

## NOTE

## Praziquantel (Droncit) is effective against diplostomosis of grasscarp *Ctenopharyngodon idella* and silver carp *Hypophthalmichthys molitrix*

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**ABSTRACT:** In laboratory experiments praziquantel (Droncit) effectively destroyed metacercariae of *Diplostomum spathaceum* (s. l.) parasitizing the eye lens of grasscarp *Ctenopharyngodon idella* and silver carp *Hypophthalmichthys molitrix*. A single treatment with Droncit fed at a dose of 330 mg kg<sup>-1</sup> body mass showed 100% efficacy against metacercariae parasitizing grasscarp. A similar, complete parasitocidal efficacy was achieved when infected grasscarp and silver carp specimens were exposed to a solution containing 1 mg praziquantel l<sup>-1</sup> for at least 90 h. Using shorter exposures (20 min to 1 h) but elevated praziquantel doses (10 to 100 mg l<sup>-1</sup>), some parasites survived but even here the intensity effect reached 67 to 97%.

Diplostomosis, which is one of the commonest fish diseases, causes substantial losses in cultures both of trout (Bylund & Sumari 1981) and of common carp, silver carp and grasscarp (Bauer et al. 1981). It is caused by the cercariae and metacercariae of *Diplostomum spathaceum* (s. l.), which migrate in the body of fish and colonise the eye lens (Molnár 1974).

So far only few researchers have dealt with the problem of medication of *Diplostomum spathaceum* infection. Ramadan (1973) tested 26 chemical compounds, 7 of which proved effective in vitro but not in vivo when used in a medicated diet. Praziquantel (Droncit) proved to be the first effective drug in vivo against *Diplostomum* metacercariae. Bylund & Sumari (1981) were the first to report on its efficacy, testing against diplostomosis of rainbow trout *Salmo gairdneri*. They mixed praziquantel with the feed at a very high dose (330 mg kg<sup>-1</sup> body mass) and fed it for 1 wk or for 1 mo at 4 d intervals. Treatment resulted in damage to and death of metacercariae.

Moser & Sakanari (1986) tested the efficacy of prazi-

quantel against *Diplostomum spathaceum* cercariae developing in snails (*Physa* and *Lymnaea* spp.), using baths containing 5.68 mg praziquantel ml<sup>-1</sup>. Exposures of 12, 24 and 48 h resulted in degeneration and destruction of the cercariae. Heckmann (1987) used praziquantel against *D. spathaceum* metacercariae parasitizing the lens of *Cottus bairdi*, and found it effective both by the intramuscular and intraperitoneal route and as a bath. Zhatkanbayeva & Heckmann (1990) reported on the efficacy of praziquantel, used as baths of different concentrations, against *Diplostomum pusillum* and *Apatemon gracilis*, 2 trematode parasites of *Nemacheilus dorsalis*.

The efficacy testing of praziquantel, applied as medicated diet or as a bath, against diplostomosis of herbivorous fishes (grasscarp, silver carp) is reported in this paper for the first time.

**Materials and methods.** Age-group 1+ grasscarp (average body mass: 16 g, SD: 3.4) and silver carp fry (average body mass: 13 g, SD: 4.6), obtained from a farm pond, were used. The fish showed 100% prevalence of infection by *Diplostomum spathaceum* metacercariae in the eye lens. The mean intensity of infection in grasscarp and silver carp was 85 (SD: 19.5) and 125 (SD: 14.4) metacercariae, respectively. The experimental fish were kept in 50 l aerated aquaria at 20 to 23 °C water temperature. During diet experiments the water was changed 2 to 3 times per week. The experiments were carried out between 8 May and 20 September 1990.

