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Field trials in Norway with SLICE® (0.2% emamectin benzoate) for the oral treatment of sea lice infestation in farmed Atlantic salmon *Salmo salar*

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ABSTRACT: Four commercial salmon farms on the West coast of Norway were recruited to a programme of field trials in which the efficacy of SLICE® (0.2% emamectin benzoate; Schering-Plough Animal Health) was compared with a commercially available product, EKTOBANN® (teflubenzuron 2 g kg^{-1}; Skretting A/S) in treating natural sea lice *Lepeophtheirus salmonis* infections in Atlantic salmon *Salmo salmar* L. At each test site, 3 fish pens were treated with each product. In total, nearly 1.2 million first-year-class fish were included in the trial, of which approximately 561 000 received emamectin benzoate at a dosage of 50 µg kg^{-1} body wt d^{-1}, while approximately 610 000 received teflubenzuron at a dosage of 10 mg kg^{-1} body wt d^{-1}. Medicated feed was provided at 0.5% body wt d^{-1} over 7 consecutive days. Feed containing emamectin benzoate was generally well accepted by the fish and no problems were encountered in feeding the medicated diet at the desired dose. Lice numbers were counted 2 d before and 1, 7, 14 and 21 d after commencement of treatment. While treatment with both substances rapidly reduced lice numbers, pens treated with emamectin benzoate were found to harbour significantly fewer lice 14 and 21 d post-treatment. Twenty-one days following treatment with emamectin benzoate the lice abundance was reduced on average by 94%. Limited sampling outside the main study period indicated that emamectin benzoate protects against sea-lice infestation over longer periods.

KEY WORDS: Sea lice · *Lepeophtheirus salmonis* · *Caligus elongatus* · *Salmo salar* · Emamectin benzoate

INTRODUCTION

Sea lice infestation remains one of the main threats to Atlantic salmon sea-cage culture. Of the 2 caligid species commonly involved, *Lepeophtheirus salmonis* and *Caligus elongatus*, the former is presently most problematical (Roth et al. 1993). If uncontrolled in a farm environment, several hundred of these ectoparasites may accumulate per fish, resulting in cutaneous damage and ultimately death of the host through osmotic loss or secondary infection. Until recently the standard treatment against these parasites has been the extensive use of organophosphate (OP) pesticides incorporated in a bath treatment, accomplished by drawing a non-permeable sheet under the cage in which the fish are held. However, the recent (and relatively rapid) development of resistance within populations of sea lice to OPs (Horsberg pers. comm.) has led to the evaluation of the therapeutic effect of many alternative compounds (Roth et al. 1993).

Whilst bath treatment incorporating novel chemical agents remains a practical option, and biological con-

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trol methods such as the introduction of cleaner-fish (wrasse) may play a role in lice management (Bjordal 1988), the advantages of oral preparations are considerable. The quantity of active substance released to the environment is much smaller than with bath treatments, the treatment is less stressful to the fish, and, the dosage is more accurately controlled and thus less likely to result in rapid development of resistance. Oral preparations are also normally relatively non-hazardous to the farmer.

A promising candidate for oral treatment of sea lice is the chemical emamectin benzoate, marketed under the trade name SLICE® (emamectin benzoate 0.2%; Schering-Plough Animal Health). Emamectin belongs to the avermectin family of compounds isolated from the micro-organism *Streptomyces avermitilis* (Burg & Stapley 1990) and is a close relative of ivermectin, an anti-parasitic drug that is unprecedentedly effective against nematode and arthropod parasites in both livestock and humans (Campbell 1990). Although ivermectin has experimentally been found to effectively reduce sea lice numbers on salmonids (Palmer et al. 1987) and has been used effectively without regulatory consent in salmon farming in Europe, it is unlikely to be licensed for use in aquaculture. This study was initiated to establish whether emamectin benzoate might emulate ivermectin in its anti-sea-lice effect in the field.

