Sonderdruck aus Journal of Applied Ichthyology
Bd. 3 (1987), H. 4, S. 183–186
VERLAG PAUL PAREY · HAMBURG 1 · SPITALERSTRASSE 12

Short Communications

Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary

Mebendazole is an efficacious drug against pseudodactylogyrosis in the European eel *(Anguilla anguilla)*

By C. Székely and K. Molnár

Received: February 11, 1987
Accepted: June 11, 1987

Summary

Our results show that mebendazole is an effective agent for controlling pseudodactylogyrosis. Exposure of the fish to a solution containing 100 mg/l mebendazole for 10 min killed *Pseudodactylogyrus bini* and *P. anguillae;* a mebendazole concentration of 1 mg/l produced the same effect after an exposure of 24 hours. Although the helminths suffered damage immediately after exposure to mebendazole, it took six days before they disappeared from the gills. For short (10 min) exposures, the efficacy of mebendazole was enhanced tenfold by previously exposing the eels *(Anguilla anguilla)* to saline (NaCl).

Zusammenfassung

Mebendazol als wirksames Medikament gegen die Pseudodactylogyrosis des Europäischen Aals (Anguilla anguilla)

Mebendazol erwies sich als wirksam gegen *Pseudodactylogyrus bini* und *P. anguillae*. Das Präparat tötete die Parasiten in folgenden Dosen: 100 mg/l im Kurzzeitbad (10 Minuten) bzw. 1 mg/l im Langzeitbad (24 Stunden). Obwohl die Parasiten durch Mebendazol sofort geschädigt wurden, waren die Kiemen der Aale erst 6 Tage nach der Behandlung parasitenfrei. Ein vorangegangenes Kochsalzbad steigerte die Wirkung des Mebendazol auf das Zehnfache im Kurzzeitbad.

Résumé

Le mebendazole comme médicament efficace contre la pseudodactylogyrose de l'anguille européenne (Anguilla anguilla)

Le mebendazole s'est révélé être efficace pour le contrôle de la pseudodactylogyrose. Un séjour de 10 minutes des poissons dans une solution contenant 100 mg/l de mebendazole a tué les parasites *Pseudo-*

U.S. Copyright Clearance Center Code Statement: 0175–8659/87/0304–0183 $ 02.50/0
J. Appl. Ichthyol. 3 (1987) 183–186

ISSN 0175–8659

dactylogyrus bini, et *P. anguillae;* un bain de 24 heures dans une concentration de mebendazole de 1 mg/l a produit le même effet. Bien que les parasites aient été immédiatement touchés par le mebendazole, cela a duré 6 jours avant qu'ils ne disparaissent des branchies des anguilles. En cas de bain de courte durée (10 minutes) l'efficacité du mebendazole a décuplé lorsque les anguilles *(Anguilla anguilla)* avaient été préalablement baignées dans une solution saline (NaCl).

Pseudodactylogyrus spp., *P. anguillae* (YIN and SPROSTON 1948) and *P. bini* (KIKUCHI 1929), parasitize the gills of different species of the eel *(Anguilla anguilla* L., *A. japonica* Schlegel, *A. australis* Richardson, *A. reinhardti* Steindachner). OGAWA and EGUSA (1976) described a third species, *P. microrchis;* however, according to MOLNÁR (1983) and OGAWA, CHUNG, KON and IMADA (1985), this latter species is synonymous with *P. anguillae*. Earlier, *Pseudodactylogyrus* spp. were known to occur exclusively on Pacific Ocean eels, but OGAWA and EGUSA (1976) and GOLOVIN (1977) found them in Japan and in the Soviet Union, respectively, on *A. anguilla* cultured together with *A. japonica*. MOLNÁR (1983) was the first to report the occurrence of *P. anguillae* and *P. bini* from *A. anguilla* cultured in Europe and living in natural waters. Later LAMBERT, LE BRUN and PARISIELLE (1984) also found *P. anguillae* on *A. anguilla*. IMADA and MUROGA (1979) investigated the therapy of pseudodactylogyrosis of the eel. Organophosphorous compounds were found suitable for controlling this helminth infection, and a 24-hour exposure to a solution containing 0.5 mg/l trichlorfon reduced the number of parasites. In our experiments the trichlorfon treatment recommended by IMADA and MUROGA (1979) proved ineffective, and complaints of the inefficacy of organophosphates against other monogeneans have become more and more frequent. Therefore, the search for a new, effective drug seemed essential. GOVEN, GILBERT and GRATZEK (1980) attributed the inefficacy of organophosphates to the resistance that had developed to these compounds. GOVEN and AMEND (1982) attempted to enhance the efficacy of trichlorfon by administering it in combination with mebendazole. This combination has proven successful against *Dactylogyrus vastator* and *Gyrodactylus elegans*.

