A field efficacy evaluation of emamectin benzoate for the control of sea lice on Atlantic salmon

Robert Armstrong, Dan MacPhee, Terry Katz, Richard Endris

Abstract — This study evaluated the efficacy of emamectin benzoate, 0.2% aquaculture premix, against sea lice on Atlantic salmon in eastern Canada. Salmon pens received either emamectin benzoate, orally, in feed at 50 µg/kg body weight/day for 7 consecutive days, or the same diet with no added medication. The site veterinarian had the option of administering a bath treatment with azamethiphos to any pen in the trial. The mean number of lice per fish was lower ($P < 0.05$) in the experimental group when measured 1, 3, 4, and 6 weeks after the start of medication. Treatment efficacy was 70%, 88%, 95%, and 61%, respectively. Three azamethiphos bath treatments were applied to each control pen during the trial, while the treatment pens received no bath treatment. No gravid female parasites were observed on any fish in the treatment group, while these life stages were observed on fish in the control group. Orally administered emamectin benzoate was palatable and highly effective for control of sea lice on salmon.

Résumé — Évaluation sur place de l'efficacité du benzoate d'emamectine dans la lutte contre le pou du poisson chez le Saumon de l'Atlantique. Cette étude avait pour but d'évaluer l'efficacité du benzoate d'emamectine, 0,2 % en prémélange d'aquaculture, contre le pou de poisson chez le Saumon de l'Atlantique dans l'Est du Canada. Les saumons en enclos ont reçu soit du benzoate d'emamectine par voie orale, via l'alimentation, à la dose de 50 µg/kg de poids corporel, pendant 7 jours consécutifs, soit la même diète mais sans médication. Le vétérinaire local avait le choix d'administrer un traitement par bains d'azamethiphos à n'importe quel enclos inclus dans l'étude. Le nombre moyen de poux par poisson était plus bas ($P < 0,05$) dans le groupe expérimental, 1, 3, 4 et 6 semaines après le début de la médication, alors que l'efficacité du traitement était respectivement de 70 %, 88 %, 95 % et 61 %. Trois traitements par bains à l'azaméthiphos ont été appliqués à chaque enclos témoin au cours de l'étude alors que les enclos traités ne recevaient pas de bains médicamentés. Aucun parasite femelle gravide n'a été observé dans l'ensemble du groupe traité alors que ces stades ont été observés sur des poissons du groupe témoin. Le benzoate d'emamectine administré oralement était d'un goût agréable et très efficace pour lutter contre le pou du poisson chez le saumon.

(Traduit par docteur André Blouin)

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Introduction

Sea lice are ectoparasitic marine copepods that can cause severe injury to farmed Atlantic salmon populations, leading to substantial economic losses for producers (1–3). *Lepeophtheirus salmonis* is the species of louse that causes the most severe problems and occurs in greatest numbers on farmed salmon. *Caligus* spp. lice may also occur in Canada (3). The parasitic phase of the life cycle consists of the 4 larval stages, 2 pre-adult stages, and 1 adult stage spent on the fish (4).

The salmon farming industry needs a variety of effective and available treatment options for successful, integrated sea lice management. The only treatment presently approved in Canada is the organophosphate insecticide azamethiphos, available under a time-limited registration from the Pest Management Review Agency (5). The temporary registration is restricted to salmon farmers in eastern Canada; therefore, no compound is approved for sea lice control on the Pacific coast. Azamethiphos is applied as a bath treatment, an administration route that is more difficult for producers than an in-feed medication. Application of tarpaulins to pens without causing injury to fish or personnel is a challenging undertaking that can be considered only in calm weather with a trained and experienced crew. Bath treatments have no residual effect on lice

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populations once the treatment has been completed. Additionally, sea lice populations have developed resistance to organophosphates in other salmon farming regions where this type of compound represented the only approved treatment option (6).

The objective of this study was to determine the efficacy of a potential alternate in-feed treatment, emamectin benzoate, against sea lice on farmed salmon in eastern Canada under commercial rearing conditions. This treatment has been reported to be highly effective in reducing numbers of pre-adult and adult lice on Atlantic salmon held in tanks (7).

