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Plasma concentrations of emamectin benzoate after Slice™ treatments of Atlantic salmon (*Salmo salar*): Differences between fish, cages, sites and seasons

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ABSTRACT

Infestations with the marine copepods *Lepeophtheirus salmonis* and *Caligus elongatus* are unavoidable in Norwegian net-pen based salmonid production and a major cause of economic loss and reduced animal welfare. Treatments are mainly performed with pyrethroids and the avermectin emamectin benzoate (Slice™). In this study, plasma concentrations of emamectin benzoate (EB) in salmon receiving standard, oral Slice™ treatments were studied in two fish farms in mid-Norway with postsmolts put to sea in the autumn of 2005. Samples were collected in the autumn of 2005 and repeated sampling was performed from the same sites in the summer of 2006. The tentative concentration of EB in the medicated feed was 10 mg/kg, resulting in a daily dosage of 50 µg/kg body weight at a feeding rate of 5 g medicated feed/kg body weight for seven days. Blood samples were collected the day after ended treatment from 50 anaesthetised fish (metacaine, 80 mg/l) in each of three separate cages, by caudal venipuncture using heparinised vacutainer tubes. Twenty-five randomly selected samples from each cage were analysed for EB by an HPLC method. The overall median concentration was 116 ng/ml, the plasma emamectin concentrations in fish varied from 6 ng/ml (autumn 2005) to 440 ng/ml (summer 2006). There were significant differences in the EB plasma concentrations among fish from the three cages at site 1, both in 2005 and in 2006. At site 2, no significant difference among the three cages could be demonstrated. There was a significant difference between pooled results between the seasons on site 2. No difference was found between the two sites in the autumn, however in the summer there was a highly significant difference between sites 1 and 2. At both sites in autumn 2005 and on site 1 in summer 2006, there were clinical outbreaks of diseases (IPN, HMSI). The multivariate ANOVA model demonstrated a highly significant influence of disease outbreaks on emamectin concentrations in plasma. The model also demonstrated tendencies that site and seasons influenced the EB concentration in blood plasma. There was no correlation between the weight of the fish and the EB concentration. The influence of feeding techniques (hand feeding versus automatic feeding) could not be tested.

1. Introduction

In 2005, 657,000 tonnes of farmed fish were produced in Norway, mainly Atlantic salmon and rainbow trout (http://www.ssb.no/english/subjects/10/05/nos_fiskeoppdrett_en/). Infestations with the marine copepods *Lepeophtheirus salmonis* and *Caligus elongatus* (sea lice) are unavoidable in the Norwegian net-pen based salmonid production, and a major cause of economic loss and reduced animal welfare (Boxaspen and Holm, 2001; Grant, 2002). Wild populations of salmonids are also infected with sea lice. The majority of these parasites on wild salmonids are believed to originate from the salmon farming industry (Bjørn et al., 2001; Bjørn and Finstad, 2002) making effective sea lice control by the aquaculture industry very important for wild stocks of salmonids as well.

Treatment strategies have been bath treatments with organophosphates and pyrethroids, and oral treatments with chitin synthesis inhibitors and avermectins. Today, the pyrethroids in combination with the avermectin emamectin benzoate (Slice™, Schering-Plough Animal Health) dominate the Norwegian market as anti-sea lice agents (Grave et al., 2004).

Organophosphates, as metrifonate, dichlorvos and azamethiphos, were used until their effect decreased in the mid 1990s due to resistance development. Reduced sensitivity to pyrethroids has also been detected in geographically isolated places (Sevatdal et al., 2005a). All these treatments were administered by bath. Bath treatments are time-consuming, labour-intensive and stressful for the fish, and may by themselves cause mortalities (Stone et al., 2002). The treatments can be difficult to administer in an optimal manner, and treatment failures may also occur due to suboptimal procedures, e.g. insufficient reduction of the cage volume during treatment.

In-feed treatments may be easily administered with less stressful husbandry practices (Stone et al., 2002). However, the success of such

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treatments depends on an even distribution of medicated feed within the cages to ensure that all fish receive a sufficient dose. This may be difficult to accomplish, as any treatment of large populations will result in large inter-individual variations in plasma- and tissue concentrations through such treatments. This was demonstrated by Coyne et al. (2006), who examined apparently healthy fish during a standard field medication with the antibacterial agent florfenicol and found plasma concentrations varying from 0.7 to 7.3 mg/l. Such variations in the concentration achieved in different members of the population result in a substantial risk of sub-optimal tissue concentrations in a large proportion of the treated population, with treatment failures and selection for resistant parasites as a possible outcome. Reduced efficacy of emamectin benzoate treatments has been demonstrated in Scotland (Lees et al., 2008) and Chile (Bravo et al., 2008).

Studies of distribution and elimination of emamectin benzoate in Atlantic salmon have been published (Kim-Kang et al., 2004; Sevatdal et al., 2005b), but we know little about the interindividual variation in emamectin concentration within groups of caged salmon on commercial farms after a standard treatment.

The aims of this study were to evaluate the variation in plasma emamectin benzoate concentration within groups of Atlantic salmon after normal, standard Slice™ treatments, to analyse which variables that influence the variations and finally to evaluate whether or not these variations resulted in differences in the clinical outcome of the treatments.