Previous controlled studies indicated that emamectin benzoate had a suitably powerful toxic effect on all life stages of sea lice and is relatively non-toxic to salmonid fish (Stone et al. 1999, Roy et. al 2000). To test the effect of emamectin benzoate under field conditions, 4 commercial Atlantic salmon sea pen sites on the west coast of Norway were selected. At each site, during a natural infestation with sea lice, 3 pens were treated with emamectin benzoate and 3 were treated with EKTOBANN® (Skretting A/S, teflubenzuron: 2 g kg^{-1}). Teflubenzeron, a chitin inhibitor, is currently available in Norway under a general exemption from registration (Norwegian Medicines Control Authority) as an oral sea lice treatment, and was included in this trial for comparative purposes. The efficacy of each treatment was estimated from the reduction in the numbers of sea lice after treatment.

MATERIALS AND METHODS

Official guidelines. This study to test the efficacy of emamectin benzoate against sea lice infestation under field conditions was performed according to the Committee of Veterinary Medicinal Products (CVMP) Note for Guidance on 'Efficacy of Veterinary Medicinal Products for Use in Aquatic Species', No. 111/8377/89 (unpubl.).

Medicated feeds. The following feeds were used: ***SLICE®:*** The emamectin benzoate medicated feed was produced by BIOMAR Denmark AS, using an ISO 9001 approved 'double coating' technique. A single size of pellet was produced (4.5 mm) appropriate to the predicted average fish size at the start of medication. A sample of the test feed was analysed for levels of the active ingredient by ABC Laboratories.

EKTOBANN®: Teflubenzuron medicated feed was produced by SKRETTING AS.

Medicated feeds were distinctively labelled (including inclusion rate) for easy differentiation between emamectin benzoate and teflubenzuron. The medicated feed was stored under cool, dry conditions at each test farm from delivery to start of medication.

Test system. Site selection, feeding, sampling, and analysis were as follows:

Site selection: Four locations met the following inclusion/exclusion criteria at the commencement of the trial: (1) The salmon were introduced to sea as smolts during spring 1997; (2) vaccination status was equal; (3) the pens were similar in regard to bacteriological, virological and parasitological diagnoses; (4) wrasse *Ctenolabrus rupestris*, if present, were present in all the test pens; (5) the net pens were similar within each replicate with respect to net type, depth and diameter; (6) no treatment against sea lice had been performed in the 3 wk preceding the trial; (7) all treatments were performed in the same year as the fish were introduced into the sea.

The allocation of pens to treatment group was decided by ballot. At each location, 3 pens were selected for treatment with each medicament. The pens uninvolved in the study received no bath treatment against sea lice during the study period.

Feeding regime: At each test site, 3 pens were treated with each product. In total, nearly 1.2 million first-year-class fish were included in the trial, of which approximately 561 000 received emamectin benzoate at a dosage of 50 µg kg^{-1} body wt d^{-1}, while approximately 610 000 received teflubenzuron at a dosage of 10 mg kg^{-1} body wt d^{-1}. Medicated feed was provided at 0.5% body wt d^{-1} over 7 consecutive days.

Before and after medication period: Before and after the medication period, all groups were fed according to individual farm practice. Feed type was chosen by the farmer and was (with the exception of Pen 1 at Site 4, where the normal ration was larger than the medicated feed) consistent in pellet size and ingredients to the medicated feed.

During medication period: The test pens were given medicated feed once a day (in the morning) at 0.5% of body weight. An afternoon feed of non-medicated diet was then given at a rate of up to 0.5% of body weight.

Sampling: Sampling at each of the participating test farms was monitored by the local veterinarian. At all experimental sites, 10 fish were selected from each pen included in the study on Day –2 (i.e. 2 d before treatment), 20 fish were then sampled on Day 1 (i.e. 1 d following commencement of treatment), and on Days 7, 14 and 21. Site 1 included an extra count on Day 36 and Site 2 included an extra count on Day 51.

