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Further evaluation of the efficacy of emamectin benzoate for treating *Pseudocapillaria tomentosa* (Dujardin 1843) in zebrafish *Danio rerio* (Hamilton 1822)

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Abstract

Pseudocapillaria tomentosa is a pathogenic nematode parasite, causing emaciation and severe inflammatory lesions in the intestines in zebrafish *Danio rerio* (Hamilton 1822). Emamectin benzoate is commercially available analog of ivermectin used for treating salmon for sea lice, under the brand name SLICE®, and we have used this for treating zebrafish with the *P. tomentosa*. Here, SLICE®, 0.2 percent active emamectin benzoate, was used for oral treatments at 0.35 mg emamectin benzoate /kg fish/day for 14 d starting at 7 d post-exposure (dpe). Another experiment entailed initiating treatment during clinical disease (starting at 28 dpe). Early treatment was very effective, but delaying treatment was less so, presumably due to inappetence in clinically-affected fish. We evaluated emamectin benzoate delivered in water, using Lice-Solve™ (Mectinsol) (1.4 % active emamectin benzoate) in two experiments. Application of four 24 h treatments, space over 7 d was initiated at 28 dpe at either 0.168 or 0.56 mg emamectin benzoate/L/bath, and both treatments completely eradicated infections. This was 3 or 10 times manufacture's recommended dose, but was not associated with clinical or histological side effects.

Keywords

zebrafish *Danio rerio*; emamectin benzoate benzoate; *Pseudocapillaria tomentosa*

1. INTRODUCTION

Pseudocapillaria tomentosa (Dujardin 1843) is a pathogenic nematode parasite of zebrafish *Danio rerio* (Hamilton1822), causing emaciation and severe inflammatory lesions in the intestine (Kent, Bishop-Stewart, Matthews, Spitsbergen 2002; Murray & Peterson 2015). The parasite has been observed in 10–15 % of zebrafish research facilities based on data

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

from the NIH Zebrafish International Resource Center (ZIRC) diagnostic service, Eugene, Oregon (Murray 2019). Approaches to control the infection first start with avoidance; - i.e., not introducing infected fish into research facilities. Once the infection occurs, gastrointestinal helminths, including those that infect fish, can be controlled with antihelminthics added to feed. Several of these have been used with considerable success with *P. tomentosa* in zebrafish (Pack, Belak, Boggs, Fishman, & Driever, 1995; Collymore et al., 2014; Maley, Laird, Rinkwitz, Becker, 2013; Samee, 2015).

About 40 years ago the macrocyclic lactone ivermectin was developed and commercialized as a very effective drug for treating parasitic nematodes and arthropods in domestic animals. (Campbell 2021). This was followed by the commercialization of several analogs (e.g., selamectin, milbemycin). Early studies showed some efficacy of ivermectin for treating fish parasites (Davies & Rodger 2000), but it can also be toxic to fish (Johnson et al. 1993; Horsberg 2012). This is likely due to a less robust blood-brain barrier in fish compared to mammals (Høy et al. 1990). Emamectin benzoate has been used against insect and nematode pests in agriculture, and is an appropriate alternative as it penetrates the blood brain barrier in fish less than ivermectin. It effective for treating external arthropod infections in fish, and is commercially available as SLICE® for treating sea lice in salmon farms (Stone et al. 1999; Hosberg, 2012). In our first studies, we found that emamectin benzoate delivered by top coating food with SLICE® was effective for treating experimentally infected fish early in the infection when at 0.25 mg emamectin benzoate/kg fish/day for 10 d was employed (Collymore et al. 2014). In regards to other parasitic nematodes, this compound has also been used for treating the nematode *Aguillicoloides crassus* in the American eel *Aguilla rostrata* by oral gavage (Larrat, Marvin & Lair, 2012). Whereas we observed no toxic side effects in zebrafish, a few *P. tomentosa* worms were still detected following treatment (Collymore et al. 2014). Here we evaluated the efficacy and general toxic effects using a higher dose (0.35 mg active drug/kg fish/day). We have documented the sequential development of worms based on several transmission studies (Kent et al. 2018). Using these data as a guide, we investigated the efficacy of initiating treatments later in the course of the infection.

