Acta Veterinaria Hungarica 36 (3—4), pp. 239—246 (1988)

EFFICACY OF FUMAGILLIN AGAINST *MYXIDIUM GIARDI* CÉPÉDE, 1906 INFECTION OF THE EUROPEAN EEL *(ANGUILLA ANGUILLA)*: NEW OBSERVATIONS ON MYXIDIOSIS OF IMPORTED GLASS EELS

Cs. Székely, K. Molnár and F. Baska

Veterinary Medical Research Institute of the Hungarian Academy of Sciences, H-1581 Budapest, P. O. Box 18, Hungary

(Received January 18, 1988)

When checked on importation in Hungary, glass eel stages of the European eel *(Anguilla anguilla)* caught at the Atlantic coast proved free from parasitic infection. After a few weeks, however, the plasmodia and cysts of *Myxidium giardi* were demonstrable in both the eels kept in a laboratory and those reared at an eel farm, indicating that the fish had been infected by the parasite latently already upon arrival in Hungary.

To prevent the development of infection, the elvers were fed fumagillin mixed in the feed at a dosage of 0.1% or 1.0%. Continuous feeding of the fumagillin-containing diet prevented the development of spores in the yet asymptomatic fish stocks, and reduced the intensity of infection in eels showing an advanced stage of infection.

Keywords: *Myxidium giardi*, *Anguilla anguilla*, renal parasite, therapy, fumagillin, biology

Until quite recently, no efficacious drug has been known to exist against myxosporeans, parasites that cause significant diseases in fishes. Finally in 1987 Molnár et al. (1987) reported that, when mixed in the feed in a concentration of 0.1%, Fumagillin DCH (CHINOIN Pharmaceutical and Chemical Works Ltd., Budapest, Hungary) prevented the spore formation of the common carp parasite *Sphaerospora renicola*. Earlier fumagillin had only been used against microsporeans, parasites taxonomically distant from myxosporeans. Katznelson and Jamieson (1952) were the first to use this antibiotic against nosema disease of the honey bee. In fish, Kano and Fukui (1982) reported successful use of fumagillin against microsporeosis of the Japanese eel *(Anguilla japonica)* caused by *Pleistophora anguillarum*.

Myxidium giardi infection of the eel is one of the most significant and best known eel parasitoses. Data on its pathology were reported by Copland (1983), Crane and Eversole (1980), Ghittino et al. (1974), Landsberg (1983), and Ventura and Paperna (1984). Many years' experience suggests that in glass eels imported to Hungary *M. giardi* infection appears within a few weeks and gives rise to substantial pathological lesions.

The favourable effect of fumagillin against renal sphaerosporosis of the common carp prompted us to test the efficacy of this antibiotic against eel

myxidiosis. The efficacy of Fumagillin DCH against myxidiosis is reported in this paper, along with observations made on *Myxidium* infection of imported glass eels.

Materials and methods

The experiments were conducted under laboratory conditions, in 401 aquaria, and at a warm-water eel farm in 1987. In the laboratory experiments we used imported glass eels that had not fed in Hungary before, and pigmented eels that had been reared at a Hungarian eel farm for 2–3 months. The glass eels had been caught in river mouths at the Atlantic coast of France. Two batches of eels were used: the first transport arrived in Hungary on 20 February 1987 and the second one month later, on 21 March 1987. Upon arrival the glass eels were just starting to feed. In the adaptation period lasting about 3 weeks prior to starting the laboratory experiment they received cut-up tubifex. Subsequently a gradual transition was made to the medicated or the control diet. The control diet used in the experiments was the special floating eel feed manufactured by Tagger and Co. GmbH (Graz, Austria). This was supplemented with Fumagillin DCH to obtain the medicated diet. The antibiotic fumagillin is produced by the fungus *Aspergillus fumigatus* and manufactured as dicyclohexylamine salt (DCH) by CHINOIN Pharmaceutical and Chemical Works Ltd. (Budapest, Hungary). Its chemical name is 2, 4, 6, 8-decatetraenedioic acid (4-(1,2-epoxy-1,5-dimethyl-4-hexenyl)-5-methoxy-1-oxaspiro (2,5)oct-6-yl) monoester. The eels were assigned to groups and fed a medicated diet containing 0.1 or 1.0% Fumagillin DCH, or the control diet, until killed. The treatment period varied between 19 and 61 days. The eels assigned to the different experimental groups varied in number (Table I).

At the end of the experiment the eels were anaesthetized and dissected. Squash preparations made from the kidney were examined for the presence of *M. giardi* plasmodia and spores by light microscopy, under a coverslip. Although *Myxidium* cysts may occur in the gills and in other organs as well, their number is negligible as compared to that found in the kidneys. On the basis of the spore counts, the intensity of infection was rated as one to three crosses (see Tables I and II), for lack of a more accurate method. Unfortunately, undoubtedly parasite-free eels cannot be used, since this fish species cannot be cultured artificially (only few promising attempts have been made at its artificial culture).

