media, and later examined for Rs (600x oil magnification objective, 50 fields). Additionally, kidney and liver tissue from 2 fish each in the Renigen, erythromycin injection, and control groups at 6 and 10 weeks post-treatment were fixed in Davidson's fixative for histological examination. Specimens were processed for 5µm paraffin sections that were later stained with Hematoxylin and eosin.

### **Results and Discussion:**

Mean weight of the trout on 01 June was 75 g (std dev. 17g). In order to obtain the standard 20 mg erythromycin / kg fish dosage, each "75 g" fish should have received an injection volume of 0.015 mL of Gallimycin 100. We were forced to use the 0.05 mL volume as it was the minimum gradation on our syringes. Our use of 0.05 mL volumes resulted in an approximately 3.3 x higher dosage (66 mg / kg) of antibiotic being delivered to the fish of this group. Only 4 fish died over the entire study (1 in the vaccine and 3 in the erythromycin IP group).

### ***Renibacterium salmoninarum* was detected in kidney imprints from all groups by the DFAT assay at both 6 and 10 weeks post-treatment (Fig 1).**

The proportion of kidney samples with higher Rs number (> 5 cells / 50 field) increased markedly in the Renigen and erythromycin injected groups between 6 and 10 weeks post-treatment. This observation suggests that Rs infection was progressing despite (or due to) the treatments. Fish fed erythromycin showed some signs of Rs reduction between the 2 sample dates while the Rs intensity of the control samples remained similar. No abnormalities were observed in the kidney or liver sections of Renigen, erythromycin injection, or control fish.

In summary, asymptomatic Rs infection was detected by standard DFAT in all groups at 6 and 10 weeks post-treatment. Rs infection in both the vaccinated and erythromycin injected trout appeared to become more severe over time. No adverse effects could be attributed to any treatment.

We thank Jerry Zinn (AquaHealth, Ltd) for his assistance in purchasing Renigen and Lisa Racliffe and Ron Stone for their help in lab processing.

Figure 1. Proportion of kidney samples assayed by DFAT that were rated as Not detected (0Rs), 1 to 5 Rs cells / 50 fields (1-5 Rs), and > 5 Rs / 50 fields (> 5 Rs) in trout sampled at 0 (time0), 6, and 10 weeks post-treatment. Treatment groups included no-treatment controls (CON), Renigen vaccinated (RENI), erythromycin injected (EIP) and erythromycin feed (EF).