Materials and methods

Experimental design

Two commercial marine sea farming sites ("site 1" and "site 2") participated in this trial, with each site providing 4 pens of fish for the study. Two of the 4 study pens at each site were randomly allocated by coin toss to a test group and 2 were allocated to a control group. Populations of sea lice in the study pens were monitored, and when lice numbers began to increase, a diet medicated with emamectin benzoate was administered to all of the study pens in the test group. The date that the test diet was first administered was labeled Study Day 0. The study pens in the control group received the same diet at the same feeding rate without the medication being added. Neither the site personnel nor the site veterinarian was informed as to the treatment status of any study pen. Data collected at either site on Study Days separated by less than 48 h were pooled as a single sampling point. On Study Days 7/8, 14/16, 22, 28/29, and 43/44, lice were counted on 10 fish from each study pen at both sites and each fish was weighed. On Study Day 35, fish were sampled at site 1 only, while on Study Days 57, 73, 92, and 115, fish were sampled at site 2 only. Neither the control nor the test group could be left untreated if sea lice populations continued to increase. Therefore, the site veterinarian had the option of administering azamethiphos (Salmosan; Novartis Animal Health Canada, Mississauga, Ontario), a sea lice bath treatment, to any study pen, if the results of lice population monitoring so indicated. The mean total numbers of lice per fish, the mean numbers of different life stages of lice per fish, and the mean fish weights in control and treatment groups were compared statistically. Divers removed and counted all dead fish from each pen twice weekly throughout the trial. The amount of feed delivered to each study pen and the seawater salinity and temperature at the 2-meter depth were recorded daily at both sites.

Trial facilities

Both farm site structures were representative of salmon farming facilities in eastern Canada. Pens of fish selected for the study were matched according to their physical structure and fish population. Each study pen was a 70-meter circumference floating circle, suspending a 7.5-meter-deep net. A total of 10 pens moored together in a floating grid system comprised the structure at one trial site, and 14 pens in an equivalent system comprised the other site. The selected farm sites were known to have received previous treatment for control of sea louse populations. Each study pen had a number code, assigned previously by site management, and a letter code, assigned during the trial to identify the treatment status of the pen.

Trial animals

A total of 151 351 Atlantic salmon were housed in the 8 study pens. The 4 study pens allocated to the test group contained 76 210 fish and the 4 study pens assigned to the control group contained 75 141 fish. The mean weight of fish at the start of the trial was 600 g at site 1 and 350 g at site 2, the latter group having been transferred to salt water more recently. The mean number of fish per study pen was 10 000 at site 1 and 28 000 at site 2. Fish at each site were derived from a single source. No fish at either site had previously been observed to have clinical indications of infectious salmon anemia (ISA) virus infection or other infectious disease, apart from louse infestation. All fish were previously vaccinated against *Vibrio anguillarum, V. ordalli, V. salmonicida,* and *Aeromonas salmonicida.* All fish had received previous treatment with ivermectin (Ivomec; Merial Canada, Baie d'Urfé, Quebec) in feed, and pens were included in the study only if, in the judgment of the site veterinarian, the previously administered treatments were having no further clinical effect.

Medicated feed

Feed for administration to fish in the test pens was prepared by coating emamectin benzoate 0.2% aquaculture premix (Slice; Schering-Plough Animal Health, Pointe-Claire, Quebec) mixed with fish oil onto pelleted feed (Signature; Shur-Gain, Truro, Nova Scotia). The inclusion rate for the premix was calculated to deliver an approximate dosage of 50 µg active ingredient per kg of fish body weight (BW), daily, for 7 consecutive days. Emamectin benzoate levels measured in feed samples collected immediately following preparation of the medicated diet were 2.77 mg/kg in feed for site 1 and 2.35 mg/kg in feed for site 2. Therefore, the actual mean total dosage delivered to fish was 361 µg/kg BW over the treatment period, or 51.5 µg/kg BW/d, at site 1, and 293 µg/kg BW over the treatment period, or 41.9 µg/kg fish BW/d, at site 2.

