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 B_1) 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 B_1) 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} 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 salmonids 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 and 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

Table 1. Results of feed analysis for treatments of Atlantic, chinook, and coho salmon and steelhead trout. Values were corrected for an average recovery of 87.7 % and are based on duplicate analyses. nd: none detected

Feed sample	Sprayed ivermectin (mg kg^{-1} feed)	Recovered ivermectin (mg kg^{-1} feed)
Untreated feed	0	nd
Atlantic carrier solution	0	nd
Atlantic 0.05 mg kg^{-1} group	10.0	6.7
Atlantic 0.10 mg kg^{-1} group	20.0	11.3
Atlantic 0.20 mg kg^{-1} group	40.0	21.0
Atlantic 0.50 mg kg^{-1} group	100.0	40.4
Atlantic 1.0 mg kg^{-1} group	200.0	97.2
Chinook 0.05 mg kg^{-1} group	10.1	9.1
Chinook 0.10 mg kg^{-1} group	19.7	13.2
Coho 0.05 mg kg^{-1} group	10.0	11.0
Coho 0.10 mg kg^{-1} group	20.0	14.9
Coho 0.20 mg kg^{-1} group	20.0	13.6
Steelhead 0.05 mg kg^{-1} group	5.0	3.6
Steelhead 0.10 mg kg^{-1} group	10.0	6.5

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 l tanks and all other species in 500 l 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 2. Mean ± SD and range in parentheses of seawater temperatures over the experimental periods

Species	Treatments	Temp. (°C)
Atlantic salmon	Controls, 0.20, 0.50 and 1.0 mg kg^{-1}	8.7 ± 0.4 (8.0 – 9.2)
Atlantic salmon	Controls, 0.05 and 0.10 mg kg^{-1}	9.0 ± 0.5 (8.0 – 10.5)
Atlantic salmon	Carrier solution and controls	12.5 ± 0.6 (11.1 – 13.7)
Chinook salmon	Controls and all treatment levels	10.3 ± 0.5 (9.4 – 13.0)
Coho salmon	Controls and all treatment levels	12.1 ± 0.8 (10.6 – 14.7)
Steelhead trout	Controls and all treatment levels	11.0 ± 0.9 (9.8 – 13.0)

of a similar size were assigned to each of the iver-
mectin-treated groups and 25 fish to each of the con-
trol 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 anesthetized with MS-222 and
weighed to determine an average weight ($\pm$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 experi-
ment and from 5 apparently healthy fish from each
treatment and control group at termination of the ex-
periments. 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 lev-
els 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 com-
pared to the other treatment levels, with values of 80,
84, and 94 %, respectively.

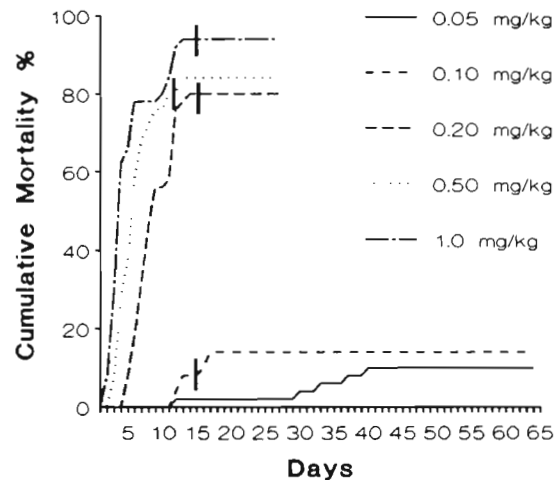Fish in the 0.05 mg kg^{-1} treatment group showed a

Fig. 1. *Salmo salar.* Toxicity of orally administered ivermectin
to Atlantic salmon. Fish were fed a commercial diet sprayed
with ivermectin in the form of a veterinary preparation of 1 %
w/v oral drench (Eqvalan; Merck Frosst Canada) at targeted
dosages of 0.05, 0.10, 0.20, 0.50 and 1.0 mg kg^{-1} fish every
second day. For the 0.20, 0.50 and 1.0 mg kg^{-1} diets the ex-
periment was terminated at Day 27. No mortalities occurred
in the control group or in the group fed the carrier solution.
Bars indicate the point at which all feeding activity ceased

reduction in their feeding activity, some loss of equilib-
rium, and a darkened colouration after 6 doses of iver-
mectin (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} treat-
ment 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 com-
pared 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 % cumula-
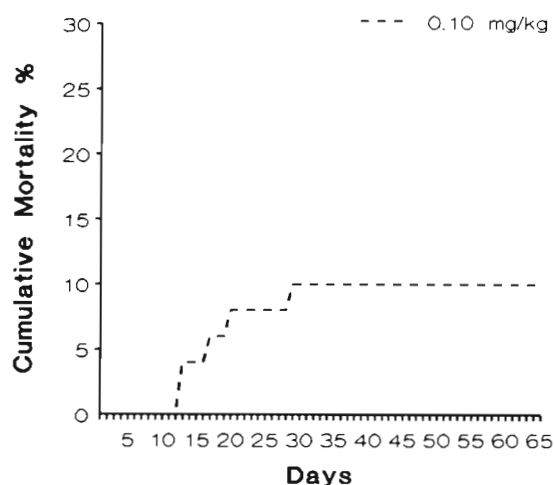tive mortality occurred in the 0.10 mg kg^{-1} treatment