The first series of experiments consisted of 4 efficacy tests of praziquantel added to the diet of grasscarp. Droncit®, a drug containing the isoquinoline pyrazine

derivative praziquantel as active compound, was used for preparing the medicated diet. Droncit® tablets (each containing 50 mg active ingredient) were ground to powder and mixed into the pulverized diet. For each feeding a fresh pellet was prepared from the dry mix by adding a small volume of water. The medicated diet was fed at a dose of 35 to 330 mg kg<sup>-1</sup> body mass d<sup>-1</sup> for different times in the treated groups. The fish were fed by hand and all consumed the feed immediately and uniformly. The experimental groups comprised 3 to 20 individuals (Table 1).

The second series of experiments consisted of 3 efficacy tests in grasscarp and silver carp, using the test compound in a bath. Praziquantel was dissolved in 2 ml ethanol and added to tap water. The treated groups comprised 3 to 12 individuals. Exposure time ranged between 20 min and 168 h and drug concentration of the bathing solution was between 0.05 and 100 mg l<sup>-1</sup> (Table 2). After treatment the fish were returned to tap water.

Treated fish were killed at different times (1 h, 4, 5, 10, or 15 d) after treatment and their eye lenses removed. The lens was first examined by stereomicroscopy for viability of metacercariae, then carefully squashed under a coverslip. Live as well as dead worms were counted in a light microscope.

**Results.** A single feeding of the medicated diet containing 330 mg praziquantel kg<sup>-1</sup> body mass was sufficient to achieve 100% efficacy (Table 1). Only dead metacercariae were found in the lens. Using doses of 35, 50 or 100 mg praziquantel kg<sup>-1</sup> body mass, no complete parasitocidal effect could be achieved even after 3 feedings and in the majority of the fish some metacercariae survived the treatment. However, the intensity effect (IE) of treatment (number of dead parasites after treatment/total number of parasites × 100) reached 93.8, 88.0 and 97.5% respectively even in these cases (Table 1).

When grasscarp and silver carp were bathed in praziquantel solutions of 1 mg l<sup>-1</sup> and 10 mg l<sup>-1</sup> concentration for 24 h and 1 h, respectively, IE values between 68.6 and 93.4% were obtained. The treatment, however, did not reduce the prevalence of infection (Table 2).

An exposure time of 20 min to solutions of 50 mg l<sup>-1</sup> and 100 mg l<sup>-1</sup> concentration showed 66.7 to 96.8% efficacy. Solutions of such high praziquantel concentration were, however, poorly tolerated: the fish turned upside down, showed reduced respiration and motility, and resumed normal behaviour only after transferral to tap water after treatment.

An exposure time of 90 h to baths of 0.5 and 1 mg l<sup>-1</sup>

Table 1. Efficacy of praziquantel given in medicated diet against *Diplostomum spathaceum* metacercariae parasitizing the eye lens of grasscarp fry

| Expt no. | No. of fish | Duration of treatment            | Dose in diet (mg kg <sup>-1</sup> body mass) | Prevalence of infection after treatment | Mortality during treatment | Average no. of larvae per fish | Efficacy against intensity of infection (%) / SD |
|----------|-------------|----------------------------------|--|---|----------------------------|--------------------------------|--|
| I        | 20          | 8 d (daily)                      | 330  | 0/17                                    | 3                          | 87.6                           | 100.0/0.0  |
|          | 20          | 1 mo (at 4 d intervals)          | 330  | 0/8 <sup>a</sup>                        | 2                          | 135.3                          | 100.0/0.0  |
|          | 20          | Control                          | 0  | 17/17                                   | 3                          | 107.8                          | - / -  |
| II       | 11          | 5 d (daily)                      | 330  | 0/10                                    | 1                          | 55.5                           | 100.0/0.0  |
|          | 10          | Twice (on Days 1 and 3)          | 330  | 0/10                                    | 0                          | 72.0                           | 100.0/0.0  |
|          | 15          | 4 times (on Days 1, 4, 8 and 12) | 330  | 0/10 <sup>b</sup>                       | 0                          | 87.4                           | 100.0/0.0  |
|          | 5           | Control                          | 0  | 5/5                                     | 0                          | 79.4                           | - / -  |
| III      | 12          | Once                             | 330  | 0/12                                    | 0                          | 55.6                           | 100.0/0.0  |
|          | 12          | 3 times (on Days 1, 2 and 5)     | 35   | 6/12                                    | 0                          | 68.2                           | 93.8/2.8   |
|          | 7           | Control                          | 0  | 7/7                                     | 0                          | 58.0                           | - / -  |
| IV       | 5           | 3 times (on Days 1, 2 and 3)     | 100  | 4/5                                     | 0                          | 80.6                           | 97.5/2.5   |
|          | 6           | 3 times (on Days 1, 2 and 3)     | 50   | 6/6                                     | 0                          | 72.0                           | 88.0/8.2   |
|          | 3           | Control                          | 0  | 3/3                                     | 0                          | 53.0                           | - / -  |