The identical diet, without medication added, was packaged in identical feed bags for the control pens to ensure that site staff was unable to distinguish between the medicated and control diets. Bags were differentiated only by a letter code that specified the pen that would receive the enclosed feed. Both test and control diets were administered in the same way to each pen at each site by using feeding practices, feeding rates, and pellet sizes already in use at the start of the trial. Fish were fed 5.0-mm pellets at 2.3% BW/d at site 1 and 3.5-mm pellets at 3.1% BW/d at site 2. Fish accepted the medicated diet at both sites with no indication of reduced palatability. Fish received no feed other than the test or control diet during the trial.

Sampling

Sea lice were counted and fish were weighed at the start of the trial and approximately every 7 d following medicated diet administration. Fish were attracted to the

Table 1. The percentage efficacy of treatment and a statistical comparison of mean total numbers of sea lice, mean numbers of copepodid/chalimus, and mean numbers of pre-adult/adult lice per Atlantic salmon for each sampling period

	Total number of lice		Copepodid/chalimus		Pre-adult/adult		Gravid females	
Study day	% efficacy	*P*-value	% efficacy	*P*-value	% efficacy	*P*-value	% efficacy	*P*-value
7/8	70	0.03	17	0.22	83	0.03	100	0.42
14/16	58	0.61	58	0.28	64	0.56	100	0.50
22	88	0.03	84	0.03	94	0.08	100	0.25
28/29	95	0.03	96	0.03	94	0.03	100	0.03
35	84	—[a]	80	—	93	—	100	—
43/44	61	0.03	0	0.31	96	0.11	100	0.08
57	96	—	93	—	100	—	100	—
73	91	—	71	—	98	—	100	—

[a]Fish sampled at only one trial site, therefore *P*-value not calculated

surface with feed; then, 10 fish were sampled from each pen with a dip net and anesthetized in a 60 mg/L tricaine methane sulfonate solution (TMS; Syndel Laboratories International, Vancouver, British Columbia) for less than 5 min. All visible sea lice were counted on each fish, categorized as copepodid/chalimus, pre-adult/adult, or gravid female life stages, and the results were recorded. The same individual, who was not aware of the treatment status of each fish pen, counted the lice throughout the trial to maximize precision. After counting, each fish was weighed, allowed to recover in a seawater bath briefly, and then returned to the pen from which it had been removed.

Statistical analysis

Sea lice count data were compared statistically by using the nonparametric, stratified, Wilcoxon exact rank-sum test. Pen means of either the total number of lice per fish or the number of lice at each recorded life stage were compared by using site as the stratification variable and pen as the experimental unit. Two-sided hypotheses were used for comparison of results obtained before treatment administration, and one-sided hypotheses that the number of sea lice per fish was lower in the test pens were used for posttreatment comparison. Louse numbers per fish were compared statistically only if data were available for both trial sites.

The percent efficacy of treatment was determined for each sampling period by Abbott's formula, which expresses the difference between mean numbers of lice per fish in the control and test groups divided by the mean number of lice per fish in the control group as a percentage (8). Fish weights were analyzed by a nested mixed model analysis of variance, controlling for fish within pen within site. Statistical significance was declared at $P < 0.05$. Analyses were performed by using commercial statistical analysis softwares (SAS v. 6.12; SAS Institute, Cary, North Carolina, USA, and StatXact Turbo v. 2.14; Cytel Corporation, Cambridge, Massachusetts, USA).

Trial termination

The trial ended on Study Day 39 at site 1, when fish in control pens were administered an in-feed treatment with emamectin benzoate, under Emergency Drug Release from the Bureau of Veterinary Drugs, to control lice levels that were increasing despite having received

