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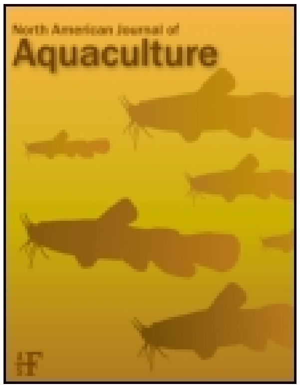

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Novel Praziquantel Treatment Regime for Controlling Asian Tapeworm Infections in Pond-Reared Fish

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ARTICLE

Novel Praziquantel Treatment Regime for Controlling Asian Tapeworm Infections in Pond-Reared Fish

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Abstract

The Asian tapeworm *Bothriocephalus achelognathii* is an intestinal fish parasite that is nonnative to but widespread throughout the southwestern United States. Praziquantel is an anthelminthic drug commonly used to treat fish for Asian tapeworm; however, it does not kill tapeworm eggs, so the water in ponds used for fish rearing must be exchanged after treatment. Our objective was to determine whether a system containing both an intermediate copepod host and a definitive fish host for Asian tapeworm could be treated without exchanging the water by using a follow-up treatment for any tapeworms that developed from eggs released before or during the first treatment. Here, we have described a new praziquantel treatment regimen to control Asian tapeworm infections in freshwater-reared fish. To evaluate the efficacy of this regimen, we stocked 50 red shiners *Cyprinella lutrensis* and an intermediate copepod host, *Cyclops vernalis*, into each of six pond mesocosms containing artificial macrophytes, sand, and gravel to simulate natural pools and provide suitable substrate for the copepod's life history. The test fish population had been naturally infected with *B. achelognathii* and had an initial infection prevalence of 14% and an infection intensity of 2.14 ± 2.19 (mean ± SD) worms per fish. Three mesocosms were treated twice, each with 2.5 mg/L praziquantel; 19 d passed between treatments to allow for possible reinfection to occur. After a 2.5-month posttreatment period to allow any remaining tapeworms to reestablish themselves, we killed and dissected all of the remaining fish. No worms were found in treated fish; however, the control group had an infection prevalence of 18 ± 6% and an infection intensity of 3.45 ± 2.1 worms per fish. Based on these results, we concluded that the praziquantel treatment regime administered was efficacious and suggest testing it on a larger scale. We caution that praziquantel has not been approved by the U.S. Food and Drug Administration for use on fish but can be used legally in some situations.

The Asian tapeworm *Bothriocephalus achelognathii* is an intestinal fish parasite that is nonnative but widespread throughout the southwestern United States (Archdeacon et al. 2010). Asian tapeworm is responsible for reduced survival, growth, condition, and fecundity in its definitive fish hosts (Scott and Grizzle 1979; Granath and Esch 1983b; Hoole and Nisan 1994; Brouder 1999; Hansen et al. 2006) and has been linked to mass mortalities in newly infected populations (Bauer 1969). Cyprinids are the fish taxon most susceptible to infection by Asian tapeworm and the largest group of threatened and endangered fishes in the southwestern United States (Clarkson et al. 1997). Furthermore, reproduction and development of Asian tapeworm are maximized in warm waters (Granath and Esch 1983c), which are typical of the small streams and ponds where most southwestern native cyprinid fishes are found.

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Praziquantel (Droncit, Bayer, Pittsburgh, Pennsylvania) has been used for decades to treat captive fish for Asian tapeworm. Praziquantel was developed as an anthelminthic treatment for trematodes and tapeworms in humans and animals (Andrews et al. 1983). Praziquantel kills a wide range of helminth parasites, but fish, amphibians, reptiles, birds, and mammals tolerate it well (Andrews and Riley 1982). Most recent studies of the effectiveness of praziquantel for fish involved adding it directly to the water (Mitchell 2004; Ward 2007; Mitchell and Darwish 2009). These simple bath treatments are effective, expose all fish to the treatment, and avoid handling stress. With adequate dosage and duration of treatment, 100% of Asian tapeworms can be eliminated from captive fish in a safe manner (Mitchell 2004; Ward 2007; Mitchell and Darwish 2009). In fact, many state agricultural and food departments have mandatory tapeworm eradication procedures that use praziquantel for the intra- and interstate movement of live fish (e.g., UDAF 1997).

