Toxicity and pathological effects of orally administered ivermectin in Atlantic, chinook, and coho salmon and steelhead trout

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ABSTRACT: Because ivermectin (22,23-Dihydroavermectin B₁) has been proposed as an oral treatment against sea lice infections of farmed salmonids, we investigated the toxicity and pathological effects of various doses of ivermectin administered orally every second day to steelhead trout Oncorhynchus mykiss, coho salmon Oncorhynchus kisutch, chinook salmon Oncorhynchus tshawytscha and Atlantic salmon Salmo salar under laboratory conditions. These species differed in their ability to tolerate ivermectin, with coho salmon the most tolerant followed by chinook, then Atlantic salmon. Unequivocal results for steelhead trout were not obtained due to a secondary disease outbreak (vibriosis). Histological examination of the major organ systems revealed no pathological changes that could be associated with ivermectin toxicity in any of the salmon species tested.

KEY WORDS: Ivermectin, Oncorhynchus kisutch, Oncorhynchus mykiss, Oncorhynchus tshawytscha, Parasite control, Parasite treatment, Pathological effects, Salmo salar, Sea lice, Sub-lethal effects, Toxicity

INTRODUCTION

Several species of marine ectoparasitic copepods including Caligus clemensi, Caligus elongatus, Caligus teres, and Lepeophtheirus salmonis commonly infect and can cause serious disease in sea-farmed salmonids (Brandal & Egidius 1979, Kabata 1979, 1988, Wootten et al. 1982, Reyes & Bravo 1983, Hogans & Trudeau, 1989a, b, Pike 1989). Epizootics of these sea lice species are most commonly treated by bath treatments of dichlorvos, which is the active ingredient in 'Nuvan 500EC' or 'Aquaguard' (Grave et al. 1991a, b, Jackson & Costello 1992, Roth et al. 1993). These treatments are labor-intensive and can cause high levels of fish mortality due either to toxicity of the dichlorvos itself or to secondary diseases brought on by physical injury and/or stress incurred during the treatment. Furthermore, populations of Lepeophtheirus salmonis, which is the most important species of sea lice with respect to disease, are reported to be developing resistance to dichlorvos (Jones et al. 1992). These problems, as well as concerns about the release of dichlorvos into the marine environment, have necessitated the development of alternative treatment methods for sea lice.

Ivermectin (22,23-Dihydroavermectin B₁) is a broad-spectrum antiparasitic drug which is effective in controlling nematodes and parasitic arthropods in a wide variety of host species when administered orally (Campbell 1989). Currently, ivermectin is used to control sea lice at some sea farm sites in Ireland and possibly Scotland (Roth et al. 1993). In preliminary studies, a single orally administered dose of ivermectin (0.20 mg ivermectin kg⁻¹ of fish weight) was determined to be effective in reducing adult sea lice numbers on Atlantic salmon Salmo salar without any treatment-associated mortality (Palmer et al. 1987). Single higher doses of 0.4 mg kg⁻¹ (fed to both Atlantic salmon, and rainbow trout Oncorhynchus mykiss) and 1.0 mg kg⁻¹ (fed only to rainbow trout) resulted in significant mortalities (Palmer et al. 1987). Ivermectin given as a single oral dose of 0.20 mg kg⁻¹ or as 2 doses of 0.05 mg kg⁻¹ given over 1 wk, gave a marked reduction in the number of Ergasilus labracis on Atlantic salmon parr (O’Halloran et al. 1992). A single dose of 0.20 mg kg⁻¹ gave a significant reduction in adult sea lice numbers on coho and chinook salmon.
mg kg$^{-1}$ resulted in a slight rise in the mortality rate, whereas the two 0.05 mg kg$^{-1}$ doses had no effect on the mortality rate. Smith et al. (1993) reported that treatments of 0.2 mg kg$^{-1}$ every 2 wk, 0.075 mg kg$^{-1}$ twice a week, 0.1 mg kg$^{-1}$ once a week, and 0.05 mg kg$^{-1}$ twice a week have no effect on morbidity or mortality of Atlantic salmon.

These aforementioned studies have provided guidelines to the toxicity of ivermectin in Atlantic salmon. However, the toxicity of this drug to other salmonid species was not determined, except in the study of Palmer et al. (1987) in which rainbow trout fed 1 dose at 1.0 mg kg$^{-1}$ fish experienced high mortality. Before the efficacy of ivermectin for the control of sea lice on Atlantic salmon or other species of salmon can be determined, additional information on its toxicity and possible side effects needs to be obtained. The aim of this study was to determine the toxicity of orally administered ivermectin to steelhead trout salmon *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar*, coho salmon *Oncorhynchus kisutch* and chinook salmon *Oncorhynchus tshawytscha*. The effects of ivermectin on fish behaviour, and the gross morphology and histopathology of the major organ systems, were also determined.