In this paper we report our results using mebendazole in controlling gill disease caused by *P. bini* and *P. anguillae*.

The eels infested by *P. bini* and *P. anguillae* used in our aquarium experiments were obtained from a warm-water eel culture farm. In this farm eels are reared in concrete ponds with continuous through flowing water and aeration. The eels were fed on a special floating diet. The eel-rearing farm raises pre-reared eels, obtained from another eel culture, to a marketable size of 200–300 g of body mass. In our medication trials we used eels from a stock showing 100% incidence of pseudodactylogyrosis. Each eel was infested by about 20–30 individual parasites. The ratio of *P. anguillae* to *P. bini* was 2 : 1. Two eels were used in each preliminary experiment; in the final experiments 10 eels were used. Solutions of MEBENVET granulatum® containing 10% mebendazole (methyl-5(6)-benzoyl-2-benzimidazolcarbamate) produced by Chemical Works of Gedeon Richter LTD, Hungary, were used during the experiments. Tests were conducted in aquaria, using 2 or 5 l of test solution on each occasion. The water of the aquarium was replaced with fresh water after a test exposure period had elapsed. Watertemperatures were held between 18 and 20°C. Success of treatment was evaluated by microscopical examination of the fish on day 7 after exposure. Control groups were also examined, comprising the same number of eels as in the treatment trials.

In the preliminary experiments, the pseudodactylogyrus-infested eels were exposed to different concentrations of the chemicals (organophosphate compounds, NaCl, ammonia) traditionally used against monogeneans. These treatments proved to be ineffective.

One method only, the mebendazole/trichlorfon combination (0.4/1.8 mg/l for 24 h) which was earlier applied successfully against the monogeneans of the common carp by GOVEN and AMEND (1982) proved to be effective in our experiment against eel pseudodactylogyrosis.

Table 1

Treatment of eel pseudodactylogyrosis by exposure of fish to different mebendazole concentrations. In the table: number of infested eels remainig positive/total number of eels treated

Mebendazole concentration (mg/l)	Exposure time							
	10 min	20 min	1 h	2 h	4 h	24 h	48 h	$\bar{C}$
0.1						3/6		9/9
0.4						0/2		2/2
0.5						4/10	0/4	11/12
0.8						0/2		2/2
1.0					3/10	1/10	0/5	11/12
2.0					2/10		0/5	11/12
4.0			2/10	0/2	0/10			11/12
10.0		6/10	0/8					10/10
40.0			0/2	0/2				2/2
50.0		7/11						10/10
100.0	0/6	0/11						10/10
200.0	0/6							9/9
500.0	0/7							9/9

$\bar{C}$ – untreated control. Each control eel was infested with 20–25 parasites.

On the basis of this experiment we set out to determine whether the high anti-pseudodactylogyrus efficacy was due to the mebendazole component. A total of 22 groups of eels infested by monogeneans were treated, using solutions of varying mebendazole concentration and different exposure times (100, 200 and 500 mg/l for 10 min; 100 mg/l for 20 min; 10 mg/l for 1 h; 4 mg/l for 4 h; 1 mg/l for 24 h). Exposures of short (10- and 20-min), medium (1- and 4-h) and long (24-h) duration were similarly effective; they produced freedom from parasites (Table 1).

In another experiment, exposure of the eels to saline (NaCl) solution was followed by exposure to a mebendazole-containing solution. The fish first were pretreated in a 5 % (w/v) NaCl solution for 2 or 5 min, then they were transferred to a mebendazole-containing solution (10 mg/l) for a short time (20 min). The saline pretreatment reduced to one-tenth the amount of mebendazole active substance necessary to produce total freedom from parasites (Table 2). In this experiment different control groups were employed: (a) untreated controls: the infection rate remained 100 %; (b) omission of pretreatment (exposure to saline): half of the eels treated with 10 mg/l mebendazole remained infected; (c) the group exposed only to 5 % NaCl solution: no efficacy was noted; (d) a further control group was that in which NaCl (5 %) and mebendazole (100 mg/l) were used simultaneously for 5 min. In this control, about half of the eels remained infested (Table 2).

A further experiment was conducted to determine why the 7-day waiting period, recommended by IMADA and MUROGA (1979), is required for the evaluation of the experiment. *Pseudodactylogyrus* monogeneans died only on days 5 and 6 after treatment in the solutions that had proven to be efficacious earlier.

Table 2

Treatment of eel pseudodactylogyrosis by exposure of fish to 5 % (w/v) NaCl solution, followed by a 20-min bath in mebendazole solutions of different concentration. In the table: number of infested eels remaining positive/total number of eels treated

Mebendazole concentration (mg/l)	Duration of exposure to 5 % (w/v) solution		
	0 min	2 min	5 min
0.0	10/10	2/2	8/10
0.1			5/10
1.0		0/2	3/12
10.0	5/10	0/2	0/12
100.0			5/10*

* Control. Simultaneous exposure to mebendazole and 5 % NaCl.