Selection of fish for sea lice counts: A maximum of 3 fish at a time were randomly selected by hand-netting from each cage. The fish were sacrificed by a blow to the head before lice were counted, and each batch of 2 to 3 fish was counted before 2 to 3 new fish were captured.

Counting of lice: As far as possible the same personnel counted each time. Only 1 person counted within a replicate. The total number of lice per fish including chalimus stages (Stages I through IV), pre-adult and adult sea lice was recorded.

Statistical analysis: Statistical analysis of treatment effects used the Stratified Wilcoxon exact-rank sum test. Site was used as the stratification variable. Analyses were performed by SAS Version 6.12 (SAS Institute and StatXact Turbo version 2.14 (Cytel Corporation).

RESULTS

Counts for each of the lice developmental stages (chalimus through adult) pooled for all 4 sites are presented in Tables 1 to 3. Counts for all parasitic stages considered as a whole are presented in Table 4.

No significant difference (between treatment groups) in numbers of any of the 3 developmental stages was identified prior to treatment. Following treatment, numbers of all stages in both treatment groups were reduced by Day 1 and further reduced by Day 7. Up to Day 14, the numbers of chalimus and adult stages continued to decline in both groups, while pre-adult numbers were maintained approximately at Day 7 levels. Up to Day 21, the chalimus stages (in both teflubenzuron- and emamectin benzoate-treated

Table 1. *Lepeophtheirus salmonis*. Number of chalimus-stage lice (mean no. per fish) on commercial salmon *Salmo salar* before and following treatment with teflubenzuron and emamectin benzoate (data pooled for all 4 sites). N: no. of pens sampled per treatment; % remaining: percent of pre-treatment level; *statistically significant

Study day	N	Teflubenzuron Mean no. lice fish^{-1} (range)	% remaining	Emamectin benzoate Mean no. lice fish^{-1} (range)	% remaining	p
–2	6	1.93 (0–5.5)	100	2.14 (0–6.4)	100	0.5267
1	6	1.45 (0.1–3.7)	75.1	1.73 (0.1–4.4)	80.8	0.3418
7	6	0.59 (0–2.5)	30.5	0.64 (0–2.0)	29.9	0.0460*
14	6	0.53 (0–1.2)	27.5	0.23 (0–0.7)	10.7	0.0013*
21	6	0.38 (0.1–0.8)	19.7	0.19 (0–0.8)	8.9	0.0021*
36	3	0.07 (0.05–0.1)	3.6	0 (0–0)	0	–[a]
51	3	10.5 (2.6–14.8)	544	0.23 (0.1–0.5)	10.7	–[a]

[a]Data from only 1 site; because of small sample size, no statistics are presented

Table 2. *Lepeophtheirus salmonis*. Number of pre-adult-stage lice (mean no. per fish) on commercial salmon *Salmo salar* before and following treatment with teflubenzuron and emamectin benzoate (data pooled for all 4 sites). N: no. of pens sampled per treatment; % remaining: percent of pre-treatment level; *statistically significant

Study day	N	Teflubenzuron Mean no. lice fish^{-1} (range)	% remaining	Emamectin benzoate Mean no. lice fish^{-1} (range)	% remaining	p
–2	6	2.32 (0.5–5.5)	100	2.34 (0.3–4.8)	100	0.6600
1	6	1.96 (0.3–5.3)	84.5	1.53 (0–4.0)	65.4	0.4949
7	6	0.13 (0–1.0)	5.6	0.09 (0–0.3)	3.8	0.6356
14	6	0.14 (0–0.5)	6.0	0.05 (0–0.2)	2.1	0.1184
21	6	0.60 (0.1–2.0)	25.9	0.07 (0–0.5)	3.0	<0.0001*
36	3	0.13 (0.1–0.3)	5.6	0.03 (0–0.1)	1.3	–[a]
51	3	3.37 (0.5–8.6)	145	0 (0–0)	0	–[a]

[a]Data from only 1 site; because of small sample size, no statistics are presented