The farm-pond trials were conducted at the eel farm of the BHG on two occasions in 1987. In the first experiment eels from the first transport were fed a diet enabling them to acclimatize, containing tubifex, then tubifex plus fish pulp, for two weeks. Subsequently the eels were assigned to two groups. Eels of the control group received a normal, unmedicated eel diet, whereas

those of the experimental group were fed the medicated diet containing 0.1% Fumagillin DCH four times daily, for 11 days. In the second experiment, lasting 76 days, eels from the second transport batch were used after the adaptation period. The eels were fed the medicated diet four times a day over the first 9 days of the experiment. Subsequently, over the remaining 67 days the fish were fed the medicated diet once and the control one three times a day. The first experiment was evaluated after 11 days, and the second one six times during the 76-day medication period by random sampling. In the field trial each experimental group consisted of eels from the same tank.

Results

Parasitological examination of the glass eels

Upon arrival in Hungary, eels of both transport batches, originating directly from the Atlantic coast, appeared to be parasite-free.

Twenty-five and 38 days after the first importation (i.e. at the beginning of the experiment, after the adaptation period), the untreated eels reared in aquaria proved free from parasites. Forty-seven days after importation, however, *Myxidium* infection became apparent and reached a prevalence of 30%. Fifty-six and 77 days after arrival the prevalence of infection reached 80 and 100%, respectively. The intensity of infection was low (+) at all times studied.

Twenty days after the second importation (i. e. at the beginning of the experiment, after the adaptation period) the untreated eels reared in aquaria already exhibited an infection of low prevalence and intensity. Forty-nine days after importation (i.e. at the end of the experiment) the prevalence of infection was 85% and its intensity was moderate (++).

Medication trials against Myxidium giardi in eels kept under laboratory conditions (Table I)

In the first experiment the eels received the medicated diet for 22 days. One out of the 17 fish fed 0.1% Fumagillin DCH showed infection of low intensity, whereas all 18 fish fed the medicated diet containing 1.0% Fumagillin DCH remained parasite-free. In the control group an infection of low intensity and 30% prevalence (4 out of 12) was found. (In the first experiment latently infected eels of the first transport batch were used.)

The second experiment was evaluated in two parts. On day 19 of medication all 8 eels fed 1.0% Fumagillin DCH were negative, whereas in the control group an infection of low intensity and 80% prevalence (8 out of 10) was demonstrated. All ten treated eels killed on day 40 of the medication trial were

Table I

Efficacy of Fumagillin DCH against the eel parasite *Myxidium giardi* (laboratory experiments performed in 1987)

Origin of the eels	Beginning of the experiment	End of the experiment	Duration of the experiment (days)	Fish treated with Fumagillin DCH		Contro l
				0.1%	1%	
Directly from the 1st transport (20 Feb.)	17 March	8 April	22	1 + / 17	0 / 18	4 + / 12
Directly from the 1st transport (20 Feb.)	30 March	17 April	19	—	0 / 8	8 + / 10
Directly from the 1st transport (20 Feb.)	30 March	8 May	40	—	0 / 10	10 + / 10
Directly from the 2nd transport (21 March)	10 April	8 May	29	—	7 + / 20	17 ++ / 20
1st transport (20 Feb.) (back from farm)*	17 April	22 May	36	—	8 + / 10	8 +++ / 10
1st transport (20 Feb.) (back from farm)*	17 April	3 June	48	—	8 + / 28	10 +++ / 11
2nd transport (21 March) (back from farm)**	25 June	10 August	47	10 ++ / 10	10 + / 10	10 +++ / 10
2nd transport (21 March) (back from farm)**	25 June	3 September	61	10 ++ / 10	10 + / 10	10 +++ / 10

+ = low intensity; ++ = medium intensity; +++ = high intensity; numerator: number of infected fish; denominator: number of fish tested ;*: the eels were reared at the farm from 20 February to 15 April; **: the eels were reared at the farm from 21 March to 23 June

negative, while in the control group the prevalence of infection was 100%. In the positive control fish the intensity of infection was low.

In the third experiment eels of the second transport batch were used. These fish had apparently been infected already at the start of the experiment. After feeding 1.0% Fumagillin DCH for 29 days, 7 out of the 20 treated eels were positive, with low intensity of infection. In the untreated controls both intensity and prevalence (17 out of 20) of infection were higher.

The fourth experiment was also evaluated in two parts. In this experiment eels of the first import batch and returned from the eel farm were used. These fish had been infected already at the beginning of the experiment. The prevalence of infection was between 40 and 50% and its intensity was low.

