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PSEUDODACTYLOGYROSIS IN *ANGUILLA ANGUILLA* (ACTINOPTERYGII: ANGUILLIFORMES: ANGUILLIDAE): CHANGE OF CONTROL STRATEGIES DUE TO OCCURRENCE OF ANTHELMINTIC RESISTANCE

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Background. The European eel, *Anguilla anguilla* (L.), is considered an endangered species and a series of protective measures have been implemented within the European community in order to enhance natural stocks. Restocking natural habitats with larger eels reared from the glass-eel stage in recirculated farming systems may be one solution. Gill diseases caused by monogenean parasites of the genus *Pseudodactylogyrus* are currently causing morbidity and mortality in these farms and previously applied standard treatments have recently failed. Therefore the applied control methods should be verified and novel solutions proposed.
Materials and methods. Eels infected by *Pseudodactylogyrus anguillae* were obtained from a typical recirculated eel-culture system which had been treated regularly but recently unsuccessfully with benzimidazole anthelmintics. In the laboratory infected eels were subjected to bath treatments with flubendazole (5 or 10 mg · L^{-1}) or praziquantel (5 or 10 mg · L^{-1}) for 24 h at 25ºC and parasite infections were recorded three days post-treatment.
Results. Gill monogeneans, *Pseudodactylogyrus anguillae*, were not controlled by the anthelmintic flubendazole at any of the dosages tested whereas praziquantel showed a significant effect when used as bath (5 and 10 mg · L^{-1}).
Conclusion. The failure of flubendazole for control of pseudodactylogyrosis may result from selection of anthelmintic resistant parasite strains due to use of benzimidazoles for decades. Future treatment regimes during acute outbreaks may be based on praziquantel. A risk for future continued selection for anthelmintic resistance exists and supplementary non-chemical methods (mechanical and biological) in rearing of European eel should be emphasized in the future management practice of eel.

Keywords: European eel, *Anguilla anguilla*, *Pseudodactylogyrus,* anthelmintic resistance, flubendazole, praziquantel

INTRODUCTION

The stocks of European eel, *Anguilla anguilla* (L.), in marine, brackish, and freshwater habitats have experienced a serious decline since the 1970s (Anonymous 2007b). Thus, eel landings in Europe fell from 20 448 t in 1968 to 1685 in 2001. Over-exploitation, habitat destruction, and diseases have been suggested as possible explanations for the problem and a series of measures have been implemented in order to enhance populations of the European eel (Anonymous 2007a). These comprise limitations in capture fisheries and management of the aquatic environment aiming at securing a 40% return of eels to the sea for spawning migration. Disregarding the primary reason for the stock decline it may be suggested that restocking of eels may be one way to improve the situation. Thus, production of juveniles and restocking of appropriate aquatic habitats have been successful measures for other teleost species (Lorenzen 2008) and it is evident that such measures may relieve the pressure from capture fisheries on wild stocks. The technology needed for rearing European eel from the glass eels stage (caught in natural waters) to market size (200–400 g) has been known for decades but the main obstacle remaining is artificial production of glass eels. Therefore the production is based on wild-caught glass eels which often carry a series of infections spreading to the eel-farm environment. Thus both parasitic infections (Køie, 1988, Molnár et al. 1994, Sures et al. 1999, Lefebvre and Crivelli 2004), bacterial diseases (Mellergaard and Dalsgaard 1987, Haenen and Davidse 2001), and viral diseases (Haenen et al. 2002, van Ginneken et al. 2004, Jakob et al. 2009) have caused problems in farms. Pseudodactylogyrosis caused by infections with gill monogeneans of the genus *Pseudodactylogyrus* is among the different diseases which hamper the production of

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young eels for stocking. These parasite infections have been treated with auxiliary substances such as formalin (Buchmann et al. 1987) or anthelmintics such as mebendazole (Buchmann and Bjerregaard 1990b). However, recent failures in the control of acute outbreaks in farmed eel by the use of both mebendazole and flubendazole (unpublished observation by the authors) suggested that anthelmintic resistance had occurred. This situation has been foreseen by Buchmann et al. (1992) following laboratory experiments and by Waller and Buchmann (2001) from field observations. These authors showed that gill parasites from a commercial eel farm, which had been treated regularly for a decade, no longer responded to mebendazole treatments which were shown to be effective during the 1980s. The present study was undertaken to test the effect on gill parasite infections of flubendazole, a benzimidazole drug (chemically related to mebendazole) which has been replacing mebendazole in recent years. A failing action would thereby indicate the occurrence of a possible anthelmintic cross-resistance. In addition a non-related drug, praziquantel, was tested as well in order to find an alternative solution to be applied for control of acute outbreaks.

