North American Journal of Aquaculture 71:30–34, 2009
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DOI: 10.1577/A07-091.1

[Article]

Efficacy of 6-, 12-, and 24-h Praziquantel Bath Treatments against Asian Tapeworms *Bothriocephalus acheilognathi* in Grass Carp

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Abstract.—Praziquantel is an effective anthelmintic treatment that has been used in dogs *Canis lupus familiaris* and cats *Felis catus*. It has also been used successfully against tapeworms and trematodes in fish. This study tested the efficacy of praziquantel bath treatments (6, 12, or 24 h) at various concentrations (0.187, 0.375, 0.75, 1.5, 3.0, 6.0, 9.0, and 12.0 mg of praziquantel/L of water) for controlling natural infestations of Asian tapeworms *Bothriocephalus acheilognathi* in grass carp *Ctenopharyngodon idella* (60–100% of the fish were infested). Treatments either were given as a single application or were applied twice (3 d apart) at fish densities of 60 or 120 g/L. The objective of the study was to find a treatment that would completely eliminate Asian tapeworms from all infested fish, as a number of states now require imported fish to be completely free of this parasite. Most of the praziquantel treatments reduced the percentage containing Asian tapeworms. Single, 24-h bath treatments at praziquantel concentrations of 0.75 mg/L or higher applied to fish held at a density of 60 g/L were the only treatments that completely eliminated Asian tapeworms. Raising the fish density from 60 to 120 g/L affected treatment success; the 24-h, 0.75-mg/L praziquantel treatment at the higher fish density did not completely eliminate Asian tapeworms (prevalence = 15%). A 12-h, 1.5-mg/L praziquantel treatment applied twice to fish held at a density of 60 g/L resulted in low Asian tapeworm prevalence (3.3%).

The Asian tapeworm *Bothriocephalus acheilognathi* is found in the intestines of freshwater fishes (usually cyprinids) throughout the world (Bauer et al. 1969; Bauer and Hoffman 1976; Hoole and Nisan 1994). In the USA, this tapeworm was first found in cultured cyprinids in 1975 and now occurs in both wild and commercial fish stocks in several states (Hoffman 1976; Heckmann et al. 1987; Choudhury et al. 2004). The Asian tapeworm has been reported to affect fish by slowing growth, lowering the ability to withstand harvesting procedures, shortening the life span, and occasionally causing death (Mitchell 2003). A number of states allow importation only of fish that are completely free of Asian tapeworms. These shipment restrictions are of a greater economic impact for commercial fish producers than are the previously mentioned health issues (Mitchell 2004). Few methods have been developed that eliminate Asian tapeworms from infested fish.

Praziquantel is an anthelmintic drug that reduces Asian tapeworm numbers in fish when injected, fed (naturally or forced), or used as a water bath treatment (Andrews and Riley 1982; Moser et al. 1986; Lewbart and Gratzek 1990; Rogstad et al. 1993; Flores-Crespo et al. 1994; Mitchell 2004). These methods of administration will usually remove enough invading Asian tapeworms to alleviate problems that affect the health of the fish. However, if Asian tapeworm eradication is the goal, then every fish must be effectively treated with the medication and every Asian tapeworm must be eliminated; this is a difficult goal to attain. Some fish may refuse to consume anthelmintic-medicated feeds, resulting in the continued presence of infested fish within a treated population. Injection and force-feeding methods target all fish in the treated population but are labor intensive and are impractical for treating large groups of fish. Application of a bath treatment is not labor intensive; bath treatments ensure that all fish are medicated, and these treatments potentially eliminate all Asian tapeworms in the treated population.

Mitchell (2004) showed that bath treatments with praziquantel greatly reduced the number and prevalence of Asian tapeworms in grass carp *Ctenopharyngodon idella*. Praziquantel treatments in 24-h exposures were tested, and only 1.5 mg praziquantel/L of water at a low fish density (6 g/L) completely eradicated Asian tapeworms from fish. Survival of a few parasites occurred when an otherwise similar treatment was given at a fish density of 60 g/L. Therefore, fish density may affect the efficacy of praziquantel treatments. Asian tapeworms were also eradicated from endangered bonytails *Gila elegans* that were subjected to a 1.5-mg/L praziquantel treatment for

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Received October 31, 2007; accepted March 13, 2008
Published online February 5, 2009

24 h (Ward 2007). Fish in these experiments were held in static water under crowded conditions for 24 h; this exposure period was stressful, partially due to ammonia accumulation in the water and low dissolved oxygen. Biologists that use such treatments, particularly on endangered fish species, are concerned about fish loss due to treatment and therefore desire a shorter exposure period.

