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Preliminary Evaluation of Praziquantel Against Metacercariae of *Nanophyetus salmincola* in Chinook Salmon (*Oncorhynchus tshawytscha*)

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ABSTRACT: Praziquantel at dosages of 10, 20 or 100 mg/kg of body weight was evaluated against metacercariae of *Nanophyetus salmincola* in chinook salmon (*Oncorhynchus tshawytscha*). Ten salmon were used in each of four treated groups and 10 salmon were nontreated controls. Three wk after treatment, viability of metacercariae was determined by histologic evaluation, and by feeding the salmon to coyotes and subsequently determining the numbers of trematode eggs/g of feces and numbers of *N. salmincola* recovered at necropsy. Results of the experiment indicated that praziquantel at the dosages and routes of administration used was not effective against metacercariae in chinook salmon.

Key words: *Nanophyetus salmincola*, chinook salmon, *Oncorhynchus tshawytscha*, metacercariae, praziquantel, drug evaluation, experimental infection.

Nanophyetus salmincola is a trematode commonly found in carnivores along coastal Washington, Oregon, and northern California in the northwestern United States (Millemann and Knapp, 1970; Gorham and Foreyt, 1984). The life cycle of the trematode involves a snail (*Oxytrema silicula*) as the first intermediate host and usually a salmonid fish as a second intermediate host. Metacercariae of *N. salmincola* have a predilection for kidney and heart tissue in fish, and when infection is massive, fish often die or are severely debilitated (Baldwin et al., 1967; Wood, 1968; Millemann and Knapp 1970; Butler and Millemann, 1971), thereby limiting the production of several salmonid species, particularly in hatcheries. Effective control of *N. salmincola* in hatcheries theoretically would reduce mortality in fish and reduce the prevalence of salmon poisoning disease in canines, a life-threatening rickettsial disease caused by *Neorickettsia helminthoeca* which is acquired when metacercariae are eaten with infected fish.

In recent experiments, praziquantel was shown to be highly effective against adult *Nanophyetus salmincola* in coyotes (*Canis latrans*) under experimental conditions (Foreyt and Gorham, 1988). The purpose of this experiment was to determine the efficacy of praziquantel against the metacercariae of *N. salmincola* in chinook salmon (*Oncorhynchus tshawytscha*).

Chinook salmon, naturally infected with metacercariae of *N. salmincola* were obtained from McAllister Salmon Hatchery (10119 Steilacoom Road, Olympia, Washington 98503, USA). The fish had a mean weight of 10.9 g, ranging from 5.1 to 14.6 g. The mean number of metacercariae in a random sample of 10 fish was 370; these ranged from 222 to 560 metacercariae/fish. Methods for determining numbers of metacercariae per fish have been reported previously (Foreyt et al., 1987). Salmon were divided randomly into five treatment groups of 10 fish per group. Each fish was treated individually on 20 May 1987. Each fish in group I was given praziquantel powdered tablets (Droncit tablets, Bayvet Division, Miles Laboratories, Shawnee, Kansas 66201, USA) orally in number 5 gelatin capsules with a forceps at a dosage of 10 mg/kg body weight (BW). Fish in group II were given praziquantel powdered tablets orally in gelatin capsules at a dosage of 100 mg/kg BW. Fish in group III were given praziquantel solution (Droncit injectable, Bayvet Division, Miles Laboratories Inc., Shawnee, Kansas 66201, USA) orally in number 5 gelatin capsules at a dosage of 100 mg/kg BW. Fish in group IV were given praziquantel solution

intramuscularly (i.m.) at a dosage of 20 mg/kg BW. Fish in group V were given no medication, but were handled physically the same as the treated fish. Fish-treatment groups were identified by various fin clippings, and were housed together in a 1 × 8 × 0.3 m tank. Natural creek water from McAllister Creek circulated through the tank. Fish were fed a commercial fish ration (Oregon Moist Diet, Moore-Clark Company, Box M, La Conner, Washington 98257, USA).