After day 36 of medication, the kidneys of 8 out of 10 eels fed 1.0% Fumagillin DCH were infected by *M. giardi* spores (the intensity of infection was low). In the control group the intensity of infection was very high and the prevalence was the same as in the treated group (8 out of 10). The remaining eels were examined after day 48 of medication. At that time 8 out of the 28 treated eels were infected (with the low intensity already demonstrable at the start of the experiment). At the same time, 10 out of the 11 control eels tested were infected, with an extremely high intensity.

In the fifth experiment, similarly to the fourth one, eels brought back from the eel farm were used. These eels, however, came from the second import batch. At the beginning of the experiment the eels showed 100% prevalence and low intensity of *M. giardi* infection. The experiment was evaluated in two parts.

By day 47 of medication with 1.0% Fumagillin DCH the intensity of infection had not changed as compared to the initial value, and the spore counts demonstrable in the kidneys already at the start of the experiment were present even at the time of evaluation. In fish fed 0.1% Fumagillin DCH, by day 47 of medication the intensity of infection somewhat increased as compared to the initial level. At the same time, in the control fish the intensity of infection increased enormously, i.e. extremely large numbers of spores were demonstrable in the kidneys. At day 61 of medication the same results were obtained as at day 47 for all groups (0.1% Fumagillin DCH, 1.0% Fumagillin DCH, control).

Field trials with eels infected by M. giardi (Table II)

In the first experiment the medicated feed containing 0.1% Fumagillin DCH was fed four times daily over a period of 11 days. The 10 eels examined were negative, whereas 4 out of the 13 control fish had *M. giardi* infection of low intensity. In this experiment the eels had been negative, or latently infected, already at the start.

In the second experiment, eels from the second import batch were used. These eels had been infected, with low prevalence and intensity, already at the beginning of the experiment. After feeding 0.1% Fumagillin DCH four times a day over a period of 9 days, one out of the 10 treated fish was found to have infection of low intensity. At the same time, 3 out of the 10 control fish were infected, with similar intensity as the treated ones. After day 9, the medicated feed containing 0.1% Fumagillin DCH was fed only once, and the normal, non-medicated eel feed an additional three times, daily. After day 21 of medication one out of the 11 treated eels had *M. giardi* infection of low intensity, whereas 5 out of the 20 fish in the two control tanks had low-intensity infection by *M. giardi* spores in the kidney. At day 36 of medication 2 out of the 15

Table II

Efficacy of a medicated diet containing 0.1% Fumagillin DCH against the eel parasite *Myxidium giardi* (field experiments performed in 1987)

Origin of the eels (transport)	Time of the examination	Duration of the experiment (days)	Group treated with Fumagillin DCH	Control group		
				1	2	3
Experiment 1						
1 (20 February)	21 March	11	0/10	4/13+	—	—
Experiment 2						
2 (21 March)	17 April	9*	1/10+	3/10+	—	—
2	29 April	21	1/11	3/10++	2/10++	—
2	14 May	36	2/15+	7/15+++	—	—
2	27 May	48	10/30+	20/20+++	—	—
2	8 June	62	20/20 15+ 5+++	20/20+++	—	—
2	23 June	76	18/20+++	20/20+++	10/10+++	10/10+++
Body mass at the end of experiment 2 ($\bar{X}$)			0.45 g	0.44 g		

+ = low intensity; ++ = medium intensity; +++ = high intensity; numerator: number of infected fish; denominator: number of fish tested; *: the medicated feed was fed four times daily (for 9 days), and subsequently only once daily

treated eels showed infection of low intensity, whereas in the control group 7 out of 15 fish were infected, with spore counts significantly increased since the previous examination. At day 49 of medication 10 out of the 30 treated fish had infection of low intensity. By that time an infection of 100% prevalence and extremely high intensity had developed in the 20 control eels examined. It should be noted that in that period, in late May, the medicated feed was probably not fed regularly: its quantity did not decrease as quickly as it should have. At day 62 of medication the prevalence of infection reached 100% also in the treated group. However, in most eels (15 out of 20) the intensity of infection was markedly lower than in the control. (Prevalence of infection was 100% in the control group as well). On day 76 of the experiment 18 out of the 20 treated eels were positive, and the intensity of infection had increased. The 50 control eels, originating from three control tanks, showed an infection of 100% prevalence and very high intensity.

In the field trial the effect of fumagillin on the eels' body mass gain was also examined. At the end of the experiment the average body mass gain of the treated and control fish was 0.45 and 0.44 g, respectively.