The objective of this study was to determine praziquantel bath concentrations that would completely eliminate Asian tapeworms from grass carp at various exposure periods (6, 12, and 24 h) and to further investigate the effect of fish density on the success of praziquantel bath treatments.

Methods

Six praziquantel bath tests were performed during April–June 2007 at the Harry K. Dupree Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas. Grass carp (average weight = 41.6 g) that were naturally infested with Asian tapeworms (prevalence [percentage of fish that contained the parasite] = 60–100%) were obtained from an infested fish lot. The fish were exposed to single, static-water treatments with praziquantel at 0.187, 0.375, 0.75, 1.5, or 3.0 mg/L for 24 h; 0.75, 1.5, 6.0, or 9.0 mg/L for 12 h; and 3.0, 6.0, 9.0, or 12.0 mg/L for 6 h (Table 1). These treatments were applied at a fish density of 60 or 120 g/L. In addition to single treatments, two 12-h praziquantel applications separated by a 3-d interval were tested at the 0.75- and 1.5-mg/L concentrations (Table 1). The treatment concentrations were based on previously published and unpublished studies (Mitchell 2004; A.M., unpublished data). Treatments in each test had three or four tank replications (Table 1). All treatments were carried out in 22-L containers with aerated static water. The amount of water in the containers was dependent on the average weight of fish and the fish stocking density (usually 60 g/L) designated for the test. A stock solution of praziquantel (100% powder formulation; Sigma Chemical Co., St. Louis, Missouri) was prepared at a concentration of 12.5 mg/mL in 70% ethanol; aliquots were added to the test water to attain the target chemical concentration. After the exposure period, fish were immediately transferred to 96-L fiberglass tanks containing 70 L of aerated, flow-through well water. At 3 d posttreatment, all fish were tested for the presence of Asian tapeworms by the squash-plate technique (Mitchell 1989, 2003).

Water quality variables were measured before all treatments. Chlorides (45–60 mg/L) and total ammonia nitrogen (TAN; <0.1 mg/L) were measured with a Hach Model FF-1A test kit (Hach Co., Loveland, Colorado). Total hardness (91.0–94.2 mg/L) and total alkalinity (206–211 mg/L) were measured by use of a standard titration method (APHA et al. 2005). Temperature (22.8–23.5°C) and pH (7.57–7.68) were measured with a Werkstätten pH/Oxi 340i/SET meter (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). During the treatment and posttreatment phases, temperature (18.7–23.5°C) was continually recorded with HOBO Water Temp Pro v2 recorders (Onset Computer Corporation, Bourne, Massachusetts). Dissolved oxygen, pH, and TAN readings were also recorded at the conclusion of treatments.

Using the MINITAB program (MINITAB 2000), Asian tapeworm prevalence data were arcsine-transformed and subjected to one-way analysis of variance (ANOVA; Zar 1984; Sokal and Rohlf 1995); differences among treatment means were determined by use of Tukey's test (1953). Treatment effects were considered significant at *P*-values less than 0.05.

TABLE 1.—Summary of praziquantel bath treatments evaluated for efficacy in controlling Asian tapeworm infestations in grass carp at the Harry K. Dupree Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas, during April–June 2007. Praziquantel concentration, bath duration, fish density, and number of replicate tanks per treatment are shown.

Test number	Praziquantel concentration (mg/L)	Bath duration (h)	Fish density (g/L)	Tank replicates
1	0, 3, 6, 9, 12	6	60	4
2	0, 0.75, 0.75 (×2),[a] 1.5, 1.5 (×2)[a]	12	60	3
3	0, 6, 9	12	60	3
4	0, 1.5, 3.0	24	60	4
5	0, 0.187, 0.375, 0.75, 1.5	24	60	4
6[b]	0, 0.75	24	120	3
	0.75	24	60	3

[a] Treatment was applied twice (3 d between applications).
[b] Two fish densities were evaluated in test 6.

Results

Most of the praziquantel treatments significantly reduced Asian tapeworm prevalence in grass carp (Tables 2–4). Single, 24-h praziquantel treatments of 0.75 mg/L and higher at a fish density of 60 g/L completely eliminated Asian tapeworms from all infested grass carp; two single-treatment tests were done at 0.75 and 1.5 mg/L, for a total of 8 replicates/treatment. A single, 24-h, 0.75-mg/L treatment applied at a fish density of 120 g/L resulted in a prevalence of 15% (6 of 40 fish). Twice-applied 12-h treatments (separated by 3 d) of 1.5 mg/L at a 60-g/L fish density resulted in a prevalence of 3.3% (1 of 30 fish). No fish died in any of the treatments. At the end of treatments,

TABLE 2.—Asian tapeworm prevalence in grass carp (percentage of fish containing the parasite) that received 6-h bath treatments (fish density = 60 g/L) with praziquantel at four concentrations. Each treatment had four replicate tanks (10 fish/tank). Means followed by different letters are significantly different (Tukey's test: $P < 0.05$).