### MATERIALS AND METHODS

#### Preparation of treated feeds.  
Ivermectin used in our experiment was in the form of a veterinary preparation of 1% w/v oral drench (Eqvalan; Merck Frosst Canada). Ivermectin carrier solution was made in our laboratory following the formulation given in the Eqvalan product information sheet. Commercial salmon pellets (White Crest) were allowed to dry for 24 h at room temperature and sprayed with either solutions of Eqvalan or the carrier solution diluted to the targeted dosage level with distilled deionized water. Diets were stored frozen at 20°C in sealed containers.

#### Feed analysis.  
Levels of ivermectin in both the control and treated diets were confirmed by the Health of Animals Laboratory, Agriculture Canada, Saskatoon, following the methods given in Doherty et al. (1990). Assayed levels in the treated feeds were generally considerably lower than the targeted levels sprayed (Table 1). The doses referred to throughout the remainder of this paper are the targeted doses.

#### Dosage levels and feeding rates.  
Atlantic salmon with an average weight of approximately 800 g were fed 5 levels of ivermectin (0.05, 0.10, 0.20, 0.50, and 1.0 mg kg$^{-1}$ fish) and carrier compound equivalent to that received in the 1.0 mg kg$^{-1}$ fish dose. Coho salmon with an average weight of approximately 50 g were fed 3 levels of ivermectin (0.05, 0.10, and 0.20 mg kg$^{-1}$ fish). Chinook salmon with an average weight of approximately 70 g and steelhead trout with an average weight of approximately 115 g were fed 2 levels of ivermectin (0.05 and 0.10 mg kg$^{-1}$ fish). Medicated diet was fed every second day for 50 d or until all the fish stopped feeding. Non-medicated diet was fed every other day and after the termination of the ivermectin feedings. Control fish were fed the untreated diet daily. Except for coho salmon, which were fed at 1.0% body weight per day, treated diets were fed at a rate of 0.5% body weight per day.

#### Sample size and monitoring.  
Atlantic salmon were maintained in 3500 1 tanks and all other species in 500 1 tanks, supplied with flowing filtered seawater. The mean and range of the seawater temperatures during the experiments are given in Table 2. Salinities ranged between 28 and 30% for each species, 50 fish

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatments</th>
<th>Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlantic salmon</td>
<td>Controls, 0.20, 0.50 and 1.0 mg kg$^{-1}$</td>
<td>8.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>0 controls</td>
<td>8.0 ± 0.5</td>
</tr>
<tr>
<td>Atlantic salmon</td>
<td>Controls, 0.05 and 0.10 mg kg$^{-1}$</td>
<td>9.0 ± 0.5</td>
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<td></td>
<td>0.10 mg kg$^{-1}$</td>
<td>8.0 ± 0.5</td>
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<tr>
<td>Atlantic salmon</td>
<td>Carrier solution and controls</td>
<td>12.5 ± 0.6</td>
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<td></td>
<td>11.1 – 13.7</td>
<td>10.8 – 10.5</td>
</tr>
<tr>
<td>Chinook salmon</td>
<td>Controls and all treatment levels</td>
<td>10.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>9.4 – 13.0</td>
<td>10.6 – 14.7</td>
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<tr>
<td>Coho salmon</td>
<td>Controls and all treatment levels</td>
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<td></td>
<td>10.6 – 14.7</td>
<td>11.0 ± 0.9</td>
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<tr>
<td>Steelhead trout</td>
<td>Controls and all treatment levels</td>
<td>11.0 ± 0.9</td>
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<tr>
<td></td>
<td>9.8 – 13.0</td>
<td>9.4 – 13.0</td>
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</table>

<table>
<thead>
<tr>
<th>Feed sample</th>
<th>Sprayed ivermectin (mg kg$^{-1}$ feed)</th>
<th>Recovered ivermectin (mg kg$^{-1}$ feed)</th>
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<tr>
<td>Untreated feed</td>
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<td>nd</td>
</tr>
<tr>
<td>Atlantic carrier solution</td>
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<td>nd</td>
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<tr>
<td>Atlantic 0.05 mg kg$^{-1}$ group</td>
<td>10.0</td>
<td>6.7</td>
</tr>
<tr>
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<td>20.0</td>
<td>11.3</td>
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<tr>
<td>Atlantic 0.20 mg kg$^{-1}$ group</td>
<td>40.0</td>
<td>21.0</td>
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<td>100.0</td>
<td>40.4</td>
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<td>Atlantic 1.0 mg kg$^{-1}$ group</td>
<td>200.0</td>
<td>97.2</td>
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<td>10.1</td>
<td>9.1</td>
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<tr>
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<td>3.6</td>
</tr>
<tr>
<td>Steelhead 0.10 mg kg$^{-1}$ group</td>
<td>10.0</td>
<td>6.5</td>
</tr>
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</table>
of a similar size were assigned to each of the ivermectin-treated groups and 25 fish to each of the control groups. A group of 20 Atlantic salmon was fed the diet containing the ivermectin carrier. Twenty-five fish from each of the control and ivermectin-treated groups, and all 20 Atlantic salmon in the ivermectin carrier group, were anaesthetized with MS-222 and weighed to determine an average weight (+SD) of fish and the total weight of fish in each tank. The amount of diet fed to the fish in each tank was based upon the initial total weight of fish in the tank.