Macrocyclic lactones are often delivered externally for treating internal parasites (Campbell 2012). Milbemycin, another macrocyclic lactone, has been used unsuccessfully as bath in attempt to treat nematode infections in angelfish *Pterophyllym scarlare* (Lichtenstein) (Killino and Bodri 1997). A commercial product of emamectin benzoate (Lice-Solve™) is available for treating external copepods by adding the product to water. Hence, we investigated the efficacy of this commercial drug for treating *P. tomentosa* using this alternative mode of delivery.

2. METHODS

2.1 Fish

Parasite-free 5D line zebrafish fish were obtained from the Sinnhuber Aquatic Resource Laboratory (SARL), Oregon State University, in which the nematode has never been observed following extensive examinations (Barton et al., 2016). This line of fish was established from outbred zebrafish from a tropical fish wholesale dealer in 2007 from about

10 adults (Kent et al., 2011). Donor fish were from a population of 5D fish in which we are maintaining the infection in our laboratory. Our vivarium contains flow through water, derived from charcoal filtered city water. Temperature was maintained at 27–28 °C, with conductivity at 115 −125 microsiemens, and at pH approximately 7.5. Light in the vivarium is provided for 14 h/day. Oral treatments were prepared by top coating a commercial diet (Adult Zebrafish Complete Diet, Zeigler Brothers, Gardners, PA) with SLICE® (Collymore et al. 2014) and fish were fed control and treated diets at 0.5% body weight/day. All experiments were performed using 2.8 L plastic zebra fish tanks (Aquaneering, San Diego CA). Fish were euthanized by hypothermia by exposure in an ice bath. They were weighed, sex determined, and worms were enumerated by examination of the entire intestine in wet mount preparations, in which the intestine was placed on a glass slide, overlaid with a 20 × 60 mm coverslip, and examined at X 100 or 200 magnification with a compound microscope (Kent et al. 2018). Mature female worms were identified by observation of eggs within the worm.

2.2 Oral High Dose - Early

Fish were exposed to *P. tomentosa* by placing them in an aquarium that previously contained infected fish as described in Collymore et al. (2014) and Kent, Gaulke, Watral, and Sharpton (2018). After exposure, fish were transferred to six tanks, each with 15 fish. Three tanks were designated as untreated fish and three tanks contained fish that were treated with oral emamectin benzoate using the commercial product SLICE® (Intervet Canada Corp., Kirland, Quebec, Canada) at 0.35 mg active drug/kg fish/day for 14 d starting at 7 d post-exposure (dpe). Four tanks (16 fish/tank) of unexposed fish (controls) were also included, in which 2 tanks were treated with the drug as described above. The intestines of half the fish from each tank was examined at either 27 and 32 dpe for exposed fish and 28 and 32 dpe for controls (Table 1). Fish were also weighed and the sex determined at necropsy.

2.3 Oral High Dose – Late.

The intent of this experiment was to determine the efficacy of emamectin benzoate delivered after the infection has progressed to a state when worms are abundant. Fish were experimentally infected by exposing them to 200 eggs/fish as described in Kent et al. (2018). Exposed fish were divided into 6 tanks, with 21 fish/tank. One fish from each exposed tank was examined at 27 dpe to establish a baseline for infections at the time of treatment, and all fish were infected. Then three tanks were treated with medicated diets at 0.35 mg emamectin benzoate/kg fish/d for 14 d starting at 28 dpe.

Two groups of unexposed controls (untreated and treated) were also included, which were also comprised of three replicates/group with 20 fish/tank. Treated and untreated exposed fish, as well as controls, were examined for worm infections at 50 and 58 dpe (Table 2). In this experiment, mortality associated with the infection was observed starting at 12 dpe, and hence fewer treated and non-treated fish were examined compared to controls (Table 2).

