Efficacy of some anthelmintics against the swimbladder nematode *Anguillicola crassus* of eel *Anguilla anguilla* under saltwater conditions

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ABSTRACT: Six anthelmintic drugs, Ivermectin, Closantel, Safewormer, Masoten, Oxfendazole and L-Levamisole HCl, were screened for their efficacy against *Anguillicola crassus*, a swimbladder nematode of European eel *Anguilla anguilla*. All experiments were carried out under saltwater conditions. Of the 6 drugs, L-Levamisole HCl used in a bath treatment gave the most promising results. Evaluation of the curative dose of L-Levamisole HCl showed that a long-term treatment (6 d) with 20 mg l⁻¹ was far more effective than a short-term treatment (24 h). Dosages lower than 20 mg l⁻¹ showed a lower efficacy against *Anguillicola crassus*. However, even with a long-term, high-dosage treatment, some recovery of adult nematodes with sublethal damage and/or migration of viable juveniles into the swimbladder lumen could not be excluded. When examined 35 d after treatment, the percentage of mobile adults had increased, while a decrease in percentage of dead worms was noticed compared to values obtained immediately after treatment.

INTRODUCTION

*Anguillicola crassus* Kuwahara, Niimi & Itagaki, 1974 is a swimbladder nematode of Japanese eel *Anguilla japonica*, indigenous to eastern Asia. For some years now, *A. crassus* has been found in European eel *Anguilla anguilla* (Paggi et al. 1982, Van Banning et al. 1985, Molnar 1986, De Charleroy et al. 1987). The nematode spread quickly through the wild eel stock in Europe due to importation of eels from the Far East, restocking of infested eels and natural dispersion (Canestri-Trotti 1987, Belpaire et al. 1989). Wild-caught fingerling eels, used for stocking several commercial eel farms in Europe, were also possibly infected (De Charleroy et al. 1990). Consequently, problems in commercial eel culture arose, and pathological effects induced by the parasite were investigated. Naturally infected eels seemed to show more severe lesions than experimentally infected eels (Haenen et al. 1989). Elevated mortalities of 10 to 20 % and loss of growth in eels by 20 to 30 % were reported (Liewes et al. 1987). Therefore, studies on possible treatments against the nematodes were strongly urged (Ghittino et al. 1989).

Basically, 2 ways of controlling anguillicolosis were proposed. Firstly, prophylactic methods were used, whereby bodies of water are treated to eliminate the intermediate hosts (copepods) so that the life cycle of the parasites cannot be completed. Complete eradication of the copepods seems virtually impossible, however (De Charleroy pers. comm.). Therefore, therapeutic treatment of infested eels with anthelmintics was considered.

Taraschewski et al. (1988) examined the effects of 5 nematocidal drugs on *Anguillicola crassus* adults and preadults, under freshwater conditions: Levamisole HCl, Metrifonate, Febendazole, Mebendazole and Ivermectin. Three different methods of administration were evaluated: force-feeding, bath treatments and intramuscular injections. Levamisole HCl seemed to be the most effective, and bath treatment with 1 mg l⁻¹ per 24 h led to a complete cure. Hartmann (1989) investigated the long-term effects of various dosages of Levamisole on the different developmental stages. Adults and preadults showed considerable loss of vitality for 3 wk after therapy. Eggs and newly hatched larvae showed no reaction to the drug. According to Hartmann (1999), a single treatment with Levamisole under freshwater conditions seemed to be ineffective.
since some nematodes were able to regenerate after suffering sublethal damage. The results mentioned above were all obtained with eels in freshwater. In the present investigation, the efficacy of 6 anthelmintic drugs (including Levamisole) on A. crassus was evaluated under saltwater conditions in a commercial eel farm.

**MATERIALS AND METHODS**

**Parasitized eels.** The experiments were conducted at an eel farm in the Netherlands which used a saltwater recirculation system (salinity 28 to 30 ppt) for stocking and rearing the eels. Naturally infected eels Anguilla anguilla (weight 15 to 30 g) from the IJsselmeer (The Netherlands) were used for the experiments.