Table 3. *Lepeophtheirus salmonis*. Number of adult-stage lice (mean no. per fish) on commercial salmon *Salmo salar* before and following treatment with teflubenzuron and emamectin benzoate (data pooled for all 4 sites). N: no. of pens sampled per treatment; % remaining: percent of pre-treatment level; *statistically significant

Study day	N	Teflubenzuron Mean no. lice fish^{-1} (range)	% remaining	Emamectin benzoate Mean no. lice fish^{-1} (range)	% remaining	p
–2	6	0.57 (0–2.3)	100	0.65 (0 –2.7)	100	0.5727
1	6	0.42 (0–1.5)	73.7	0.41 (0–1.1)	63.1	0.7900
7	6	0.17 (0–1.0)	29.8	0.20 (0–0.6)	30.8	0.1522
14	6	0.11 (0–0.7)	19.3	0.13 (0–0.6)	20.0	0.8900
21	6	0.21 (0–0.7)	36.8	0.06 (0–0.3)	9.2	0.0140*
36	3	0.17 (0–0.5)	29.8	0.03 (0–0.1)	4.6	–[a]
51	3	0.02 (0–0.1)	3.5	0 (0–0)	0	–[a]

[a]Data from only 1 site; because of small sample size, no statistics are presented

Table 4. *Lepeophtheirus salmonis*. Number of chalimus (mean no. per fish) on commercial salmon *Salmo salar* before and following treatment with teflubenzuron and emamectin benzoate (data pooled for all 4 sites). N: no. of pens sampled per treatment; % remaining: percent of pre-treatment level; *statistically significant

Study day	N	Teflubenzuron Mean no. lice fish^{-1} (range)	% remaining	Emamectin benzoate Mean no. lice fish^{-1} (range)	% remaining	p
–2	6	4.82 (0.9–11.2)	100	5.13 (2.1–11.5)	100	0.7292
1	6	3.83 (0.7–10.1)	79.4	3.66 (0.9–9.3)	71.3	0.9126
7	6	0.88 (0–2.6)	18.26	0.93 (0.2–2.3)	18.1	0.3338
14	6	0.78 (0.1–1.7)	16.2	0.40 (0 –0.9)	7.8	0.0038*
21	6	1.18 (0.2–3.0)	24.5	0.32 (0–0.2)	6.2	<0.0001*
36	3	0.37 (0.2–0.8)	7.7	0.07 (0–0.2)	1.4	–[a]
51	3	13.9 (11.2–15.9)	288	0.23 (0.1 0.5)	4.5	–[a]

[a]Data from only 1 site; because of small sample size, no statistics are presented

groups) continued to decline, while adult lice continued to decrease only in the emamectin benzoate-treated group (an increase was recorded in the teflubenzuron-treated group). While pre-adult lice numbers had increased significantly in teflubenzuron-treated fish by Day 21, the increase in the emamectin benzoate group was marginal.

Only at Site 1 were lice numbers evaluated on Day 36 and only at Site 2 were lice numbers counted on Day 51. The sample size of 3 pens in each case was insufficient to evaluate statistical significance, but in each survey a numerical advantage was found in favour of the emamectin benzoate-treated groups for all parasitic stages.

DISCUSSION

The results obtained in the present study confirm preliminary evaluation by Stone et al. (1999) that emamectin benzoate is effective against sea lice infestation in Atlantic salmon. Emamectin benzoate successfully reduced the level of infestation of all life stages of *Lepeophtheirus salmonis* under field conditions. Response to treatment was relatively rapid, the majority of lice being removed from the fish within the 7 d treatment period and lice abundance reduced by an average of 94% 21 d post-treatment. Results following treatment with emamectin benzoate were equal to and often significantly better than treatment with teflubenzuron. Although the study lacked negative controls (on animal welfare and commercial grounds), the extremely large numbers of fish involved (over 1.1 million) combined with the use of the 'positive' control i.e. teflubenzuron, strengthen confidence in the findings.