Six coyote pups, <2 wk old, were obtained as a result of a predator control program in Whitman County in eastern Washington. Pups were bottle fed a milk formula (369 ml of evaporated milk diluted 1:1 with water, 30 ml corn syrup and two whole eggs mixed in a blender). After weaning at 5 wk of age, pups were maintained in individual kennels and fed a commercially dry dog ration (Purina Puppy Chow, Ralston Purina Company, St. Louis, Missouri 63164, USA). Food and water were available at all times. The ears of the coyotes were tattoed with a number for identification. Coyotes were approximately 16 wk old when the experiment began. A fecal sample was obtained prior to fish-feeding and examined for trematode eggs using a modified fecal sugar flotation method (Foreyt, 1986). Three wk after treatment, eight fish from each treatment group were fed to five coyotes, one coyote per fish-treatment group. Kidneys from one or two of the remaining fish from each treatment group were examined histologically for viable metacercariae. Additional pieces of kidney were squashed between two glass slides and examined with a dissecting microscope (20×) for movement within metacercariae. Two wk after the coyotes had eaten the fish, all coyotes were euthanized with pentabarbitol sodium (Pentabarbitol Sodium Euthanasia Injection, Anthony Products Company, Arcadia, California 91006, USA). At necropsy, a fecal sample was obtained and examined by fecal flotation for trematode eggs, and the small intestine was removed. After being opened longitudinally and washed, the intestinal mucosa was scraped with a knife; contents, washings, and scrapings were mixed and examined for *N. salmincola* with a dissecting microscope at 15×; and trematodes were counted.

All fish ate regularly, remained healthy and survived the experiment except for two fish (one control and one from group I) that jumped out of the tank. Two gelatin capsules were found in the tank during the first 8 hr observation period. The fish that vomited the capsules could not be identified.

Coyote feces were negative for trematode eggs prior to fish-feeding. At necropsy, numbers of *N. salmincola* adults recovered and eggs per gram (EPG) of feces were similar between the treated coyotes and the untreated control coyotes (Table 1).

Histopathologic examination of the randomly selected salmon kidneys revealed large numbers of intact *N. salmincola* metacercariae with normal cellular morphology in all treatment groups. Live metacercariae were observed in kidney squashes from fish in all treatment groups.

Praziquantel at dosages of 4 to 25 mg/kg BW is used widely in human and veterinary medicine for the effective treatment of adult trematode and cestode infections, including species of *Clonorchis, Diphyllobothrium, Echinococcus, Hymenolepis, Opisthorchis, Paragonimus, Schistosoma* and *Taenia* (Gemmel et al., 1977; Kirkpatrick and Shelly, 1985; Pearson and Wilson, 1986).

In this experiment, praziquantel was ineffective at eliminating or reducing *N. salmincola* infection in salmon in all groups. Circulation of natural stream water through the salmon tank may have increased the infection of metacercariae in fish because cercariae are common in the stream water. It is possible that additional live metacercariae may have been acquired after treatment; however, the results between the treated groups and the

TABLE 1. Numbers of adult *Nanophyetus salmincola* and eggs/g/feces recovered from coytoes fed salmon treated with praziquantel.

Coyote number	Treatment group number	Drug treatment	Eggs/g/feces	Numbers of *Nanophyetus salmincola*
3	I	Praziquantel powder, 10 mg/kg, orally	492	70,350
71	II	Praziquantel powder, 100 mg/kg, orally	425	12,311
1	III	Praziquantel solution, 100 mg/kg, orally	1,875	36,242
35	IV	Praziquantel solution, 20 mg/kg, intramuscularly	1,063	33,236
78	V	Control	556	10,466

control group did not differ, indicating the drug did not affect the viability of metacercariae (Table 1).

Infection of metacercariae of *N. salmincola* in fish is a serious problem in several hatcheries in the northwestern United States because of the debilitating effect on fish (Wood, 1968; Millemann and Knapp, 1970; Butler and Millemann, 1971). Some hatcheries have had to terminate production during certain seasons because of the magnitude of this problem, or hatcheries release fish from the hatchery at an early date to reduce mortality in the hatchery. Control of the trematode in hatcheries that use stream water is limited. Electrical grids (Combs, 1968) and ultrasonic generators (Farrell et al., 1973) which destroy cercariae, are too expensive and impractical for use with large volumes of water. Chemical control of snails is not used because of potential toxicities in the aquatic ecosystem. Although control measures for *N. salmincola* are needed in hatcheries that use natural stream water in indigenous areas, praziquantel was ineffective in eliminating viable *N. salmincola* metacercariae in salmon at the dosages and routes used in the present study. Fish were examined 3 wk after treatment to allow sufficient time for the drug to effectively affect metacercariae, but no dead or decomposing metacercariae were observed on tissue squashes or on histologic sections. It is also possible that the drug was not absorbed by the fish. Drug therapy or other control methods are needed to prevent *N. salmincola* infections of fish in hatcheries, and further research for control strategies are warranted.

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