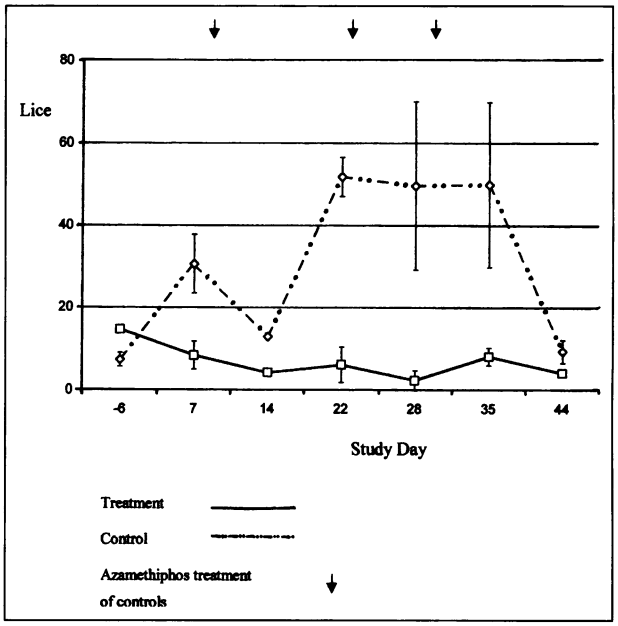

Figure 1. The mean and pen level standard deviation for the total number of sea lice (*Lepeophtheirus salmonis*) per Atlantic salmon (*Salmo salar*) per Study Day at site 1 before and after the administration of emamectin benzoate between Study Days 0 and 6.

3 treatments with azamethiphos. The trial concluded on Study Day 73 at site 2, when deteriorating weather conditions made further scheduled fish sampling difficult and potentially dangerous. However, farm site personnel were able to complete 2 additional 5-fish samples for sea lice counting on Study Days 92 and 115.

Results

The mean total number of lice per fish was similar in the test and control groups at both trial sites before the start of emamectin benzoate treatment. Following the medication period, the mean total number of lice decreased in the test group, while fluctuating and generally increasing in the control group (Figures 1 and 2). This treatment effect was consistent across all life stages of lice on fish at each Study Day until the last sampling period that included both sites (Study Day 28/29) before the trial terminated (Figures 3 to 5; Table 1). The treatment effect was also significant at multiple sampling

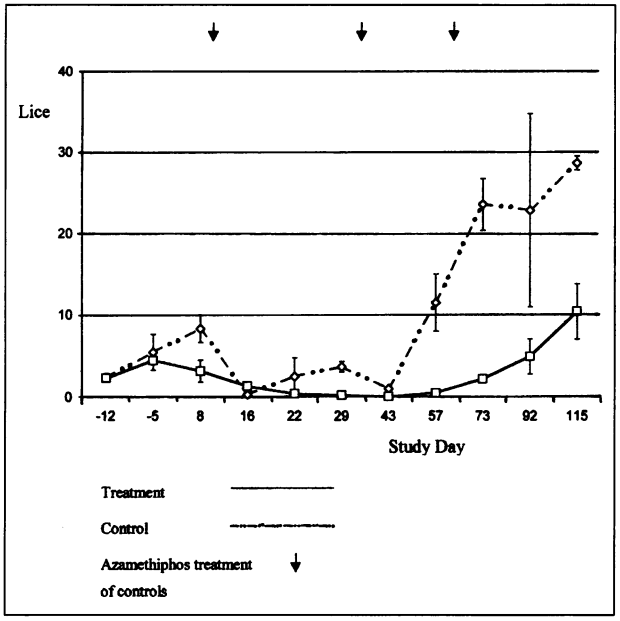

Figure 2. The mean and pen level standard deviation for the total number of sea lice (*Lepeophtheirus salmonis*) per Atlantic salmon (*Salmo salar*) per Study Day at site 2 before and after the administration of emamectin benzoate between Study Days 0 and 6.

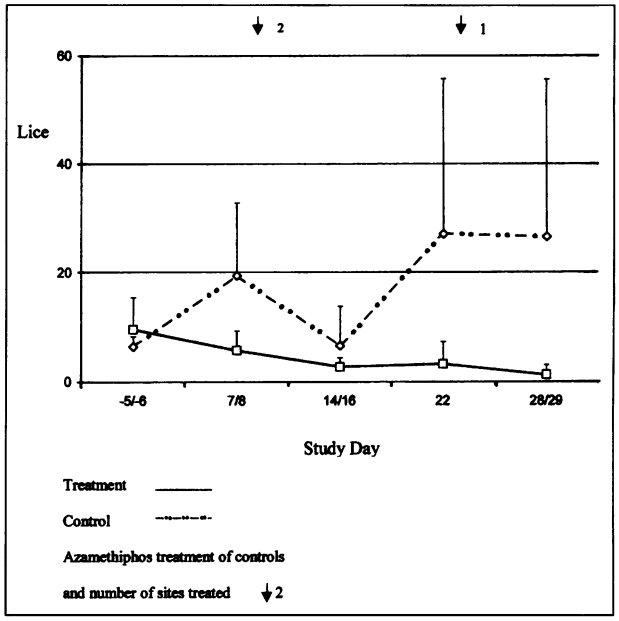

Figure 3. The mean and pen level standard deviation for the total number of sea lice (*Lepeophtheirus salmonis*) per Atlantic salmon (*Salmo salar*) per Study Day in treatment and control groups summarized across both trial sites.

