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TREATMENT OF *PSEUDODACTYLOGYRUS* INFESTATIONS OF *ANGUILLA ANGUILLA* I

TRIALS WITH NICLOSAMIDE, TOLTRAZURIL, PHENOLSULFONPHTHALEIN and RAFOXANIDE.

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Introduction

One of the main problems associated with commercial eel-farming in intensive systems is infestations with the gill parasitic monogeneans *Pseudodactylogyrus bini* and *P. anguillae* (Buchmann et al. 1987).

The only drug shown to be effective against pseudodactylogyrosis under practical eel-culture conditions is the benzimidazole mebendazole (Szekély & Molnar 1987, Buchmann & Bjerregaard 1989) and other benzimidazoles are liable to have effect too. However a risk for selection of drug resistant parasites exists when the same type of anthelmintics are used continously. A shift to drugs with other mechanisms of action will counteract this selection (see Anderson & Waller 1985). There is therefore a continuing need to search for other anthelmintics effective against pseudodactylogyrosis.

The present paper reports the results from trials with niclosamide, toltrazuril, phenolsulfonphthalein and rafoxanide. Niclosamide and toltrazuril have been reported by others to be effective against monogenean infections (Schmahl & Taraschewski, 1987 and Schmahl & Mehlhorn, 1988, respectively). Phenolsulfonphthalein is the water soluble piperazine salt of niclosamide reported to be cestocidal (Roberson 1988) and rafoxanide has been widely used to treat infections with *Fasciola, Bunostomum* and *Haemonchus* Roberson 1988).

If drugs are to have practical importance for use in eel-farms based on recycled water, which have a daily water renewal of about 10%, they should be tolerated by eels for an extended period of time. Therefore these trials were conducted with drug exposures of 24 hours.

Material and methods.

Batches of 9 to 11 specimens of pigmented eels *(Anguilla anguilla)*, (body length 9.5 cm to 18.5 cm, body weight 1.0 g to 9.3 g) were used for the experiments. The eels had been fed and infected (infection level before experiments in Table 1) in a commercial eel-culture system (25°C). Eels were exposed at 24-27°C for 24 hours to different drug concentrations and controls were exposed to appropriate solvent concentrations and pure tap water (Tables 2 and 3). Fish were kept in plastic aquaria (17 l volume) containing 7 l aerated solution. After drug exposure for 24 hours, eels were transferred to similar aquaria with clean aerated water which was replaced every 24 hours until eels were examined 3-4 days after treatment. Total number of *Pseudodactylogyrus*-parasites were recorded for each eel in addition to recording of the eel's body length and weight. Prevalence and abundance were calculated according to Margolis et al. (1982) (Table 2). Mortality and behaviour of eels during and after treatment were recorded (Table 3).

Results

From Table 2 it can be seen that niclosamide in a concentration of 1 mg/l eradicated the parasites. Unfortunately eels showed a mortality of 36% in this drug concentration (Table 3). In a concentration of 10 mg/l all eels died within 30 minutes and in 0.1 mg/l parasites survived. These were undamaged and produced viable eggs. Toltrazuril in a concentration of 20 mg/l was lethal for all eels within 18 hours, and 10 mg/l for 12% of the eels within 48 hours. In the latter concentration and in 0.1 mg/l the drug had no effect on the parasites. Both

parasite species survived and eggs obtained from these parasites developed and hatched. Both eels and parasites survived in phenolsulfonphthalein concentrations of 10 and 100 mg/l.

Rafoxanide was lethal for all eels within 3.5 hours in a concentration of 10 mg/l and lethal for 8 of 10 eels within 48 hours in 1 mg/l. One of the two surviving eels was still lightly parasitized in the latter group (Table 2). In 0.1 mg/l rafoxanide all eels survived the drug exposure but eels were still infected (Table 2).

Discussion.

Treatment of pseudodactylogyrosis in eel-farms based on recycled water with a water renewal of 5 - 10% per day results in the drug remaining in the system for many hours. Thus short term treatments are not practical in recycling systems.

Niclosamide was shown to eradicate all *Pseudodactylogyrus*-parasites in a concentration of 1 mg/l during 24 hours of treatment but with a relatively high eel mortality. A concentration of 0.1 mg/l, reported as effective against *Gyrodactylus* by Schmahl & Taraschewski (1987), was not toxic to eels but was without effect on *Pseudodactylogyrus*-parasites. Thus because of the high toxicity this drug should not be recommended as a treatment for pseudodactylogyrosis. Toltrazuril was ineffective in treatment of *Pseudodactylogyrus*-infestations when concentrations of 1 and 10 mg/l were used for 24 hours, as parasites survived and produced viable eggs. In addition eel mortality was observed in drug concentrations of both 20 and 10 mg/l. Thus the recommendation of this drug against *P. bini* by Schmahl & Mehlhorn (1988) was not confirmed. Their recommendation was based on TEM-findings of vacuolizations in the tegument of the parasites after drug exposure. Before drug treatments are recommended, trials with accurate recordings of parasite elimination should be conducted.

Although phenolsulfonphthalein is nontoxic even at concentrations as high as 100 mg/l, it has no parasitocidal effect.