Toxicity data are available for various concentrations of praziquantel in water. Mitchell and Hobbs (2007) determined the 24- and 96-h LC50 values (i.e., concentrations lethal to 50% of the test fish) for golden shiners *Notermigonus crysoleucas* were 55.1 and 49.7 mg/L, respectively, and for grass carp *Ctenopharyngodon idella* were 63.4 and 60.6 mg/L, respectively. The 24- and 96-h no observable effect concentrations (NOEC), i.e. the highest levels that will kill none of the fish, were 50 and 45.0 mg/L for golden shiners, respectively, and 60.0 and 60.0 mg/L for grass carp, respectively (Mitchell and Hobbs 2007). No mortality or side effects resulting from praziquantel treatments of up to 36 mg/L were observed in bonytail (*Gila elegans*; Ward 2007). However, in acute toxicity tests on fry of the African sharptooth catfish *Clarias gariepinus*, Obiekezie and Okafor (1995) determined the 24-h LC50 of praziquantel to be 13.4 mg/L.

Existing protocols for using praziquantel to treat fish have only been applied to captive fish in tanks where fish are held for 24 h in treatment water, which is then exchanged with clean water before fish are returned to their natural habitat. The exchange of water is necessary because treatment with praziquantel does not kill eggs in the expelled tapeworms (Pool 1985; Kline et al. 2009). Instead, the eggs hatch into free swimming coracidia that are eaten by the intermediate host, cyclopoid copepods, within which coracidia develop into procercoids (Liao and Shih 1956). If infected copepods are eaten by a suitable definitive fish host, procercoids develop into tapeworms in the fish intestine (Liao and Shih 1956; Körting 1975). The Asian tapeworm can complete its development in less than 2 weeks, depending on water temperature. At warm temperatures, maturation of the adult tapeworms and egg development and hatching are stimulated, and ingestion of coracidia by copepods is enhanced (Granath and Esch 1983a; Hanzelová and Žitnan 1987). Rates of both egg development and hatching peak at 30°C (Granath and Esch 1983c; Hanzelová and Žitnan 1987). Below 20°C, tapeworms take longer to develop into gravid adults (Oskinis 1994).

Because praziquantel does not kill Asian tapeworm eggs, fish may become reinfected if treated only once in the presence of the copepod intermediate host. Existing methods for treating ponds involve removing fish, treating them with praziquantel in captivity, draining and drying the pond, disinfecting the pond with calcium hydroxide or exposing the pond substrate to winter conditions to destroy parasite eggs and the intermediate host, and then restocking the pond with tapeworm-free fish (UDAF 1997). These methods are costly and time-consuming, particularly when multiple or large ponds are infected.

In this paper, we have described and evaluated a new praziquantel treatment regimen for controlling Asian tapeworm infections in pond-reared fish. Briefly, we created replicate pond mesocosms that contained a definitive fish host, an intermediate copepod host, and Asian tapeworms and administered two praziquantel bath treatments, each separated by 19 d. We hypothesized that the first treatment would kill adult tapeworms and the second treatment would kill any tapeworms that developed from eggs released before or during the first treatment.

METHODS

We used six mesocosms, each consisting of a 1,098-L, circular, flat-bottomed fiberglass tank to simulate conditions in a natural pool. Tanks were 112 cm in diameter, 114 cm high, and had a water depth of 86 cm. Mesocosms were located within a greenhouse at the University of Arizona Agricultural Center, Tucson. We equipped each tank with a trickling biofilter containing bioballs to circulate water and remove ammonia. Artificial macrophytes in each mesocosm simulated natural vegetation and provided cover for fish and copepods. Sand and gravel provided substrate for benthic phases of the copepod's life history. We recorded water temperature in each tank daily. To simulate natural ponds, we inoculated each mesocosm with water obtained from ponds at San Bernardino National Wildlife Refuge, Arizona. We maintained the mesocosms for 2 months before stocking fish and copepods.