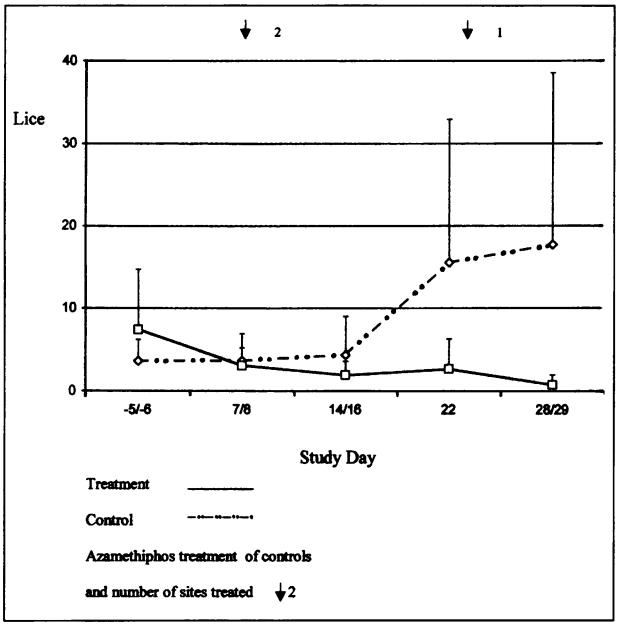

Figure 4. The mean and pen level standard deviation for the number of juvenile (attached) sea lice (*Lepeophtheirus salmonis*) per Atlantic salmon (*Salmo salar*) per Study Day in the test and control groups at both sites.

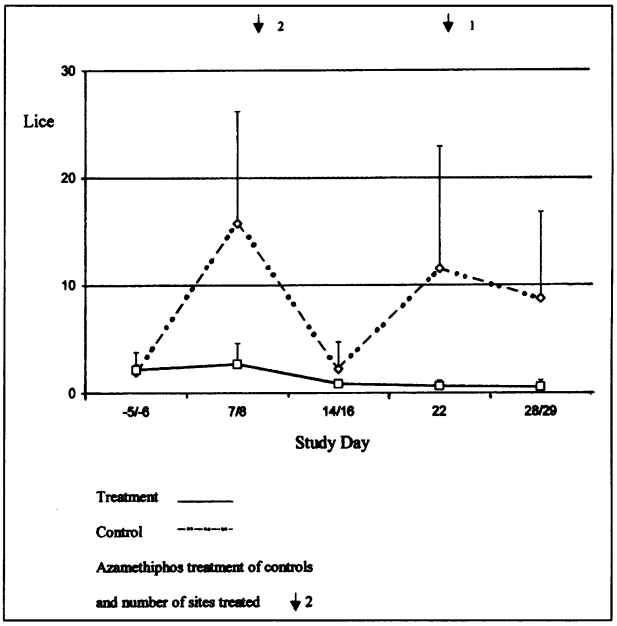

Figure 5. The mean and pen level standard deviation for the number of pre-adult and adult (motile) sea lice (*Lepeophtheirus salmonis*) per Atlantic salmon (*Salmo salar*) per Study Day in the test and control groups at both sites.

points despite application of azamethiphos to the control pens. The difference between test and control groups continued during the extended observation period at site 2 until the last sample collection on Study Day 115 (Figure 2), although the number of lice in the medicated group began to increase after Study Day 43. No gravid female lice were found on fish from the test group at either site throughout the entire study, including the extended observation period at site 2, while a low number of gravid females were found on fish in the control group.