Fig. 2. *Oncorhynchus tshawytscha*. Toxicity of orally adminis-tered ivermectin to chinook salmon. Fish were fed a commer-cial diet sprayed with ivermectin in the form of a veterinary preparation of 1 % w/v oral drench (Eqvalan; Merck Frosst Canada) at targeted dosages of 0.05 and 0.10 mg kg^{-1} fish every second day. No mortalities occurred in the control group or in the 0.05 mg kg^{-1} treatment group

group (Fig. 2). Both treatment groups consumed their full treated food ration throughout the experiment. A reduction in the feeding activity was noted after 13 doses in both treatment groups. At the end of the ex-periment the 0.10 mg kg^{-1} group was darker in colour and was feeding much more slowly than the 0.05 mg kg^{-1} treatment group. Down-turned eyes, as seen in the Atlantic salmon that were fed the higher doses, were not observed in chinook salmon.

Coho salmon

The coho control group had an 8 % cumulative mor-tality over the experimental period. No mortalities oc-curred 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 experi-ment (Fig. 3).

Although feeding activity was reduced when com-pared 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 approxi-mately 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 steel-head 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
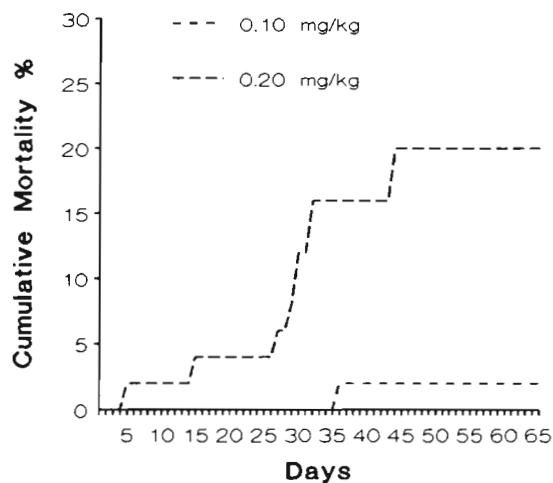

Fig. 3. *Oncorhynchus kisutch*. Toxicity of orally administered ivermectin to coho salmon. Fish were fed a commercial diet sprayed with ivermectin in the form of a veterinary prepara-tion of 1 % w/v oral drench (Eqvalan; Merck Frosst Canada) at targeted dosages of 0.05, 0.10, and 0.20 mg kg^{-1} fish every second day. No mortalities occurred in the 0.05 mg kg^{-1} treat-ment group
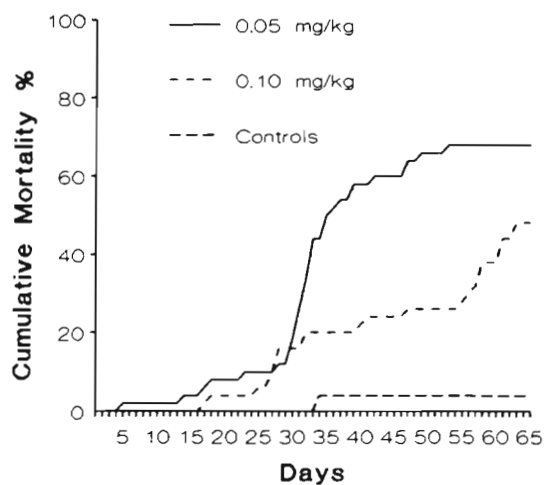

Fig. 4. *Oncorhynchus mykiss*. Toxicity of orally administered ivermectin to steelhead trout. Fish were fed a commercial diet sprayed with ivermectin in the form of a veterinary prepara-tion of 1 % w/v oral drench (Eqvalan; Merck Frosst Canada) at targeted dosages of 0.05 and 0.10 mg kg^{-1} fish every second day. A vibriosis outbreak occurred in all groups

end of the experiment. In all treatment groups most of the dead fish were heavily infected with *Vibrio ordalli*. 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 26 % mortality of Atlantic salmon given a single oral dose of 0.75 mg kg^{-1}. Palmer et al. (1987) reported that 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} had a 38 % cumulative mortality (O'Halloran et al. 1992).

Ivermectin, when used for the control of sea lice, is normally fed at 0.05 mg kg^{-1} fish every third day (Jackson & Costello 1992). In our study, ivermectin was fed at this level every second day and resulted in a low cumulative mortality (10 %) in Atlantic salmon, and no mortality in chinook or coho salmon. Smith et al. (1992, 1993) reported that oral treatments with ivermectin 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 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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