Praziquantel concentration (mg/L)	Prevalence per replicate (%)	Mean prevalence (%)
0[a]	57,[b] 80, 70, 70	69.2 z
3	20, 0, 40, 0	15 y
6	10, 20, 10, 10	12.5 y
9	10, 20, 0, 30	15 y
12	10, 10, 0, 20	10 y

[a] Control.
[b] Three fish jumped out of this tank.

pH was 7.6–8.7 and dissolved oxygen was 4.2–8.7 mg/L; TAN was 0.7–1.3 mg/L at the end of the 6-h treatment, 1.0–3.4 mg/L at the end of the 12-h treatment, and 4.0–13.0 mg/L at the end of the 24-h treatment.

Discussion

To eliminate Asian tapeworms from grass carp, it was necessary to apply a single, 24-h bath treatment of praziquantel at 0.75 mg/L or higher for a fish density of 60 g/L. The 60-g/L fish density (~0.5 lb/gal) is a high stocking rate for grass carp in holding tanks. For 24-h exposures to concentrations less than 0.75 mg/L at a fish density of 60 g/L and for all 6- and 12-h exposures regardless of concentration, Asian tapeworm numbers were reduced but the parasites were not completely eliminated. These treatments would be unacceptable if the goal is to transport fish to states that prohibit the importation of Asian tapeworm-infested individuals. All single applications of praziquantel at shorter exposures (including 12 mg/L for 6 h and 9 mg/L for 12 h; both at 60-g/L fish density) resulted in at least a 10% prevalence of Asian tapeworms. The high praziquantel concentrations used in the 6- and 12-h treatments were more than 10 times the concentrations that were effective in the 24-h treatments; testing even higher concentrations of the chemical was not considered practical. The use of two 12-h applications of 1.5-mg/L praziquantel at a 60-g/L fish density is a promising approach, because only two medium-sized Asian tapeworms were found in 1 of 30 fish (prevalence = 3.3%); the parasites were found in the posterior region of the intestine and probably would have been passed in another 24 h. Normally, healthy Asian tapeworms attach in the anterior third of the fish's intestine. No further testing was done on this treatment group, because insufficient numbers of infested fish remained.

TABLE 3.—Asian tapeworm prevalence in grass carp (percentage of fish containing the parasite) that received 12-h bath treatments (fish density = 60 g/L) with praziquantel at four concentrations. Each treatment had three replicate tanks (10 fish/tank). Within test 2 or 3, means followed by different letters are significantly different (Tukey's test: $P < 0.05$).

Test number	Praziquantel concentration (mg/L)	Prevalence per replicate (%)	Mean prevalence (%)
2	0[a]	100, 100, 100	100 z
	0.75	80, 90, 90	86.7 y
	0.75 (×2)[b]	30, 20, 20	23.3 x
	1.5	80, 50, 50	60 y
	1.5 (×2)[b]	0, 0, 10	3.3 w
3	0[a]	50, 80, 50	60 z
	6	20, 50, 40	36.7 z
	9	40, 30, 30	33.3 z

[a] Control.
[b] Treatment was applied twice (3 d between applications).

TABLE 4.—Asian tapeworm prevalence in grass carp (percentage of fish containing the parasite) that received 24-h bath treatments (fish density = 60 or 120 g/L) with praziquantel at five concentrations. Each treatment had four replicate tanks (10 fish/tank). Within a test, means followed by different letters are significantly different (Tukey's test: $P < 0.05$).

Test number	Praziquantel concentration (mg/L)	Fish density (g/L)	Prevalence per replicate (%)	Mean prevalence (%)
4	0[a]	60	100, 100, 90, 100	97.5 z
	1.5	60	0, 0, 0, 0	0 y
	3	60	0, 0, 0, 0	0 y
5	0[a]	60	100, 100, 100, 100	100 z
	0.187	60	40, 40, 10, 40	32.5 y
	0.375	60	10, 10, 0, 10	7.5 x
	0.75	60	0, 0, 0, 0	0 w
	1.5	60	0, 0, 0, 0	0 w
6	0[a]	60	70, 60, 70, 90	72.5 z
	0.75	60	0, 0, 0, 0	0 y
	0.75	120	30, 10, 0, 20	15 y

[a] Control.