<sup>a</sup> 10 fish killed later to monitor eye regeneration; <sup>b</sup> 5 fish killed later to monitor eye regeneration

Table 2. Efficacy of praziquantel given as a bath against *Diplostomum spathaceum* metacercariae parasitizing the eye lens of herbivorous fish (grasscarp, silver carp) fry

| Expt no. | No. of fish tested | Fish species | Duration of treatment | Concentration (mg l <sup>-1</sup> ) | Prevalence of infection after treatment <sup>a</sup> | Total no. of larvae | No. of surviving larvae | Efficacy against intensity of infection (%) / SD |
|----------|--------------------|--------------|-----------------------|-------------------------------------|--|---------------------|-------------------------|--|
| I        | 9                  | Grasscarp    | 24 h                  | 1                                   | 8/9  | 787                 | 156                     | 80.2/13.8  |
|          | 6                  | Silver carp  | 24 h                  | 1                                   | 6/6  | 812                 | 255                     | 68.6/5.4   |
|          | 9                  | Grasscarp    | 1 h                   | 10                                  | 9/9  | 498                 | 39                      | 92.2/4.0   |
|          | 8                  | Silver carp  | 1 h                   | 10                                  | 7/8  | 862                 | 53                      | 93.4/5.5   |
|          | 5                  | Grasscarp    | 20 min                | 50                                  | 3/5  | 360                 | 20                      | 94.4 <sup>b</sup> /4.3                           |
|          | 12                 | Silver carp  | 20 min                | 50                                  | 12/12  | 1384                | 44                      | 96.8 <sup>b</sup> /2.0                           |
|          | 4                  | Grasscarp    | 20 min                | 100                                 | 4/4  | 223                 | 55                      | 75.3 <sup>c</sup> /2.2                           |
|          | 8                  | Silver carp  | 20 min                | 100                                 | 8/8  | 1236                | 411                     | 66.7 <sup>c</sup> /10.0                          |
| II       | 4                  | Grasscarp    | 90 h                  | 0.5                                 | 3/4  | 312                 | 4                       | 98.7/0.9   |
|          | 8                  | Silver carp  | 90 h                  | 0.5                                 | 6/8  | 1034                | 16                      | 98.5/1.3   |
|          | 4                  | Grasscarp    | 90 h                  | 1                                   | 0/4  | 326                 | 0                       | 100.0/0.0  |
|          | 8                  | Silver carp  | 90 h                  | 1                                   | 2/8  | 960                 | 2                       | 99.8/0.3   |
| III      | 6                  | Grasscarp    | 168 h                 | 0.05                                | 6/6  | 545                 | 545                     | 0.0/0.0  |
|          | 3                  | Grasscarp    | 168 h                 | 0.1                                 | 3/3  | 186                 | 182                     | 2.2/3.5  |
|          | 3                  | Silver carp  | 168 h                 | 0.1                                 | 3/3  | 360                 | 244                     | 32.2/33.6  |
|          | 8                  | Grasscarp    | 168 h                 | 1                                   | 0/8  | 531                 | 0                       | 100.0/0.0  |
| IV       | 10                 | Silver carp  | – (control)           | 0                                   | 10/10  | 1263                | 1263                    | – / –  |
|          | 10                 | Grasscarp    | – (control)           | 0                                   | 10/10  | 749                 | 749                     | – / –  |