The test group received no treatment other than oral emamectin benzoate throughout the trial, while the control group was treated with azamethiphos on Study Days 9, 23, and 31 at site 1, and Study Days 10, 33, and 58 at site 2. The mean weekly mortality rates during the

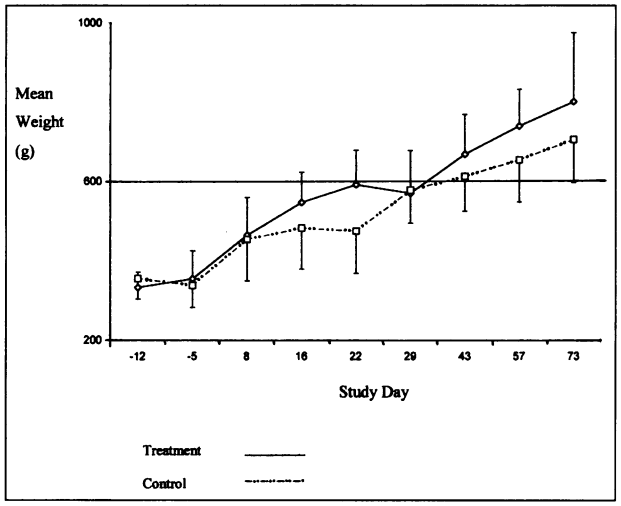

Figure 6. The mean and fish level standard deviation for the weight of Atlantic salmon per Study Day at site 2.

period between the end of treatment administration and termination of the trial were 0.06% in the test group and 0.09% in the control group at site 1, and 0.07% in the test group and 0.06% in the control group at site 2. These data were not compared statistically, because differences between mortality rates in the test and control groups were slight and inconsistent. The mean weight of fish in the test group was observed to be greater than that of fish in the control group at site 2 at almost every sampling period after Study Day 8 (Figure 6). No differences in mean fish weight were seen at site 1 at any sampling period (data not shown). Water temperature decreased steadily during the trial from 15°C to 11°C at site 1 and from 14°C to 9°C at site 2, while salinity levels fluctuated between 31 and 32.5 parts per thousand at both sites.

Discussion

Emamectin benzoate 0.2% aquaculture premix was effective for control of all life stages of sea lice when administered to Atlantic salmon. Treatment efficacy reached 95% on Study Days 28/29 (2 sites) and 57 (1 site), indicating a strong clinical effect. There was no factor other than application of the test treatment that could have accounted for the observed differences in lice populations between the test and control pens.

The clinical effect of emamectin benzoate treatment was consistent across all test pens with very little variability in the population of any sea lice life stage lice on treated fish. However, there was considerable variability in mean sea lice population counts in the control pens. Despite this variability and the low number of replicates, significant differences were observed between treatment and control groups on multiple Study Days following treatment administration.

Two factors likely account for most of the variability in mean lice populations on control fish. The first factor is that the louse population increased more rapidly at site 1 than at site 2; therefore, the calculated mean has greater variability. Secondly, azamethiphos bath treatments of control pens on both sites at different dates decreased the number of adult sea lice. This effect can be seen most strongly following the first azamethiphos treatment at site 1 on Study Day 9. Subsequent bath treatments had inconsistent impacts on treatment efficacy throughout the trial (Figures 1–4), because organophosphate compounds are primarily effective against adult lice (4) and the proportion of adult lice in the control population varied during the trial (data not shown). However, the reduced mean louse populations on control fish at both sites following bath treatments on Study Days 9 and 10 reduced the calculated percentage efficacy of the test treatment on Study Day 14/16 to 58%, a difference that was not significant. This was the lowest treatment efficacy percentage observed during the postmedication period. The fluctuations in the number of lice per fish that followed azamethiphos treatment further increased the variability of the calculated mean number of lice.

The investigator counting lice noted on data collection sheets used for the trial during the postmedication period that the attached stages of lice on fish in the treatment groups at both sites were primarily copepodids, the first stage of this parasite to attach to the fish (9). This investigator estimated that the proportion of the lice population comprised of copepodids was 100% in 1 test pen at site 2 on Study Day 57. A likely explanation for this observation is that these life stages do not feed (9) and, therefore, are not exposed to emamectin benzoate administered systemically to the fish. It is difficult to distinguish juvenile life stages of lice reliably without microscopic examination; therefore, differential counting of juvenile stages was not undertaken.