Fish were fed once a day before they were disturbed by removal of dead or moribund fish. The amount of treated feed eaten was estimated and observations of feeding activity and other behaviours (e.g. lethargy, hyperactivity) were made. The weight, fork length, and presence or absence of food in the stomach were recorded for all sampled fish.

Atlantic salmon treated at levels of 0.2, 0.5, or 1.0 mg kg$^{-1}$ were monitored for mortality and behaviour over 27 d after which time the experiments were terminated due to high mortalities. The remaining groups were monitored for 64 d.

**Statistical analysis.** Mortality rates were compared between ivermectin doses using G-tests (Sokal & Rohlf 1981).

**Histology.** Samples of liver, spleen, kidney, pyloric caeca, posterior intestine, gill, brain, and eye were taken from the moribund fish throughout the experiment and from 5 apparently healthy fish from each treatment and control group at termination of the experiments. In addition, samples of 5 Atlantic salmon from the 1.0 mg kg$^{-1}$ treatment group were collected weekly after the cessation of feeding. All tissues were fixed in Davidson's solution, wax-embedded, cut to a thickness of 5 μm, and stained with hematoxylin and eosin.

**RESULTS**

**Atlantic salmon**

The cumulative mortality of Atlantic salmon to 5 levels of orally administered ivermectin is given in Fig. 1. There were no mortalities in the control group or in the group fed the ivermectin carrier solution. Cumulative mortalities in the 0.05 mg kg$^{-1}$ and the 0.10 mg kg$^{-1}$ treatment groups were 10 and 14 %, respectively, at the end of the experiment. Cumulative mortalities were significantly higher (G-test; p < 0.05) in the 0.20, 0.50, and 1.0 mg kg$^{-1}$ treatment groups when compared to the other treatment levels, with values of 80, 84, and 94 %, respectively.

Fish in the 0.05 mg kg$^{-1}$ treatment group showed a reduction in their feeding activity, some loss of equilibrium, and a darkened colouration after 6 doses of ivermectin (12 d). On average 66 % of the daily ration was eaten after this time. Feeding activity ceased after 7 doses of ivermectin (14 d) in the 0.10, 0.20, and 1.0 mg kg$^{-1}$ treatment groups, and after 6 doses (12 d) in the 0.50 mg kg$^{-1}$ treatment group. With the exception of an additional 4 % mortality in the 0.50 mg kg$^{-1}$ treatment group and a 6 % mortality in the 0.10 mg kg$^{-1}$ treatment group, mortalities ceased after the fish stopped feeding. Fish showed a loss of equilibrium after 1 dose in the 0.50 and 1.0 mg kg$^{-1}$ treatment groups and after 3 doses in the 0.20 mg kg$^{-1}$ treatment group. All fish in these groups darkened in colour, and the eyes of the moribund and surviving fish were rolled ventrally so that the lenses were no longer visible. The position of the eyes returned to normal after death.

The 0.10 mg kg$^{-1}$ treatment group was fed untreated food from Day 19 (5 d after cessation of feeding on treated diet) to the end of the experiment. However, feeding activity of this group was reduced when compared to that of the controls.

**Chinook salmon**

There were no mortalities in the chinook control or in the 0.05 mg kg$^{-1}$ treatment group, and a 10 % cumulative mortality occurred in the 0.10 mg kg$^{-1}$ treatment
Coho salmon

The coho control group had an 8% cumulative mortality over the experimental period. No mortalities occurred in the 0.05 mg kg⁻¹ treatment group, and only a 2% cumulative mortality in the 0.10 mg kg⁻¹ treatment group. In the 0.20 mg kg⁻¹ treatment group there was a 20% cumulative mortality at the end of the experiment (Fig. 3).