Discussion

Until now, glass eels caught from river mouths at the Atlantic Coast have been thought to be free from infection by the parasite *M. giardi* (Crane and Eversole, 1980). It was assumed that these eels contracted infection only later, at the eel farm, after they had started to feed. This was supported by negative results of the diagnostic examination of eel transports arriving in Hungary. The present experiments have revealed that glass eels are latently infected by certain, hitherto unknown, developmental stages of *M. giardi* which developed into mature stages even under laboratory rearing conditions (where the eels could not contract infection) after some time. It was found that the glass eels had been infected latently (with 100% prevalence) already at the time of importation. There was a difference between the individual eel specimens in the time of spore formation, presumably depending on the time of their infection at the Atlantic Coast. Eels arriving in Hungary on 20 February developed a less intensive infection over a period of 77 days than those arriving a month later, on 21 March, and kept under the same laboratory conditions, in 48 days. A probable explanation for this fact is that, before having been caught, eels of the second transport had spent a longer time in the river mouths of the Atlantic Coast, and were thus longer exposed to the risk of contracting infection from mature eels living there.

Medication trials conducted in aquaria have shown that, when mixed in the feed in 1.0% concentration, Fumagillin DCH successfully prevented the plasmodium and spore formation of *M. giardi* in the latently infected fish. In experiments in which the eels had shown signs of infection already at the start, medication failed to eliminate from the kidney spores that had been present there earlier. Nevertheless, fumagillin did prevent the formation of new spores. This was confirmed by the rise in the prevalence and intensity of infection in the control group. Fumagillin blocks the development of *M. giardi* and prevents the formation of new spores; however, it has no effect on the already existing ones. It should be pointed out that fumagillin prevents the spore formation of the common carp parasite *Sphaerospora renicola*, another myxosporean, as well (Molnár et al., 1987).

Unfortunately, the first field trial, conducted with latently infected eels of the first import batch, was called off after 11 days for technical reasons. The results of this short experiment are promising (the treated group was negative, and the control showed an infection of 30% prevalence and low intensity). However, from these results no far-reaching conclusions can be drawn.

The first three samplings of the second field trial showed that feeding of 0.1% Fumagillin DCH kept *M. giardi* infection at a lower level, in both prevalence and intensity, than in the control groups. In late May, the farm staff's

failure to feed fumagillin regularly resulted in a rise of infection in the treated group: first the prevalence, then also the intensity of infection reached the values obtained for the control groups. These not fully convincing results may be accounted for by the improper feeding of Fumagillin DCH. It is also possible that a single daily dose of Fumagillin DCH in the feed was not sufficient to control the infection. When the normal diet was offered three times a day and the medicated diet once daily, at 11 a.m., the eels presumably failed to consume sufficient amounts of it because of its slightly unpleasant taste. Unfortunately, we could not extend the experiment to more tanks and, thus, were unable to test the effect of other doses.

We have been unable to determine with certainty whether, besides their latent infection brought from the Atlantic Coast, the eels contract infection at the farm and, if yes, how severe this infection is. We do not know whether the substantial increase observed in the intensity of infection can be explained by the gradual development of latent forms, or by the eels' continuous reinfection at the farm.

We can conclude that fumagillin does not have any adverse effect on the body mass gain. However, in toxicity experiments (to be reported later on) we arrived at the conclusion that at a high concentration (5–10%) Fumagillin DCH exerts a negative effect on body mass gain as compared to the control.

References

Copland, J. W. (1983): The pathology of *Myxidium giardi* Cépéde, 1906 infections in wild and cultured eels, *Anguilla anguilla* L. J. Fish Dis. **6,** 451–460.

Crane, J. S. and Eversole, A. G. (1980): Ectoparasitic fauna of glass eel and elver stages of the American eel *(Anguilla rostrata)*. Proc. World Maricul. Soc. **11,** 275–280.

Ghittino, P., Smith, F. G. and Glenn, J. S. (1974): A case report of Myxosporidia *(Myxidium giardi)* in the dermis of an American eel *(Anguilla rostrata)*. Riv. It. Piscic. Ittiop. **9,** 13–17.

Kano, T. and Fukui, H. (1982): Studies on *Pleistophora* infection in eel *(Anguilla anguilla)* I, Experimental induction of microsporidiosis and fumagillin efficacy. Fish Pathol. **16.** 193–200.

Katznelson, H. and Jamieson, C. A. (1952): Control of Nosema disease of honey bees with fumagillin. Science **115,** 70–71.

Landsberg, J. (1983): Preliminary report on the occurrence of *Myxidium giardi* Cépéde, 1906 in cultured elvers *Anguilla anguilla* L. BAMIDGEH **35,** 18–27.

Molnár, K., Baska, F. and Székely, Cs. (1987): Fumagillin, an efficacious drug against renal sphaerosporosis of the common carp, *Cyprinus carpio*. Dis. Aquat Org. **2,** 187–190.

Ventura, M. T. and Paperna, I. (1984): Histopathology of *Myxidium giardi* Cépéde, 1906 infection in European eel, *Anguilla anguilla* L., in Portugal. Aquaculture **43,** 357–368.