The 24-h, 0.75-mg/L praziquantel treatment (fish density = 60 g/L) is not only effective but should be safe for fish that, like grass carp, can withstand a temporary rise in TAN up to 13 mg/L (un-ionized ammonia up to 1 mg/L) and possibly a decline in dissolved oxygen to about 4 mg/L by the end of the treatment period. In the 24-h exposures, only four tanks had dissolved oxygen readings of less than 6.2 mg/L (i.e., 4.2, 5.6, 5.8, and 5.9 mg/L); all fish survived and none showed obvious sign of stress. If necessary, additional air stones could be added. For grass carp, the highest 24-h nonlethal concentration of praziquantel is 60 mg/L (Mitchell and Hobbs 2007); this is 80 times higher than the 0.75-mg/L concentration.

Contrary to the results presented here, Mitchell (2004) indicated that praziquantel treatments (single,

24-h exposures of 0.7 and 1.5 mg/L at a fish density of 60 g/L) did not eliminate all Asian tapeworms. However, after the completion of that study, it was discovered that the praziquantel powder was not properly stored (the chemical was stored in a cabinet at room temperature instead of being held at −20°C); this probably resulted in testing of a weakened product. The 24-h treatment results from the present study (using properly stored praziquantel) demonstrate that a 24-h exposure to praziquantel at a concentration as low as 0.75 mg/L for a fish density of up to 60 g/L effectively eliminates Asian tapeworms from grass carp. Thus, it is important to use only properly stored praziquantel when treating infested fish.

Higher fish densities appear to lessen the efficacy of praziquantel treatments (e.g., 0.75 mg/L for 24 h). In the present study, raising the fish density from 60 to 120 g/L (from 0.5 to 1.0 lb/gal) increased the Asian tapeworm prevalence from 0% to 15%. A similar situation was noted by Mitchell (2004). Asian tapeworms in grass carp treated with a single, 24-h application of 1.5-mg/L praziquantel at a fish density of 6 g/L were completely eliminated, but when the same treatment was given to the same group of infested fish held at a density of 60 g/L, the prevalence of the parasite was 13.3% (4 of 30 fish). Even though improper storage probably weakened the efficacy of praziquantel used in that study, the effect of fish density was still evident.

In fisheries, parasite and disease treatments are often given in terms of chemical concentration and exposure period. Praziquantel bath treatments should be defined by at least the chemical concentration, exposure period, number of exposures, and fish density. Only if these four components are defined can effective treatments be applied. Three other factors are also important when considering the use of these bath treatments. First, some anecdotal evidence suggests that the concentration of Asian tapeworms in the intestinal tracts of fish affects the efficacy of treatments (more parasites = less-effective treatment). Our tests were done with fish that individually contained high numbers of Asian tapeworms, and the parasite's prevalence among these fish was also high. Prevalence of Asian tapeworms in control fish in the two tests with the best praziquantel treatment (a single, 24-h application of 0.75-mg/L praziquantel at a fish density of 60 g/L) was 86.25% (69 of 80 fish); 32 of the 80 control fish (40%) were considered to have massive infestations (clearly visible, white to yellow expanded area in at least the anterior intestine). We are confident that the bath treatments that eliminated Asian tapeworms in this study will eliminate these parasites from most, if not all, infested grass carp. Second, it is important to use only properly stored praziquantel (storage according to the label); freezing is required for at least some of the powdered formulations. Third, results should not be assessed until 3 d posttreatment, as earlier assessments will give false positives. Based on one of our tests (a twice-applied, 12-h, 1.5-mg/L praziquantel treatment given at a fish density of 60 g/L), it is possible that 4 d posttreatment is better than 3 d; however, we seldom observed dying Asian tapeworms after 3 d. From our observations, it takes 2 d for most of the treated parasites to die and then 1 d for their elimination from the intestines.

Although some agencies have recommended the use of praziquantel on fish to be shipped into their states, readers should note that praziquantel is not approved by the U.S. Food and Drug Administration for use as a therapeutant on fishes. At the present time, the use of praziquantel against Asian tapeworms in grass carp can only be authorized if the fish producer has established a client relationship with a veterinarian and if an extra-labeled prescription is given by that veterinarian. The fish cannot be consumed as food after treatment with this chemical.

Acknowledgments

The authors acknowledge Matthew Barnett and Bradley Farmer for their superior and dedicated technical assistance throughout the course of the study. Mention of trade names of commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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