To ensure that the Asian tapeworm was capable of completing its life cycle, we stocked cultures of the known intermediate copepod host, *Cyclops vernalis*, in all tanks on alternate days throughout the experiment. On days when copepods were not added to the tanks, we fed fish a supplemental diet of pellet food (ZP3 pellets, Aquatic Eco-Systems, Apopka, Florida).

We used red shiner *Cyprinella lutrensis* as the Asian tapeworm host in mesocosms. The red shiner is nonnative and ubiquitous in southern Arizona and is a known host for the Asian tapeworm. Consequently, many red shiner populations in this area are infected with the tapeworm. We hand-seined approximately 400 red shiners from an infected population at the confluence of the Verde and Salt rivers, Arizona. We randomly assigned 50 of these fish to each of the six mesocosms and dissected an additional 50 fish at the start of the experiment to assess the initial Asian tapeworm infection rate. Total length (TL, mm) of all red shiners was measured and averaged by group.

The experiment started in August 2006 when greenhouse temperatures were above 20°C to ensure that no infected

TABLE 1. Mean (SD) prevalence and intensity of infection of Asian tapeworms in red shiners held in control and treatment mesocosms at the end of experiment. A random subsample of 50 fish that were dissected to assess initial prevalence and intensity in all fish at the start of the experiment found 14% of the fish were infected (prevalence) with a mean intensity of 2.14 (2.19) tapeworms per fish. Mean water temperature among tanks for the duration of the experiment, and initial and final mean fish total lengths (TL) among tanks are also listed.

Group	*n*	Prevalence (% fish infected)	Mean intensity (tapeworms per fish)	Mean water temperature (°C)	Initial TL (mm)	Final TL (mm)
Control	3	18 (6)	3.45 (2.1)	21.0 (1.4)	50.0 (0.3)	53.5 (0.7)
Treatment	3	0 (0)		20.1 (0.7)	49.9 (0.7)	54.1 (2.3)

copepods would be in diapause only to emerge and reinfect fish. Fish were in mesocosms for 1 week before the first praziquantel treatment was administered. Fish that died before the first praziquantel dose were replaced. For the first treatment, we randomly chose three of the tanks and added a powdered formulation of praziquantel (100%; AquaScience, North Kansas City, Missouri). Praziquantel was added according to the manufacturer's dosage recommendations at a concentration of 2.5 mg/L in water. Doses were administered nominally, i.e., water samples were not collected from each tank and doses verified analytically. After 19 d, we repeated the treatment in the same tanks to kill any tapeworms that may have developed from eggs released before or during the first treatment. We based the time interval of 19 d between treatments on previous studies of the rate of tapeworm development in warm water (Oskinis 1994). Because praziquantel is only slightly soluble in water, we dissolved it in 70% isopropyl alcohol before adding it to tanks following the procedures of Mitchell (2004) (5 mg praziquantel/mL isopropyl alcohol). The other three tanks, which served as controls, were not treated with praziquantel but had the same amount of 70% isopropyl alcohol added. After the treatment cycle, we left mesocosms undisturbed for 2.5 months to allow any remaining tapeworms to reestablish. Any fish that died during the experiment were measured for TL and examined for the presence of Asian tapeworm and other diseases or parasites. At the conclusion of the experiment, we killed all fish and measured TL. We then removed and opened the intestines from each fish and examined each intestine for Asian tapeworms under a Zeiss Stemi 2000 dissecting microscope with a power range of 6.5–50 × .

Response variables measured were fish length (TL), Asian tapeworm prevalence, and Asian tapeworm infection intensity. We used one-way analysis of variance (ANOVA) to confirm there were no differences in TL among fish from each control mesocosm, treatment mesocosm, and the initial dissected subsample. We used two-sample *t*-tests to test the hypothesis that fish length and Asian tapeworm prevalence and infection intensity did not differ between treatment and control mesocosms at the end of the experiment. Two-sample *t*-tests were also used to test for differences in average water temperature between control and treatment mesocosms during the experiment. A one-sample *t*-test was used to test the hypothesis that Asian tapeworm prevalence and infection intensity did not differ between the control mesocosms at the end of the experiment and the initial values determined from the dissected group.