<sup>a</sup> Expt I the fish were examined on post-treatment Day 10 and in Expts II and III immediately after treatment  
<sup>b</sup> At about the 18th minute the fish began to whirl but quickly resumed normal behaviour after having been transferred to clear water  
<sup>c</sup> The fish turned upside down and showed reduced respiration and motility during treatment but recovered strength about 30 min after having been transferred to clear water after treatment

praziquantel concentration resulted in 98.5 and 100% efficacy. Doses reduced to 0.1 and 0.05% gave very poor efficacy (0 to 32.2%) even if an exposure time as long as 168 h was used. In grasscarp exposed to a solution of 1 mg l<sup>-1</sup> praziquantel concentration for 168 h an IE of 100% was achieved.

After treatment the dead, but often also the surviving, metacercariae exhibited striking morphological and functional changes. The cuticle was shrunken and wrinkled, the shape distorted and, in many cases, the tegument had become vacuolated, which was visible by light microscopy. Degenerated but still living metacercariae showed considerably reduced motility as compared to controls.

Metacercariae killed by praziquantel treatment remain in the eye lens for a long time (i.e. become conserved) and disintegrate structurally only 4 to 5 mo later.

**Discussion.** Although praziquantel was reported to be efficacious against the metacercariae of *Diplostomum spathaceum* (Bylund & Sumari 1981, Heckmann 1987), it has not previously been tested for efficacy against diplostomosis in herbivorous fishes (grasscarp, silver carp).

When kept in aquaria without vegetable food, grasscarp accepts granulated food and could easily be accustomed to consuming the medicated diet in our experiments. Praziquantel fed at a dose of 330 mg kg<sup>-1</sup> body mass for 1 wk or 1 mo, in the same way as recommended by Bylund & Sumari (1981) for trout, effectively killed *Diplostomum spathaceum* metacercariae. However, our experiments showed that even a single feeding of the medicated diet containing 330 mg praziquantel kg<sup>-1</sup> is sufficient to kill all *Diplostomum* metacercariae parasitizing the lens of grasscarp.

Medicated diets of lower drug concentrations (35, 50 or 100 mg kg<sup>-1</sup> body mass) and fed 3 times were also effective (IE: 93.8, 88.0 and 97.5%, respectively) but some parasites survived in the treated fish. Thus, these doses fail to give complete efficacy.

As it is hardly possible to accustom silver carp to feedstuffs (as also for grasscarp kept in ponds), praziquantel cannot be safely introduced into these fish by feeding. This prompted us to use the drug in the form of bath against diplostomosis of herbivorous fishes (Table 2).

Of the exposure times and concentrations tested, a quite long (more than 90 h) exposure to a solution

containing 1 mg praziquantel  $l^{-1}$  proved to be the best, as it gave 100 % efficacy. With shorter exposures complete parasitocidal activity could not be achieved but the intensity of infection was markedly reduced. For this a 1 h exposure to a solution of 10 mg  $l^{-1}$  praziquantel concentration seems to be the most suitable. This gives 93 to 94 % efficacy; however, some surviving, in many cases degenerated, metacercariae remain in all fish specimens treated. Exposures to high (50 or 100 mg  $l^{-1}$ ) concentrations of praziquantel are expensive and poorly tolerated by fish. As praziquantel treatment is very costly, we tested baths of very low praziquantel concentration (0.1 and 0.05 %); however, these were ineffective even after an exposure time of 168 h (1 wk).

Although the high price of praziquantel often prevents its use even in human medicine, its use in valuable stocks (spawners, exhibition specimens) is practicable. A 90 to 168 h exposure to baths of 1 mg  $l^{-1}$  praziquantel or a single feeding of medicated diets containing 330 mg praziquantel  $kg^{-1}$  body mass are the recommended routes of application.

As *Diplostomum spathaceum* cercariae continuously infect fish kept in ponds, valuable fish treated with

praziquantel should be transferred to an environment where reinfection cannot occur.

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