On Study Day 39, site 1 withdrew from the trial and emamectin benzoate was administered to the control group. The mean number of lice per fish in the site 1 control group then decreased rapidly, presumably a result of the in-feed treatment. Withdrawal of site 1 precluded further statistical analysis, and it was not possible to calculate the duration of treatment efficacy. However, the extended observation period at site 2 provides some insight, because the mean number of sea lice per fish began to increase slowly in the test groups after Study Day 43. The mean total number of lice increased more slowly on fish in the test group than on fish in the control group during this extended observation period at site 2. The increase in the mean total number of lice per control group fish in site 2 between Study Day 43 and 73 was slower than the increase in the site 1 control group fish between Study Day 14 and 22. Cooler water temperature in the late autumn lengthens the sea lice generation time and likely reduced the rate of louse population increase in the site 2 control group (10).

The percentage efficacy level against gravid female lice was 100% (Table 1) at every sampling period (significant on Study Days 28/29 and 43/44). Fish in the test group remained completely free of gravid females throughout the trial. This suggests that the test treatment should prove highly effective for sea lice management under field conditions by preventing lice from reproducing during the postmedication period.

The cost of the azamethiphos required for bath treatment of a typical net pen of salmon is approximately $420.00. Results of this trial suggest that approximately 3 bath treatments would be required during the period of

effective louse control provided by a single emamectin benzoate treatment in feed. These 3 bath treatments would, therefore, cost the producer $1260, exclusive of labor costs. The cost of treatment of the same group of fish with the test treatment is estimated to be $1020.

It is possible that the observed weight differences at site 2 are a consequence of effective sea lice treatment. Sea lice may induce osmotic stress, suppress the host immune response, and transmit infectious agents; therefore, a reduction in the number of lice on treated fish could increase energy utilization efficiency (11,12). The significance and long-term effects of this observation require further investigation.

Acknowledgments

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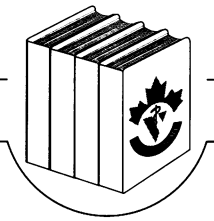
BOOK REVIEW



COMPTE RENDU DE LIVRE

Gotthelf LN. ***Small Animal Ear Diseases: An Illustrated Guide.*** WB Saunders, Philadelphia, 2000, 280 pp, ISBN 0-7216-750-9.

This 15-chapter handbook is a marvellous vehicle for marketing the videographic otoscope. The videoscopic pictures are of excellent quality and certainly give those of us restricted to old-fashioned otoscopic examination a new and wondrous view of the ear canal. Several chapters are devoted to detailed anatomy of the ear canal and methods of examination, including computed tomography and magnetic resonance imaging. Although these advanced techniques provide clear visual images of the canals and bullae, I wonder if they are justified in most circumstances.

The 15 chapters vary in subject matter, from predisposing factors of otitis externa and primary causes of ear disease to marketing of ear care. I found the material offered to be quite repetitive, with many of the chapters covering the same material with only slight variations (predisposing factors, causes, perpetuating factors, ceruminous otitis, and ceruminoliths). Other chapters do offer some variation, with subjects such as inflammatory polyps, allergic otitis, otitis media, and ototoxicity of topical preparations.

While most pathogenic organisms on epithelial surfaces are secondary to upsets in integrity of normal flora, they have rated mere mentions rather than chapters, as in the case of the treatment of chronic recurring *Malassezia* and bacterial otitis. Most practitioners would be appreciative of an easily located reference on these conditions. None of the statements are referenced, but a short list of "suggested readings" ends each chapter. The paucity of good scientific literature on canine ears should be a signal to us all to "get busy." The best feature of this book is the quality and number (> 100) of outstanding photographs captured by the videographic otoscope. The book might better have been titled "A Color Atlas of Small Animal Ear Diseases." I would recommend it for the pictures alone, but I was also captivated by the 11-page formulary of common otic preparations listed by function. Also included were generic and brand names, as well as pharmaceutical suppliers. These 2 features, the pictures and the formulary, are the biggest enticement to purchase the book.

Reviewed by ***Wendy Parker, DVM,*** *Associate Professor, Department of Clinical Sciences, Ontario Veterinary College, Guelph, Ontario N1G 2W1.*