2.4 Bath Treatment - Toxicity

We evaluated the toxicity of bath exposure of Lice-Solve™ (Vetpark Professional, Winchester Hants, England) at both 5 and 10 times the manufacture’s recommended dose.

This product contains 1.4 % active emamectin benzoate, and the recommended dose for carp and goldfish infected with external arthropods is 4 mg product/L pond water, equivalent to 0.056 mg emamectin benzoate/L water. Fish were treated at 0.28 mg/L or 0.56 mg/L for four times. First they were treated for 24 h in a static tank, the water flow was then resumed for 1–2 h to flush the tank, and then fish were treated again for 24 h, and then the water flow was resumed. The procedure was repeated 4 days later. Fish were euthanized after 21 days post treatment (i.e., 21 d after initial treatment) and preserved in Dietrich's fixative, processed for histology, and visceral organs and gills were examined for pathological changes using sagittal sections (Spagnoli et al. 2011).

2.5 Bath Treatment - Efficacy

Fish were infected by exposure of 200 eggs/fish as described in Kent et al. (2018). Following the toxicity experiment, we evaluated the efficacy of Lice-Solve™ by treating exposed fish at both 3 and 10 times the manufacture's recommended dose, equivalent to 0.168 or 0.56 mg emamectin benzoate/L, respectively using the four treatments as described above. This was conducted with two separate experiments for each concentration, with very similar design (Table 3).

Each experiment contained a total of eight tanks of exposed fish, four treated and four untreated, and each tank contained 15 fish. Fish were treated by turning off inflow water for 24 h, at 28, 39, 33 and 34 dpe. Presence of worms were evaluated in 5 fish from each tank at immediately before the first treatment, and also at 42 dpe and 56 dpe, which was 14 and 28 d post-treatment, respectively. Three tanks of controls in (untreated and unexposed) were included for each experiment, with 10 fish/tank. These were examined at 28 and 56 dpe as designated for exposed groups. Water flow to tanks, including controls, was turned off during treatment, and all tanks had air stones for oxygenation.

2.6 Statistics

The association between the prevalence of worms, which is measured as the number of individuals that carry at least one worm, and study or individual parameters was measured using logistic regression. Specifically, for each trial, a model was constructed to quantify the association between worm prevalence and drug exposure status; days post exposure, tank, and fish sex:

$$\text{Worm prevalence} = \text{exposure} + \text{dpe} + \text{tank} + \text{sex} \quad (1)$$

Similar models were constructed to measure the prevalence of mature female worms, which is measured as the number of individuals that carry at least one mature female worms. However, in the case of the 2Oral High Dose - Early experiment this regression approach did not return significant associations between mature worm prevalence and study parameters, which we suspected was due to insufficient power for the model to uncover associations given the low rate at which mature worms were observed even in untreated fish. In this case, we used a Pearson's Chi-Squared test to measure the association between mature worm prevalence and drug exposure to improve the power of discovering an association.

Consistent with prior work (Kent et al. 2018) worm abundance, which is measured as the total number of worms observed in an individual, manifested a negative binomial distribution across individuals, wherein few individuals carry most of the worms. Accordingly, negative binomial generalized linear models were used to measure the association between worm abundance (numbers of worms/fish including negative fish within exposed groups) and experimental or individual parameters. Specifically, for each trial, models were constructed to quantify the association between worm abundance, drug exposure status, days post exposure, tank, and fish sex:

$$\text{Worm abundance} = \text{exposure} + \text{dpe} + \text{tank} + \text{sex} \quad (2)$$

Similar models were constructed to measure the corresponding associations for mature female worm abundance, which is the number of mature female worms carried by an individual. However, in this case the High Dose Oral - Early and Bath treatments at the lower dose groups did not manifest patterns of overdispersion and so normal generalized linear regression was used instead to construct these models.