Reinfestation of the eel stock did not occur since saltwater is lethal for the larval stages of Anguillicola crassus, and no marine crustaceans are known to function as first intermediate hosts for this nematode (De Charleroy et al. 1989). Since heavily infested eels died and there was no reinfection of theStocked fish, the percentage of infestation of the stock of eels used for experiments declined from 78% in July 1987 to 35% in March 1988. The mean number of adult nematodes per eel during the experiments was 2.85, which represents a rather low level of infection.

**Selection of antiparasitical drugs.** Since no anthelmintics specific for fish were known at the time our research was conducted, anthelmintics developed for veterinary use in farm animals were evaluated. Drugs were selected using the following criteria: the drug should (1) be effective against haematophagous nematodes; (2) attain a substantial level in the blood; (3) be easy to administer and be known to have a low toxicity; (4) be easily resorbed through the digestive tract or the skin; (5) be known to leave no unacceptable residues.

On this basis, 6 drugs were retained (asterisks indicate the names used in this report): (1) Ivermectin (1% solution of Ivermectine*; M.S.D.-Agrivet); (2) Flukiver drench (5% solution of Closantel*; Janssen Pharmaceutica); (3) Safewormer powder (containing a mixture of pyranteltartrate and flubendol; Colombi, Hoevelaken); (4) Masoten powder (Bayer Leverkusen); (5) Syanthic (2.265% solution of Oxendazole*; Syntex International, Animal Health Division); (6) Ripercol (10% solution of L-Levamisole HCl*; Janssen Pharmaceutica).

**Treatment methods.** In most experiments, bath treatments were used. This proved to be the best method, since eels that did not feed could also be treated. In addition, a force-feeding experiment was carried out with Closantel.

Information on use of the drugs for treatment of fishes was minimal. Therefore, each drug was screened first on its toxicity to eels. Next, its efficacy against Anguillicola crassus was investigated. For the force-feeding experiment, the given dosage was based on the prescription for farm animals.

**Experimental conditions.** Since the eels were stocked in a saltwater recirculation system, most treatments were carried out in saltwater (salinity 28 to 30 ppt). Only for the bath treatment with Closantel was freshwater used, since this drug seemed to be insoluble in saltwater. About 1 wk before the experiment with Closantel started, the eels were transferred to freshwater to adapt to the freshwater environment.

For the first set of experiments, on toxicity and efficacy of the drugs (see Table 1), a minimum of 10 eels per treatment was used. They were kept in aerated aquaria containing 8 l saltwater. In addition, aquaria (40 l) with about 30 eels per experimental condition were used in experiments to evaluate the curative dose of L-Levamisole HCl (see Table 2). In both these sets of experiments, the eels were killed and examined immediately after treatment.

In a final experiment, in which long-term effects of L-Levamisole HCl on adult nematodes were evaluated, 2 tanks of 30 m³ were stocked with 334 and 322 kg of eels respectively (mean individual weight = 32 g). One tank was treated on 6 consecutive days with 20 mg l⁻¹ L-Levamisole HCl. The other tank was used as a control. The water in both tanks was drained and changed daily during treatment. Care was taken not to flush water containing L-Levamisole HCl into the recirculation system. In this experiment, 30 eels were investigated from each tank 35 d after finishing the therapy, in order to evaluate the long-term effects of the treatment. During treatment the eels were not fed.

**Parasitological examination.** After all treatments, eels were anaesthetized and killed with an overdose of Hypnodil (20 mg l⁻¹) before the swimbladder was removed for examination. The swimbladder was dissected and examined for Anguillicola crassus. Adults, preadults, larval stage 1 (L1, surrounded with egg membrane) and larval stage 2 (L2, hatched and very mobile, without egg membrane and with a long thin tail) were distinguished. Since we used drugs during the experiments which cause paralysis in nematodes (e.g. L-Levamisole HCl), 3 different states of vitality of adult and preadult A. crassus were distinguished: 'mobile', 'immobile' (worms paralyzed but still intact) and 'dead' (worms disintegrated).

The mobility of Anguillicola crassus adults and preadults was determined as follows: (1) the nematode was put in a drop of freshwater, or (2) the bottom of the petri dish containing the nematode was slightly heated. If the nematode was intact but did not respond to these stimuli by showing wriggling movements, it was consi-
ordered 'immobile'. Occasionally, worms which had disintegrated were found; these were recorded as dead.