MATERIALS AND METHODS

Fish. Infected European eels, *Anguilla anguilla* (L.), used for this study were fingerlings with body lengths varying from 18 to 29 cm and body weights within 11–30 g. Eels were obtained from a production system, which has been in continuous action for several years with a low-grade infection of *Pseudodactylogyrus* spp. Eels were brought to the university fish keeping facility and kept in pure aerated tap water at 25ºC until commencement of the experiment.

Parasites. One week before experimentation a subsample of five of these were tested for infection which showed a 100% prevalence and a mean intensity (mean number of parasites per infected host) of 77.6 (±45.21) (Bush et al. 1997). Only *P. anguillae* were found.

Production system. The farm delivering fish produces near 200 t of European eels (live body weight) per year in an indoor facility based on a total water volume of 1200 m^3. Water temperature is kept at 25˚C. The standing stock is 100 t and the stocking density is 15 kg · m^{-2} (for glass eels) and 150 kg · m^{-2} (for larger eels). The facility comprises mechanical filtering using 40-µm nylon mesh, biofilters, trickling filters, sedimentation facility, oxygen cones supplying pure oxygen under pressure, pumps, electronic alarm systems (for low/high water level and low oxygen level), pumping reservoir, and automatic feeders supplying pelleted dry feed. The main function of the biofilter is the oxidation of ammonia to nitrite by *Nitrosomonas* and further on to nitrate by *Nitrospira* and *Nitrobacter* bacteria (Pedersen et al. 2009). However, a microscopic fauna comprising several species of ciliates, rotifers, oligochaetes, free-living nematodes, and turbellarians may colonize the filter and take part in degradation of organic particles (Buchmann 1988).

Anthelmintics tested. Flubendazole for oral use (Flubenol®Vet., Janssen Pharmaceutical N.V. Beerse, Belgium) was prepared in a stock solution (500 mg · L^{-1}) with 1 mL 96% ethanol in 1000 mL deionized water. Praziquantel (Droncit®Vet., Bayer Health GmbH, Leverkusen, Germany) was prepared with 1 mL 96% ethanol as a stock solution of 450 mg · L^{-1} in de-ionized water. From these stocks bath solutions of 5 and 10 mg · L^{-1} were prepared in municipal tap water.

Experimental design. A total of 50 eels were divided into groups of five fish each and two trials were performed (Table 1). Individual eels were placed in plastic aquaria (total tank volume 6 L) containing 1 L water of drug solution (trial 1, December 2010) or containing 2 L solution (January 2011) in a thermostat-controlled room with a temperature of 25.1ºC. These eels were exposed in static bath to a solution of 0 mg · L^{-1} drug (control), praziquantel 5 or 10 mg · L^{-1}, flubendazole 5 or 10 mg · L^{-1}. Following a 24-h exposure period eels were transferred to non-medicated fresh tap water at the same temperature every day until parasite examination at day 3 post-exposure.

Table 1

Trials against *Pseudodactylogyrus* infections in eels using bath treatments for 24 h. Flubendazole and praziquantel were used in concentrations of 5 and 10 mg · L^{-1} at 25.1ºC; Eels were examined 3 days post-exposure; The non-parametric rank sum test Mann–Whitney *U*-test was used for analysis and data considered significantly different when $P < 0.05$

	Anthelmintic	Dose [mg · L^{-1}]	No. of eels	Body length [cm]	Prevalence [%]	Abundance (mean ± SD)	*P*
Trial 1	Control*	0	5	22–29	100	111.8 ±109.9	—
	Praziquantel	5	5	19–27	60	5.2 ± 4.5	<0.05
	Praziquantel	10	5	18–26	60	1.4 ± 1.5	<0.05
	Flubendazole	5	5	22–24	100	47.6 ± 20.2	>0.05
	Flubendazole	10	5	21–26	100	114.8 ± 52.5	>0.05
Trial 2	Control*	0	5	18–29	100	10.0 ± 6.3	—
	Praziquantel	5	5	15–26	0	0	<0.05
	Praziquantel	10	5	15–21	60	1.0 ± 0.5	<0.05
	Flubendazole	5	5	15–23	100	15.0 ± 8.3	>0.05
	Flubendazole	10	5	15–26	100	16.0 ± 7.4	>0.05

*no drug

It was noted if any behavioural changes of eels occurred during the treatment. Thus, balance disturbances, escape reactions, and excessive ventilation movements were recorded.

Parasite-examination. Eels were sacrificed by decapitation. Subsequently, the operculae and gill arches removed to a petri dish containing 5 mL tap water. Gill filaments were cut and all parts of the gills were scrutinized under the dissection microscope (Leica, Germany) (sub-illumination) (7–40× magnification) and the number of parasites counted.

Ethics and legal aspects. The present investigation was performed under the experimental animal license 2006/561-1204 of the committee for animal experimentation, The Danish Ministry of Justice, Copenhagen, Denmark.