2. Materials and methods

Two fish farms in mid-Norway were included in this study, both with postsmolt Atlantic salmon (*Salmo salar*) put to sea in autumn of 2005. The study period was from October 2005 to August 2006. In both farms, standard treatments with emamectin benzoate against sea lice were conducted, both in the autumn of 2005 and in the summer of 2006.

Emamectin benzoate (Slice™, Schering-Plough Animal Health) was used to prepare medicated feed for salmon by a licensed, commercial feed-mill. The batches of medicated feed were analysed for the active ingredient by the producer before being released. The tentative concentration of the active compound in the medicated feed was 10 mg/kg, resulting in a daily dosage of 50 µg/kg body weight at a feeding rate of 5 g medicated feed/kg body weight. This treatment was administered daily for seven days. The two fish farms were followed through each treatment.

Blood samples were collected from 50 randomly sampled fish in three randomly selected cages at each site in autumn of 2005 and then again in the summer of 2006. The samples were collected the day after the treatment ended. The blood samples were collected from anaesthetised fish (metacaine, 80 mg/l) by caudal venipuncture using heparinized vacutainer tubes, centrifuged and the plasma was immediately frozen at −20 °C. They were kept frozen until analysed.

The weight and trunk length of each fish were measured when the samples were collected. The water temperature was also recorded. Sea lice counts were performed before initiation of the treatment and then at 1, 4 and 8 weeks after the end of the treatment.

Out of the 50 samples collected from each cage, 25 randomly selected samples were analysed by an in-house method based on De Montigny et al. (1990). Two hundred fifty microliter plasma was spiked with 50 µg doramectin (internal standard), then 3 ml of acetonitril: purified water (30:70) containing 0.1% triethylamine were added. After mixing, the samples were cleaned up using a Bond Elute C8 solid phase extraction column. The extracts were evaporated to dryness using nitrogen gas and then gently mixed with the derivatisation reagents (200 µl 1-methylimidazole: acetonitril, 50:50 and 225 µl trifluoroacetic anhydride:acetonitrile, 67:33). The samples were derivatized at 75 °C for 90 min, allowed to cool to room temperature and immediately analysed by high-performance liquid chromatography (HPLC) using an ODS Hypersil analytical column (Agilent, USA), 2.0 × 250 mm with 5 µm particles, a mobile phase of methanol:water (95:5) and a fluorescence detector operating at an excitation wavelength of 364 nm and an emission wavelength of 470 nm. The flow rate was 0.3 ml/min, and the injection volume was 2 µl. The calculation of the concentration of emamectin benzoate was based on the ratio between peak heights of emamectin and the internal standard doramectin. Emamectin concentrations above 5 ng/ml could be detected with the method.

The distribution of emamectin benzoate concentrations among all samples collected at both sites in both seasons significantly differed from a normal distribution, as determined by the Shapiro test. The distribution also deviated from a normal distribution after logarithmic- or *Z*-transformation (standardisation to the same scale with a mean of zero and a standard deviation of one), thus the nonparametric Friedman's ANOVA test was used to evaluate differences among cages, sites and seasons, and Spearman's rho was used to calculate correlations. The statistical analyses were performed using the SPSS software (Version 15.0).

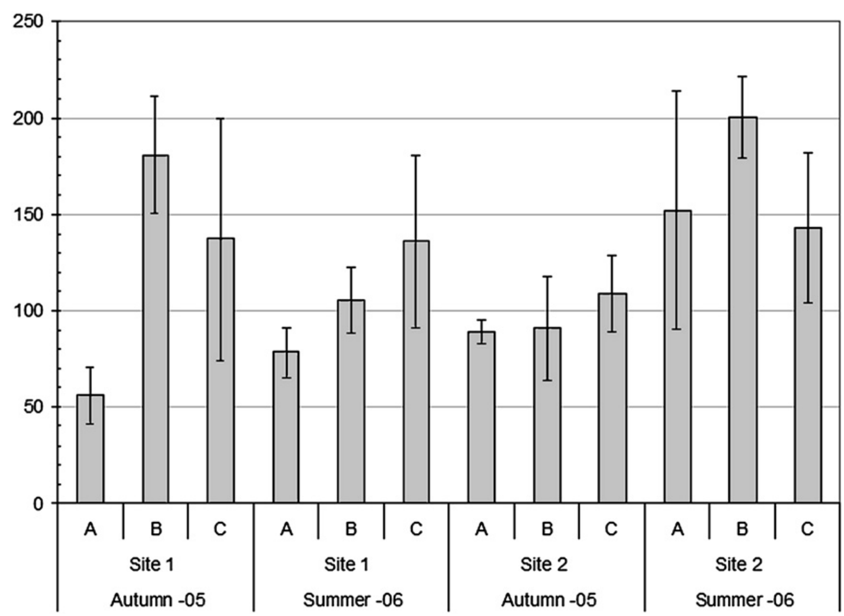

Fig. 1. Concentrations of emamectin benzoate (EB) in plasma from Atlantic salmon the day after a tentative dosage of 50 µg EB/kg body weight daily for seven days. Fish were sampled from three cages (A, B and C) at two sites (1 and 2) in autumn 2005 and summer 2006. The median values and the 95% confidence interval are given. The grand median was 116 ng/ml.