The results of our experiments indicate that the conventional agents trichlorfon-, NaCl- and ammonia-containing solutions, are ineffective against pseudodactylogyrosis. On the other hand, in our experiment the solution containing 0.4 mg mebendazole and 1.8 mg/l trichlorfon per litre, successfully used by GOVEN and AMEND (1982) against the monogeneans of *Cyprinus carpio*, gave good results in controlling pseudodactylogyrosis when used as a 24-h treatment. This preliminary experiment was performed with the two drugs in combination. However, we presumed from the fact that trichlorfon alone was inefficacious against *Pseudodactylogyrus* parasites, that the parasiticidal effect could be attributed solely to mebendazole. Indeed the efficacy of mebendazole used alone against *Pseudodactylogyrus* was excellent. Depending on the concentration, a short or long exposure can produce a 100% antiparasitic effect. Of the doses applied, 0.4–1.0 mg/l, 4 mg/l and 10 mg/l concentrations gave 100% efficacy when applied in a 24-h, 4-h and 1-h exposure respectively. If the drug was used at a concentration of 100 mg/l, a 10- to 20-min exposure time was sufficient to produce complete freedom from the parasite. AMEND and FENDER (1976) developed a new method whereby antigens were infiltrated into fish during immersion of the animal in hyperosmotic solution containing the antigen. Utilizing this method, the efficacy of mebendazole may be improved considerably by pretreatment in 5% (w/v) NaCl solution; thus, the required quantity of this rather expensive drug can be reduced. The potentiating effect of NaCl can be explained by the fact that there is an osmotic outflow of water from the tissues of the ectoparasite when it is placed in saline solution. When the fish is transferred into a mebendazole-containing solution, the reverse process takes place. There will be an influx of water containing mebendazole through the epidermis. Pre-exposure of the fish to a NaCl solution enhanced the efficacy of mebendazole about tenfold.

Our experiments showed that complete parasiticidal effect of the drug requires six days to manifest itself, irrespective of long or a short exposure time. Since the development of resistance to trichlorfon derivatives which are frequently used in fish farms can be expected, the widespread use of mebendazole exhibit an excellent treatment of *Pseudodactylogyrus* parasites. The substance could be included in the technology of fish culture, primarily in the form of short treatments.

References

AMEND, D.; FENDER, D. C., 1976: Uptake of bovine serum albumin by rainbow trout from hyperosmotic solutions: a model for vaccinating fish. Science, New York. **192,** 793–794.

GOLOVIN, P. P., 1977: Monogeneans of eel during its culture using heated water. pp. 144–150. In: O. A. Skarlato (ed), Research of Monogenea in the USSR. Leningrad, USSR, (in Russian).

GOVEN, A.; AMEND, F., 1982: Mebendazole/trichlorphon combination: A new anthelmintic for removing monogenetic trematodes from fish. J. Fish Biol. **20,** 373–378.

GOVEN, A.; GILBERT, P.; GRATZEK, B., 1980: Apparent drug resistance to the organophosphate dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate by monogenetic trematodes. J. Wildl. Dis. **16,** 343–346.

IMADA, R.; MUROGA, K., 1979: *Pseudodactylogyrus microrchis* (Monogenea) on the gills of cultured eels – III. Experimental control by trichlorphon. Bull. Jap. Soc. Sci. Fish. **45,** 25–29.

LAMBERT, A.; LE BRUN, N.; PARISIELLE, A., 1984: Presence en France de *Pseudodactylogyrus anguillae* (Yin et Sproston, 1984) Gussev, 1965 (Monogenea, Monopisthocotylea). Ann. Parasitol. Hum. Comp. **60,** 91–92.

MOLNÁR, K., 1983: Das Vorkommen von parasitären Hakensaugwürmern bei der Aalaufzucht in Ungarn. Ztschr. Binnenfisch. DDR. **30,** 341–345.

OGAWA, K.; CHUNG, H.; KON, G.; IMADA, R., 1985: On the validity of an eel monogenean *Pseudodactylogyrus microrchis* (Ogawa et Egusa, 1976). Bull. Jap. Soc. Sci. Fish. **51,** 381–385.

OGAWA, K.; EGUSA, S., 1976: Studies on eel pseudodactylogyrosis – I. Morphology and classification of three eel Dactylogyrids with a proposal of a new species, *Pseudodactylogyrus microrchis*. Bull. Jap. Soc. Sci. Fish. **42,** 395–404.

Authors' address: CSABA SZÉKELY and KÁLMÁN MOLNÁR, Veterinary Medical Research, Hungarian Academy of Sciences, P.O.B. 18, H-1581 Budapest