Finally, normal linear regression was used to model the relationship between fish weight, drug exposure status, worm exposure status, sex, or the interaction between these parameters:

$$\text{Weight} = \text{drug exposure} * \text{worm exposure} * \text{sex} \quad (3)$$

For all models, forward and backward stepwise regression optimized the selection of model parameters based on Akaike Information Criterion (Bozdogan, 1987). All statistical analyses were conducted in R (v3.4.1). Generalized linear models were implemented using the glm and glm.nb functions through the MASS library (v7.3–47). Stepwise regression and optimization were implemented through the step function in the stats package (v3.6.0). Chi square analysis was implemented using the chisq.test function in the stats package (v3.6.0) using default parameters. All linear model outputs required $p < 0.05$ to be deemed statistically significant.

3 RESULTS

Using the generalized linear models constructed for each trial, we found that all trials manifested a significant association ($P < 0.05$) and a negative slope between drug exposure and total worm abundance as well as mature worm abundance. There were no tank replicate effects in all trials and comparisons. Sex was occasionally a confounding variable with comparing weights, but had no effect on other analyses (e.g., worm abundance).

3.1 Oral High Dose - Early

Fish were examined at two different time points, about 5 d apart (Table 1). We summarize the results here together as the collection time points were close together and there was no statistical difference between mean worm abundance at these time points within the different

treatment regimens (P = 0.081) (Table 1). Untreated fish showed heavy infections, with an prevalence of overall infection at about 90%, while 22% of the treated fish were infected. Hence, treatment was strongly correlated with reduced prevalence of total worms ($P = 1.82 \times 10^{-8}$). Within the infected fish, untreated fish had a significantly greater abundance of worms (P = 0.0001); a mean abundance between 8 and 12 worms/fish, while untreated fish had far fewer than 1 worm /fish. Likewise, about half the untreated fish were infected with mature females, with slightly more than 1 worm/fish, while none were detected in the treated fish. Hence, treatment significantly reduced mature female worm abundance ($P = 7.97 \times 10^{-7}$) according to normal generalized linear regression and as well as the abundance of female worms both at 28 days ($P = 1.004 \times 10^{-6}$) and 32 days ($P = 6.12 \times 10^{-6}$) according to a Chi-Square test of independence.

Regarding effects on weight, fish exposed to the parasite were on average lighter than unexposed ($P = 6.2\ X10^{-13}$). Within exposed fish groups, untreated fish were significantly lighter than treated fish (about 20%) (P = 0.0380), but there were fewer males in the treated group (P = 0.0400). Drug treatment had no impact on mean fish weight of unexposed fish (P=0.8460).

3.2 Oral High Dose – Late

Treatment of infected fish with oral emamectin benzoate at 0.35 mg drug/kg fish after infections were fully established was less efficacious than initiation of treatment earlier in the course of the infection (Table 2), and both groups of infected fish in this experiment suffered mortality over the course of the experiment. Mortality in treated fish was not significant less than in untreated fish (Chi Square test of independence, P= 0.09821). In the untreated group, 7 of 60 fish died before the first sampling time (50 dpe), with an additional 21 of the remaining 33 fish dying by 58 dpe (Table 2). With the treated group, 2 fish died before 50 dpe, and an additional 15 fish dying between 50 and 58 dpe. Although mortality was similar, combining the two time points, for the two treated and untreated fish, the treated fish showed a lower prevalence of infection than untreated exposed fish ($P = 1.82 \times 10^{-8}$), with about 60 vs over 90% (Table 2). Treated fish also had a lower abundance of worms (P=0.00015). The treatment was also associated with reduced infection with mature female worms (Table 2), with a reduction in mean prevalence (P = 0.005) and treated fish had about a third the abundance of mature female worms compared to untreated fish (P = 0.00472).

3.3 Bath Treatment

Toxicity trials with Lice-Solve™ in which fish were treated at 5 and 10 times the manufacture's recommended dose multiple times did not result in significant morbidity or any mortality. At the higher concentration, the water was turbid and fish appeared stressed as the accumulated near the bottom of the tank and exhibited more rapid respiration during treatment. However, fish appeared normal and resumed feeding after each treatment, and histological examination of all major organs of the fish (e.g., gills, liver, kidney and intestine) did not reveal any lesions consistent with toxicant exposure.