The mobility of larval stages 1 and 2 was examined with a light microscope. When there was any doubt about the vitality (especially for stage L1) they were kept in petri dishes with freshwater for 1 to 3 d and examined again. Under these conditions, viable larvae hatch and are very mobile.

RESULTS
Toxicity of drugs to eels

Ivermectin and Closantel, administered by means of a bath treatment, were found to be toxic in low concentrations. For Ivermectin, concentrations between 2 × 10⁻⁴ mg l⁻¹ and 15 × 10⁻³ mg l⁻¹ were tested. Closantel was tested in concentrations between 25 × 10⁻² mg l⁻¹ and 100 mg l⁻¹. Ivermectin induced mortality in 50% of the eels at a concentration of 2 × 10⁻⁴ mg l⁻¹ after 24 h. The LC₅₀ value for Closantel, screened in freshwater, was 2.5 mg l⁻¹ after 24 h. The drench form of Closantel, however, was not toxic when a dose of 10 mg kg⁻¹ body weight was administered orally in the force-feeding experiment. Masoten was found not to be toxic in low concentration (0.5 mg l⁻¹) as a bath treatment. Concentrations above 2.0 mg l⁻¹ should not be used, according to the instructions for farm animals distributed with the drug.

Table 1. Experiments on efficacy of 5 anthelmintic drugs against Anguillicola crassus adults infecting Anguilla anguilla. Concentrations in mg l⁻¹ unless otherwise indicated. BW: Body weight; Inf.: infested; Mob.: mobile nematodes; Imm.: immobile but intact nematodes; Dead: decomposed nematodes; Total: % Imm. + % Dead

<table>
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<th>Drug</th>
<th>Method</th>
<th>Time</th>
<th>Conc.</th>
<th>No. of eels</th>
<th>% Inf.</th>
<th>No. of worms</th>
<th>% Mob.</th>
<th>% Imm.</th>
<th>% Dead</th>
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³ Control for the treatments with Closantel, Masoten, Oxfendazole and Levamisole.
Since L-Levamisole · HCl is easy to administer (bath treatment) and has a very low toxicity for eels (LC₅₀ = 250 mg 1⁻¹, 24 h; Taraschewski et al. 1988), and considering the rather promising results with this product, all further experiments were focused on the use of this drug as a treatment for anguillicolosis.

**Evaluation of curative dose of L-Levamisole · HCl**

Further experiments were set up to determine the optimal curative dose of L-Levamisole · HCl, using bath treatments in a saltwater system.

Firstly, a short-term bath treatment (24 h, 1 administration) with 20 mg 1⁻¹ L-Levamisole · HCl was compared with a long-term treatment (6 d, 6 administrations) (Table 1). The dose of 20 mg 1⁻¹ was selected based on our previous findings which showed that higher doses of L-Levamisole · HCl did not result in higher immobilization of Anguillicola crassus (data not shown). The long-term treatment clearly gave better results than the short-term treatment. When treated with 20 mg 1⁻¹ L-Levamisole · HCl for 6 consecutive days, all eels were free of mobile A. crassus adults. In contrast, 15% of the nematodes were still mobile when only a short-term treatment of 24 h with the same concentration was carried out.

Secondly, long-term bath treatments (6 d) with varying concentrations of L-Levamisole · HCl (2.5, 5.0, 10.0 and 20 mg 1⁻¹) were tested to determine if concentrations lower than 20 mg 1⁻¹ would be sufficient to paralyze the nematodes (Table 2). Treatments of 6 d with concentrations lower than 20 mg 1⁻¹ showed a much lower efficacy against Anguillicola crassus. When treated with 20 mg 1⁻¹ L-Levamisole · HCl, 95% of A. crassus adults were immobile or dead, whereas only up to 78.6% of the adults were immobilized or dead after treatment with lower concentrations.

Unfortunately, the prevalence of preadult and larval stages during the Levamisole experiments was so low that the impact of the drug on these nematode developmental stages could not be determined clearly in this study.

**Long-term effect of L-Levamisole · HCl treatment on Anguillicola crassus adults**

In a final experiment, the long-term effects of L-Levamisole · HCl treatment on adult nematodes were evaluated. Thirty-five days after treatment, 30 eels were examined for viability of Anguillicola crassus adults. Again, L-Levamisole · HCl demonstrated effectiveness: when treated, 85% of the adult A. crassus were paralyzed or dead, compared with only 20% in the control group (Table 3).