Because of the high toxicity for eels and low effect on *Pseudodactylogyrus* parasites rafoxanide cannot be recommended for treatment of pseudodactylogyrosis. The low abundance in some groups (e.g. toltrazuril 10 mg/l) should not be interpreted as a result of the drug exposure. The mean body length of eels in this group was relatively low (Table 2) and as a positive correlation exists between body size and infection level in the *Pseudodactylogyrus/Anguilla* system (Buchmann 1989) a low abundance should be expected. This relationship was also reflected in this study: in control eels (pure water and toltrazuril solvent) the correlation between body size of eels and parasite number was 0.48 (N=14), in rafoxanide solvent the correlation was 0.55 (N=10) and in phenolsulfonphthalein treated eels (ineffective) the correlation was 0.84 (N=19).

In conclusion mebendazole (Vermox) in a concentration of 1 mg/l is still the preferred treatment of pseudodactylogyrosis in intensive eel culture systems based on recycled water because of its low toxicity to eels and its high parasitocidal effect (Buchmann & Bjerregaard 1989).

Niclosamide in a concentration of 1 mg/l seemed to be the most effective drug in our screening procedures, but due to its high toxicity it should not be used in commercial eel farms. Future screening studies must reveal alternatives to benzimidazoles.

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Summary

In search for alternatives to benzimidazoles (until now the only effective drug against pseudodactylogyrosis in recycled eel-culture systems) a number of drugs with other mechanisms of action were tried. Toltrazuril and phenolsulfonphthalein were without any effect and the former drug was toxic to eels in concentrations of 10 and 1 mg/l and in 0.1 mg/l the parasitocidal effect if any was minimal. Niclosamide was toxic in concentrations of 10 and 1 mg/l. The latter concentration had a clear parasitocidal effect but 0.1 mg/l although untixoc for eels had no effect on the parasites. None of the screened drugs are recommended for the treatment of *Pseudodactylogyrus*-infestations in eels in recycled eel-plants.

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Tabel 1. Data for eels before experiments.

No. of eels examined	14
Body weight	
$\bar{x}$	5.4 g
S.D.	2.7
Body lenght	
$\bar{x}$	14.9 cm
S.D.	2.9
P. anguillae (PA)	
prevalence	100 %
abundance	9.4
S.D.	6.9
P. bini (PB)	
prevalance	64.3 %
abundance	2.7
S.D.	1.3
Postlarvae (PL)	
prevalance	50 %
abundance	1.4
S.D.	0.5
PA+PB+PL	
prevalance	100 %
abundance	11.8
S.D.	8.2

Table 2. Effects of drug on parasitization level. Only survived eels examined.

Drug	Concentration	No. of eels examined	Weight (g) $\bar{x}$/ S.D.	Length (cm) $\bar{x}$/ S.D.	Infection level Prevalance(%)	abundance/S.D
Niclosamide	0.1 mg/l 1.0 mg/l	10 7	4.9/2.8 1.7/0.5	14.8/2.6 11.1/1.0	100 0	13.8/7.9 0/0
Phenolsulfon-phthalein	10.0 mg/l 100.0 mg/l	10 9	4.2/3.2 3.5/1.9	13.6/3.6 13.4/2.2	100 100	12.4/11.5 10.2/ 5.7
Toltrazuril	1.0 mg/l 10.0 mg/l	10 7	3.5/2.5 2.1/0.7	12.7/3.2 11.4/1.31	100 86	7.2/3.6 3.3/2.6
Rafoxanide	0.1 mg/l 1.0 mg/l	10 2	2.7/0.8 3.4/0.5	12.9/1.24 14.3/0.4	90 50	5.8/4.0 0.5/0.7
Control-pure water	-	8	2.8/0.9	12.5/1.9	88	7.9/5.2
Control for toltra-zuril trietthanolamine: macrogol:	240.0 mg/l 0.8 ml/l	6	2.6/1.0	11.9/1.6	100	6.7/4.1
Control for rafox-anide acetone:	0.28 ml/l	10	3.2/1.4	12.9/1.5	100	8.2/5.1

Table 3. Toxicity for eels of the screened drugs.

Drug	Concentra-tion	Mortality 0 - 24 hours	24 - 48 hours	Behaviour
Niclosamide	0.1 mg/l 1.0 mg/l 10.0 mg/l	0 4 of 11 (36%) within 18 hours 10 of 10 (100%) within 30 min.	0 0 -	Normal 3 eels upside down Spasms upside down
Phenolsulfon-phthalein	10.0 mg/l 100.0 mg/l	0 0	0 0	1 with unstable balance 2 with unstable balance
Toltrazuril	1.0 mg/l 10.0 mg/l 20.0 mg/l	0 0 10 of 10 (100%) within 18 hours	0 1 of 8 (12%) -	Normal Sluggish eels, upside down Eels upside down, spasms
Rafoxanide	0.1 mg/l 1.0 mg/l 10.0 mg/l	0 7 of 10 (70%) within 14.5 hours 10 of 10 (100%) within 3.5 hours	0 1 of 10 (10%) -	Normal Sluggish eels Upside down
Control-pure water	-	0	0	Normal
Control for tol-trazuril; triethanolamine macrogol (solvents)	240.0 mg/l 0.8 ml/l	0	0	Normal
Control for ra-foxanide; acetone (solvent)	0.28 ml/l	0	0	Normal