The two experiments evaluating efficacy at 3 and 10 times manufacturer's recommended concentrations showed that the drug was effective at both 0.168 and 0.56 mg emamectin

benzoate/L delivered in four 24 h treatments. With the high dose experiment, the treated and untreated fish had identical prevalence and abundance of infections before treatment was initiated at 28 dpe (Table 3). Then no worms were observed in any of the 40 treated fish at the two later examination times, while the untreated fish had a significantly greater prevalence ($P=1.82 \times 10^{-8}$) and abundance (P = 0.00001) for all worms and mature female worms (P=0.007 and 0.004, respectively).

The second experiment using 3 times the recommended concentration (0.168 mg emamectin benzoate/L) (which was three times the recommended treatment for a single dose) had similar results. Again, immediately before treatment (28 dpe), the treated and untreated fish showed a similar level of infection. However, both groups had less severe infections at this time compared to the previous experiments (Table 1 – 3), with 35 % prevalence. Following treatment, the abundance of worms was statistically different in the treated fish compared to untreated fish (P=0.000456), with complete eradication of the infection in the former (Table 3).

Bath exposures did not appear to effect weight independent of infection. In the bath experiments using 3 or 10 times the recommended concentration, unexposed controls were examined at 28 and 56 dpe, and controls in neither experiment were infected. Exposed fish that were not treated were about 22% lighter at end of the experiment compared to the time that treatment was started, while exposed fish that were treated lost only 7% of their weight, but this was not statistically significant (P = 0.543). Moreover, there was a higher ratio of male fish in the untreated population at the end of the experiment, and males tend to be smaller than females. Hence there was an effect of sex on fish weight ($P =1.11 \times 10^{-5}$), with males being lighter. Drug treatment was only marginally associated with mean weight (P = 0.0785), while exposed fish (combining treated and untreated groups) were significantly lighter than controls (P = 0.018). Although the bath exposure experiments did not include uninfected and treated fish, the weights of treated and exposed fish were similar to untreated controls at the end of the experiment (408 vs 426 mg, respectively), and importantly, their sex ratios were similar.

The second bath experiment (3 times manufacturer's recommendation), had relatively lighter infections than the other trials. While the drug was effective (Table 3), treated and untreated fish had similar weights before and after treatment (P = 0.5211).

4 DISCUSSION

Compared to our previous study (Collymore et al., 2014), increasing the concentration of emamectin benzoate in the diet from 0.25 mg active drug/kg fish feed for 7 d to 0.35 mg active drug/kg fish and extending treatments to 14 d improved efficacy with no apparent side effects. However, in both of these studies, treatment was initiated early in the infections. Worms are most abundant with experimental exposures of zebrafish to *P. tomentosa* at about 3.5 – 6 wk after exposure (Kent et al., 2018). Initiation of oral treatment at this higher dose after the infection had progressed to this high level of infection showed some efficacy, particularly with reducing the numbers of mature female worms. However, the fish mortality rate was about the same with between the treated and untreated groups. Noga (2010)

provides a general review on advantages and disadvantages of different methods for applying chemotherapeutics to fish. Oral drugs (e.g., incorporated in feeds) may have advantages to other methods as the precise dose is controlled and fish do not have to be handled. A disadvantage with treatment by this method is that fish with the most severe infections often do not feed, and hence these individuals often do not receive the appropriate therapeutic dose. This provides a likely explanation for the reduced efficacy in the present study when oral emamectin benzoate was administered after the infection had progressed to a disease state, and severely affected fish that ultimately died probably did not eat adequate amounts of the treated feed.