Although feeding activity was reduced when compared to the controls, full treated feed rations were eaten in the 0.05, and 0.10 mg kg⁻¹ treatment groups throughout the experiment. A reduction in the feeding activity of the 0.20 mg kg⁻¹ treatment group was noted after 5 doses of ivermectin (Day 11). On average the 0.20 mg kg⁻¹ treatment group consumed approximately 68% of their treated food ration after this time. Fish in the 0.05 and 0.10 mg kg⁻¹ groups began to darken after about 16 doses of ivermectin. Those in the 0.20 mg kg⁻¹ group darkened after 2 doses. Down-turned eyes, as seen in the Atlantic salmon, were not observed in coho salmon.

Steelhead trout

There was a 4% cumulative mortality in the steelhead control group over the experimental period (Fig. 4). Cumulative mortality in the 0.05 mg kg⁻¹ treatment group was 68% at the end of the experiment. The cumulative mortality in the 0.10 mg kg⁻¹ group was significantly lower (48%) (G-test; p < 0.05) by the
end of the experiment. In all treatment groups most of the dead fish were heavily infected with Vibrio ordalii. After 8 doses of ivermectin a reduction in feeding activity in both the 0.05 and 0.10 mg kg$^{-1}$ treatment groups was noted. On average the 0.05 and 0.10 mg kg$^{-1}$ treatment groups consumed 74 and 57 % of their full treated feed ration, respectively, after this time.

**Gross pathology and histology**

Gross examination of moribund Atlantic salmon in the 0.50 and 1.0 mg kg$^{-1}$ fish treatment groups revealed some congestion of their intestines. Many of the moribund steelhead salmon had enlarged spleens and ascites fluid in their body cavities. Such gross pathology is commonly associated with vibriosis outbreaks. Examination of histological sections of gill, heart, intestine, kidney, liver, pyloric caeca, and spleen revealed no pathological changes that could be associated with ivermectin toxicity in any species.

**DISCUSSION**

There are few data available on the toxicity of orally administered ivermectin to fish. Over the first 2 d of this study, single doses of ivermectin at 0.2, 0.5, and 1.0 mg kg$^{-1}$ administered to Atlantic salmon caused 0, 8, and 38 % cumulative mortality, respectively. Smith et al. (1993) reported 25 % mortality of Atlantic salmon given a single oral dose of 0.75 mg kg$^{-1}$. Palmer et al. (1987) reported a single oral dose of 0.2 mg kg$^{-1}$ administered over 24 h did not appear to be toxic to either Atlantic salmon (12.5 to 85 g average weight) or rainbow trout (36 g average weight). O'Halloran et al. (1992) reported a slight elevation in mortality (0.6 %) and lethargy in Atlantic salmon smolts (35 g average weight) fed a single dose of 0.20 mg ivermectin kg$^{-1}$ fish. They also reported that mortality rates were elevated (10 to 24 % cumulative mortality) in Atlantic salmon (151 to 221 g average weight) fed a single dose of 0.4 mg ivermectin kg$^{-1}$ fish. Rainbow trout (36 g average weight) fed a single dose of 1.0 mg ivermectin kg$^{-1}$ fish. They also reported that mortality rates were elevated (10 to 24 % cumulative mortality) in Atlantic salmon, and no mortality in chinook or coho salmon. Smith et al. (1992, 1993) reported that oral treatments with ivermectin at 0.2 mg kg$^{-1}$ every 2 wk, 0.075 mg kg$^{-1}$ twice a week, 0.1 mg kg$^{-1}$ once a week, and 0.05 mg kg$^{-1}$ twice a week administered over long periods of time (9 to 42 wk) caused no treatment-associated mortalities, no detectable chronic toxicity, and no adverse effects on weight gain.

Results of our experiments indicate that salmon species differ in their ability to tolerate orally administered ivermectin, with coho salmon being the most tolerant followed by chinook and then Atlantic salmon. The reason for this difference remains to be determined. It is possible that these species may differ in their efficiency of ivermectin uptake across the intestine. The results for steelhead trout are difficult to interpret because they were complicated by the development of vibriosis in the experimental groups. Vibriosis was most likely responsible for the high levels of mortality seen in the control and both treatment groups.

Assayed levels of ivermectin in the medicated feeds were generally considerably lower than the targeted levels. Uneven spraying of the feed or loss of ivermectin during frozen storage are possible reasons for these discrepancies. The use of other formulations of ivermectin or methods of incorporation into feeds might influence the level of ivermectin obtained in the feeds and/or its bioavailability. Such changes could have important effects with respect to the toxicity of ivermectin to salmonids.

In our study no pathological changes were associated with ivermectin toxicity. Palmer et al. (1987) also reported no pathological changes in the organs of seawater salmon and freshwater rainbow trout that survived treatment with ivermectin.

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