**DISCUSSION**

Out of 6 anthelmintics which were tested as possible drugs against Anguillicola crassus under saltwater conditions, L-Levamisole · HCl seemed the most effective. The other drugs were either found to be too toxic for...
eels (Ivermectin and Closantel, bath treatment) or too difficult to administer (Closantel, force-feeding), or had too low an efficacy against A. crassus [Safewormer, Masoten, Oxfendazole (bath treatments), Closantel (force-feeding)]. Taraschewski et al. (1988) also noted the high toxicity of Ivermectin. They used intramuscular injections, but the eels died within 2 wk, whereas the worms survived. These investigators found L-Levamisole HCl to be the most effective drug for treatment of eels in a freshwater environment.

To assess the optimal curative dose of L-Levamisole HCl, it was necessary for us to focus on various dosages and times of exposure. Taraschewski et al. (1988) also tested different dosages and exposure times. Although their results served as a useful basis for further research, caution had to be exercised in drawing conclusions because of the low number of eels used per experimental condition. Our experiments showed that a long-term bath treatment of 6 consecutive days with 20 mg l⁻¹ L-Levamisole HCl was much more effective than a short-term treatment (24 h) with the same concentration. When different dosages were tested in a long-term treatment of 6 d, all dosages lower than 20 mg l⁻¹ showed lower efficacy against Anguillicola crassus. Under saltwater conditions, a bath treatment of 6 consecutive days with 20 mg l⁻¹ seemed to give optimal results. This contradicts results of Taraschewski et al. (1988) for freshwater experiments in which a 24 h bath treatment of 1 mg l⁻¹ led to a complete cure. According to Hartmann (1989), who also conducted his study in freshwater, a higher concentration of L-Levamisole HCl (5 mg l⁻¹, 24 h) worked faster and effected higher short-term nematode mortality than a lower dosage (2 mg l⁻¹, 24 h), which was in accordance with our findings. The use of a high dosage (20 mg l⁻¹), such as in our experiments, will obviously cause high short-term mortality among A. crassus adults. However, one should be cautious not to draw quick conclusions about the optimal curative dose, because the effect of a therapy, checked immediately after treatment, can differ completely when rechecked some weeks later. Hartmann (1989) showed that some of the nematodes are able to regenerate after suffering sublethal damage. He noticed an increase in the number of intact nematodes about 3 wk after treatment with L-Levamisole HCl. This would be due to the recovery of preadults and adults and the migration of surviving juveniles into the lumen of the swimbladder. As indicated by our experiments, long-term treatment (6 d) with a high dosage (20 mg l⁻¹) will most probably have a long and severe impact on the viable nematodes, more nematodes will suffer lethal damage and, subsequently, recovery from sublethal damage will be much lower than after a short-term, low-dosage treatment.

Nonetheless, the percentage of mobile worms found 35 d after treatment was higher than when checked immediately after therapy. These results suggest that, even after long-term high-dosage treatment, a degree of recovery and migration of viable juveniles into the swimbladder lumen cannot be excluded.

Furthermore, the number of dead worms 35 d after treatment was lower than that immediately after treatment. Hartmann (1989) explained this decrease in dead, disintegrated worms as a result of ejection of their remains from the eels through the ductus pneumaticus. This could not, however, be verified in our experiments, since we used commercial tanks with a high density of eels for this experiment.

Our results show that, although a long-term treatment using a high dosage of L-Levamisole HCl seems quite efficacious against adult Anguillicola crassus initially, it will probably be ineffective if not followed by subsequent treatments. In accordance with Hartmann’s recommendation (1989), a second treatment scheduled about 3 wk after the first, and possibly other treatments later at longer time intervals, seem advisable. The advantage of a saltwater system (as in our experiments) over a freshwater system is that no reinfection of the eel stock occurs, since nematode larvae do not survive saline conditions and no suitable intermediate hosts are present.

Although L-Levamisole HCl seems to be a very promising drug in the treatment of anguillicolosis, more data should be gathered about the residues of the drug in the fish flesh, and its effects on consumability of the eels, before it can be used in commercial eel farms on a large scale.

LITERATURE CITED


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