Bath treatment offers an alternative for treating anorexic fish. Emamectin benzoate, in the commercial formulation SLICE®, was developed as a commercial feed additive to treat *Lepeophtheirus salmonis*, an external copepod infection of salmon (Hosberg, 2012). This drug is a macrocylic lactone, and this class of drug (e.g., ivermectin) has a great ability to be absorbed and distributed in tissues in vertebrates, and hence there are many commercial products used for treating internal helminths in veterinary medicine in which this class of drugs is applied to the external surfaces of the host (Campbell, 2012). This concept lead us to investigate a bath exposure with emamectin benzoate to treat *P. tomentosa*. Killino and Bodri (1997) unsuccessfully attempted to treat nematode infections angelfish *Pterophylllum scalare* with bath delivery of milbimycin at 0.065 or 0.125 ppm. The identity of the intestinal nematodes was not confirmed, but likely was *Neocapillaria pterophylii* (Heinze, 1933) as this is common in freshwater angelfish (Moravec, 2001).

Emamectin benzoate in the form of Lice-Solve™ (also known as mectinsol) is marketed as an external bath treatment with for copepods and *Argulus* spp. infecting koi carp and goldfish in ponds. We therefore used this product in our study. First, we found that fish could tolerate multiple treatments even at ten times the recommended dose for LiceSolve™, although the water became cloudy at this dose and the fish accumulated near the bottom during treatment. Killino and Bodri (1997) observed a similar transient morbidity response in angelfish when exposed to a bath of millbemycin at 0.125 ppm or higher concentrations. Reducing the concentration by about a third in our study eliminated the water quality issues, and still was 100% effective. We are aware of one other report evaluating the toxicity of bath administration of macrocyclic lactones to fish. Ivermectin, which is more toxic to fish than emamectin benzoate when delivered orally, showed moderate toxicity at 1.8 ppm with *Sparus aurata* (Mladineo, Marsic-Lucic & Buzancic, 2006). Whereas often impractical or expensive with extensive aquaculture, administration of drugs in the water may be appropriate with small aquaria such as used in zebrafish facilities.

There was no apparent effect of the Lice-Solve™ on weight. Uninfected treated controls were not included in the bath efficacy studies as toxicopathic effects were addressed in the prelimary toxicity study – i.e., food consumption and behavior in general was not altered following the bath treatment. Moreover, at end of high dose bath experiment fish that were exposed and treated where similar in weight to untreated controls (408 vs 426 mg), and we conclude that any temporary weight loss that may occur from short duration treatment is compensated by clearing infection. Given that the drug was effective at 3 times

recommended dose, future studies should evaluate the efficacy at even lower doses or with fewer baths. Also, other endpoints, such as effects on fecundity, should be considered.

Following our findings here, and our other recent studies (Kent et al., 2018; Kent, Watral, Villegas & Gaulke, 2019), we offer the following recommendations for controlling the infection in zebrafish facilities. First, as with any captive fish, it is important to closely monitor fish, especially moribund fish and fish from facilities without accurate disease history. With *P. tomentosa*, it is important to identify infections early. Whereas very variable, morbidity can be significant within a few weeks of exposure, and based on the appearance of gravid females and egg development, the life cycle can be completed in about a month. Oral treatment will be more effective if initiated early. The concentration of the drug is better controlled with drug delivered in the diet, but fish with active infections and clinical disease often are anorexic. Hence, oral treatment during outbreaks may not target the most severely affected and infected individuals. Bath treatment provides an alternative route of delivery. Along with disinfection methods for *P. tomentosa* eggs in water or surfaces without fish (Kent et al., 2019), the emamectin benzoate bath delivery approach provide yet another method for reducing or eliminating this important parasite in zebrafish facilities. Moreover, these approaches, bath or oral treatment with emamectin benzoate, and killing parasite eggs with various dissinfectants could be extended to other captive fish systems suffering from nematode, or perhaps arthropod, infections.

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Table 1.

Efficacy of high dose oral treatment with emamectin benzoate (SLICE®) for *Pseudocapilliaria tomentosa* administered early in infection. Fish were held in three replicate tanks/group for exposed groups. Four tanks (16 fish/tank) of unexposed fish (controls) were also included, in which 2 tanks were treated with the drug. Approximately half the fish from each tank were examined at each sampling date.