Emamectin benzoate treatment led to a reduction in lice abundance which was maintained to the end of the trial (day 21) in 11 of 12 test pens. Although the number of cages sampled does not allow statistical comparison, the counts made on Day 51 at Site 2 indicated that emamectin benzoate may confer protection over a longer period than first thought. The data from the remaining post-trial count (Day 36, Site 1) was influ-

enced by an additional introduction of wrasse and high rainfall, which led to a uniform low prevalence of lice throughout the site. Given the levels of effectiveness found in the present trial, combined with its effect on all parasitic stages of lice, repetition of treatment with emamectin benzoate should be infrequent.

Examination of feeding records established that medicated feeds were not always fed at strictly 0.5% body wt d^{-1}, but ranged between 0.45 and 0.70 with an average of 0.54%. This was caused by error in biomass estimation. Such small discrepancies between estimated biomass and actual biomass are unavoidable under commercial conditions, and the relatively large sample numbers used in the present trial to predict total biomass held this variation to a minimal level. While undesirable in a controlled trial, these variations are typically found on every commercial fish farm and represent the environment in which the product will be used when licensed. No deleterious effects were associated with the provision of medicated feed at any of the levels tested.

Emamectin benzoate medicated feed was generally satisfactorily accepted by the fish. Although slight appetite depression was reported in some pens at 2 of 4 experimental sites, there was no problem in feeding fish at 0.5% body wt d^{-1}. Appetite was reported as normal when top-up rations were supplied.

Although *Caligus elongatus* was present at each of the experimental sites, its prevalence was low. Nevertheless, it is considered that emamectin benzoate was probably effective in treating these lice also. Absolute conclusions relating to *C. elongatus* are difficult, as this species is highly mobile and non-specific in its choice of host.

To summarize: (1) Emamectin benzoate administered via feed at a dosage of 50 µg kg^{-1} body wt daily for 7 d was highly efficacious in the treatment of natural infestations of *Lepeophtheirus salmonis* in Atlantic salmon at 4 farms in Norway under summer conditions. (2) Based on lice counts, treatment with emamectin benzoate compared favourably at all experimental sites to treatment using a commercially available anti-sea lice treatment (teflubenzuron). Lice populations (all parasitic stages) on treated fish were reduced by over 90%, 21 d post-initiation of treatment. (3) Feed surface-dressed with emamectin benzoate and fed at 0.5% body wt d^{-1} was generally well accepted by salmon. Some appetite depression was observed, but no problems were reported in feeding at 0.5% body wt. (4) Emamectin benzoate was well tolerated. No adverse reaction or mortality was associated with its use.

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LITERATURE CITED

Bjordal Å (1988) Cleaning symbiosis between wrasses (Labridae) and lice infested salmon (*Salmo salar*) in mariculture. ICES CM/1988

Burg RW, Stapley EO (1990) Isolation and production of the producing organism. In: Campbell WC (ed) Ivermectin and abamectin. Springer-Verlag, New York, p 24–32

Campbell WC (1990) Preface. In: Campbell WC (ed) Ivermectin and abamectin. Springer-Verlag, New York, p vii–viii

Palmer R, Rodger H, Drinan E, Dwyer C, Smith PR (1987) Preliminary trials on the efficacy of ivermectin against parasitic copepods of Atlantic salmon. Bull Eur Assoc Fish Pathol 7:47–54

Roth M, Richards RH, Somerville C (1993) Current practices in the chemotherapeutic control of sea lice infestations in aquaculture: a review. J Fish Dis 16:1–26

Roy WJ, Sutherland IH, Rodger HDM, Varma KJ (2000) Tolerance of Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhyncus mykiss* (Walbaum), to emamectin benzoate, a new orally administered treatment for sea lice. Aquaculture 184:19–29

Stone J, Sutherland IH, Somerville CS, Richards RH, Varma KJ (1999) The efficacy of emamectin benzoate as an oral treatment of sea lice, *Lepeophtheirus salmonis* (Krøyer) infestations in Atlantic salmon, *Salmo salar* L. J Fish Dis 22:261–270

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