Statistics and calculations. The prevalence (percentage of hosts infected) and abundance (mean number of parasites in both infected and uninfected hosts) was calculated according to Bush et al. (1997). Due to the fact that parasite infection data were highly overdispersed and not normally distributed differences of means were tested using a non-parametric rank sum test Mann–Whitney *U*-test. A 5% probability level was applied in all cases.

RESULTS

Bath treatments for 24 h using flubendazole in concentrations of 5 and 10 mg · L^{-1} had no effect on prevalence or abundance on gills of eels (Table 1). When treating a high-grade infection (trial 1) and a low-grade infection (trial 2) no change of prevalence (still 100%) and abundance was found. In contrast praziquantel used similarly had a significant effect both in 5 and 10 mg · L^{-1}. Prevalence decreased to 60% (trial 1) and even 0% (trial 2) and abundances fell to less than 5 parasites per host. However, it was noted that in three out of four trials surviving parasites (although few) were detected (Table 1). No obvious adverse effects on eels were recorded. Both non-medicated control eels and treated eels showed regular ventilation movements of mouth and opercula. No balance disturbances were noticed.

DISCUSSION

The present investigation has shown that a previously effective drug flubendazole (Buchmann and Bjerregaard 1990a) no longer is able to eliminate the infections. Due to our knowledge on how fast anthelmintic selection can occur under controlled subtherapeutical benzimidazole treatments (Buchmann et al. 1992) these results suggest that resistance to flubendazole has arisen in the farmed *Pseudodactylogyrus* population following regular use of flubendazole or the closely related mebendazole drug. Resistance against this latter compound in these gill parasites on farmed eel was previously reported by Waller and Buchmann (2001) and the flubendazole-resistance reported in the present study may be interpreted as cross-resistance of parasites towards closely related benzimidazole drugs. *Pseudodactylogyrus bini* and *P. anguillae* colonize gills of eels and may result in severe infections if left uncontrolled and it is problematic that mebendazole and flubendazole which previously were efficacious (Buchmann and Bjerregaard 1990a, b) now have proved to be without any effect. Praziquantel (10 mg · L^{-1}) can be used for bath treatment as previously suggested (Buchmann et al. 1990) and auxiliary substances such as formalin have been widely applied (Buchmann et al. 1987, Mellergaard and Dalsgaard 1987). The drug praziquantel, which has not yet been used regularly in farms, was in this study shown to possess a high effect and this anthelmintic may be used for future treatment of acute outbreaks. The better cure rate seen in trial 2 may be explained on the fact that the general infection level of eels used was lower compared to trial 1. However, in both trials some parasites were found to survive praziquantel treatment and a risk exists for selection of resistance also against this drug. The surviving parasites may be expected to possess some resistance genes which could accumulate following repeated treatments in farms (Buchmann et al. 1992). Therefore it is recommended to establish alternative management practice based on sustainable principles for future control of this gill parasitosis. Mechanical measures based on water filtration may be a possibility. The sizes of eggs from these monogeneans are around 50–60 × 60–80 μm and the larvae (oncomiracidia) have dimensions of 160–193 × 49–63 μm (Buchmann et al. 1987). Therefore the 40-μm micro-screen in the mechanical filter removes some of these stages and can keeps the infection at a low and tolerable level. In case the filters fail (occasional filter wreckage and screen-damages) infections may build up and more traditional methods need to be applied for control of the acute infections. Also UV-irradiation should be considered due to the lethal effect on waterborne pathogens (Gratzek et al. 1983, Templeton et al. 2005). Biofilters contain a range of faunal elements (turbellarians, copepods, ciliates, oligochaetes) some of which may eliminate eggs and larvae of the parasites (Buchmann 1988) and this suggests that bio-control should be included in future management programmes.

CONCLUSIONS

The problematic situation of the European eel requires a multitude of actions including restriction of fishery efforts, re-establishment of natural habitats and restocking. The on-growing of eels in recirculated farming systems allows 85%–90% of the glass-eels to survive to large pre-adult stages which are suited for restocking purposes. The exact survival rate of glass eels under natural conditions is not known but may be considered to be very low due to predation and disease. Therefore it is reasonable to increase restocking efforts based on fingerlings and older eels following rearing in recirculated farming systems. Optimization of farming systems, including their health status, is therefore crucial. Future farming of Japanese eel *Anguilla japonica* may be based on artificially produced fry (Kagawa et al. 2005) and may be run as pathogen-free systems. However, it may take decades before the European eel can be reproduced artificially and existing farms are based on introduction of wild-caught glass eels

including their pathogens which are able to propagate under farm conditions. Gill monogeneans elicit some of the worst problems and the present report has provided evidence that some control methods (benzimidazole treatments) are no longer effective and that new methods must be applied at an existing farm. Praziquantel treatments may be applied for treatment of acute outbreaks but due to the risk of anthelmintic selection alternatives measures should be introduced. Some of these measures are based on environmentally friendly techniques such as filtration using microscreens, UV-irradiation, and biocontrol.

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