	Untreated Exposed		Treated Exposed		Untreated Control		Treated Control	
DPT† (DPE‡)	20(27)	25(32)	20(27)	25(32)	21(28)	25(32)	21(28)	25(32)
Number	23	22	24	23	16	16	16	16
Prevalence (%)	96	86	22	22	0	0	0	0
Abundance§	12.8	8.4	0.26	0.30	0	0	0	0
Prevalence (%) Mature¶	56.5	54.5	0	0	0	0	0	0
Abundance Mature	1.26	1.41	0	0	0	0	0	0
Weight (mg)	303	291	378	336	459	422	475	428
Male/Female Ratio	13/10	9/12	7/17	9/14	4/12	9/7	6/10	7/9

†DPT = days post treatment

‡DPE = days post exposure

§abundance = worms/fish, including negative fish within the population.

¶adult female worms with eggs

Table 2.

Efficacy of high dose oral treatment with emamectin benzoate (SLICE®) for *Pseudocapilliaria tomentosa* with treatment initiated during active infection. Fish were held in three replicate tanks/group with 20 fish/tank. Approximately half the fish from each tank were examined at each sampling date. Controls represent unexposed fish.

	Untreated Exposed		Treated Exposed		Untreated Control		Treated Control	
DPT[†] (DPE[‡])	22(50)	30(58)	22 (50)	30 (58)	22(50)	31(58)	22(50)	31(58)
Number	20	12	25	15	27	28	27	28
Prevalence (%)	95	92	67	60	0	0	0	0
Abundance[§]	6.45	3.00	2.46	2.20	0	0	0	0
Prevalence (%) Mature [¶]	55	42	21	13	0	0	0	0
Abundance Mature	1.00	0.67	0.33	0.13	0	0	0	0
Weight (mg)	188	196	223	235	354	308	324	334
Male/Female Ratio	9/11	5/7	12/13	4/10	8/19	11/16	10/17	10/18
Mortality (%) [#]	11.7	63.6	3.4	44.1	0	0	0	0

[†]DPT = days post treatment

[‡]DPE = days post exposure

[§]Abundance = worms/fish, including negative fish within the population.

[¶]Adult female worms with eggs

[#]Percent mortality between 0–50 dpe or 51–58 dpe

Table 3.

Bath Treatment.

Efficacy of bath treatment with emamectin benzoate (Lice-Solve™) for *Pseudocapilliaria tomentosa*. Exposed fish were held in four replicate tanks/group with 15 fish/tank. Controls were in held in three replicate tanks, with 10 fish/tank.

	X 10 recommend dose								X 3 recommend dose							
	Untreated exposed			Treated Exposed			Control		Untreated Exposed			Treated Exposed			Control	
DPT[†] (DPE[‡])	0(28)	14(42)	28(56)	0(28)	14(42)	28(56)	0(28)	28(56)	0(28)	14(42)	28(56)	0(28)	14(28)	28(56)	0(28)	28(56)
Number	20	20	20	20	20	20	15	15	20	20	20	20	20	20	15	15
Prevalence (%)	80	80	65	80	0	0	0	0	35	40	45	35	0	0	0	0
Abundance[§]	2.30	1.60	1.95	2.35	0	0	0	0	0.8	0.95	1.05	0.85	0	0	0	0
Prevalence Mature (%)[¶]	35	20	35	20	0	0	0	0	5	20	15	0	0	0	0	0
Abundance Mature	0.40	0.35	0.50	0.30	0	0	0	0	0.10	0.35	0.25	0	0	0	0	0
Weight (mg)	443	399	348	436	447	408	482	426	338	348	335	332	386	339	327	350
M/F ratio	9/11	10/10	13/7	11/9	10/10	9/11	6/9	8/7	7/13	4/16	7/11	8/12	6/14	7/13	6/9	7/8

[†]DPT = days post treatment

[‡]DPE = days post exposure

[§]Abundance = worms/fish, including negative fish within the population.

[¶]= Adult female worms with eggs