RESULTS

The random subsample of 50 fish that were dissected to assess initial prevalence and intensity in all fish at the start of the experiment found 14% of fish were infected (prevalence) with *B. achelognathii* and had a mean ± SD infection intensity of 2.14 ± 2.19 tapeworms per fish. At the end of the experiment, no Asian tapeworms were found in any of the fish from the three treatment tanks, but 18% of fish were infected in the control tanks. As such, there was a highly significant difference between control and treatment mesocosms in the percent prevalence of Asian tapeworm ($t = 5.1962$, df $= 4$, $P = 0.007$; Table 1).

There were no differences in percent prevalence ($t = 1.1547$, df $= 4$, $P = 0.368$) or mean intensity ($t = 1.0721$, df $= 4$, $P = 0.396$) of Asian tapeworm infection between control fish and the fish dissected at the start of the experiment to determine initial infection rates of red shiners.

There was no difference in initial TL of red shiners among the six mesocosms or the seventh group that was assessed for initial tapeworm infection rate (mean TL of all fish at start of experiment, 50.7 ± 9.4 mm; one-way ANOVA: $F_{2,4} = 2.60$, $P = 0.19$). At the end of the experiment, there was no difference in mean TL between treatment and control groups ($t = -0.4448$, df $= 4$, $P = 0.679$; Table 1).

Although there was a 3°C temperature gradient across the greenhouse where mesocosms were held, there was no difference in mean water temperature between treatment and control tanks because treatments were randomly assigned to tanks ($t = 0.9785$, df $= 4$, $P = 0.383$; Table 1).

All fish survived the experiment except for 16 early mortalities in one treatment tank. These mortalities were detected 1 week after fish received the first praziquantel dose and occurred simultaneously with an increase in ammonia, which occurred only in this tank. We subsequently added oxidizing bacteria to all tanks to help break down ammonia and nitrite (Cycle by NUTRIFIN, Hagen, Baie d'Urfé, Quebec) and no further deaths occurred.

DISCUSSION

Our treatment regimen, which used praziquantel to eliminate the Asian tapeworm in the presence of the tapeworm's intermediate host, the cyclopoid copepod *Cyclops vernalis*, was effective. This regimen has the potential to be much less costly and time-consuming than are existing methods for treating ponds containing intermediate hosts; current methods involve removing fish and treating them in captivity in addition to draining and drying the pond, and treating the pond substrate.

There was no evidence of Asian tapeworm recruitment in treatment tanks. However, there appeared to be tapeworm recruitment in control tanks. During the final fish dissection, we noted many immature, nonsegmented tapeworms in control fish that were little more than scolexes. Marcogliese and Esch (1989) considered such small tapeworms to be recently recruited (within the previous 9 d).

Although our results are encouraging, further research is necessary before implementing praziquantel in the field. Chronic physiological effects and ecotoxicity effects of praziquantel exposure on nontarget aquatic organisms, and its effectiveness against the Asian tapeworm procercoid life stage need to be determined. Negative effects of praziquantel treatment would need to be weighed against the potential negative effects of not treating fish.

We saw no long-term effects on growth of red shiners, and no deaths could be attributed to the praziquantel treatment; however, a more thorough examination of chronic physiological and sublethal effects of praziquantel on all life stages of fish to be treated is needed. Although a few studies have examined the pharmacokinetics of praziquantel orally administered to fish (Rogstad et al. 1993; Tubbs and Tingle 2006), no studies on the bioavailability and tissue concentration of praziquantel have been done for bath-treated fish. This is particularly important for bath treatments in which water is not exchanged, because fish would be exposed to the drug until it degrades.

Little is known about degradation products of praziquantel or its rate of biodegradation. Because of the broad-spectrum activity of praziquantel against many parasitic trematodes and cestodes (Andrews et al. 1983), we advise caution when administering it in natural ecosystems, as it is likely to affect native platyhelminths.

We also recommend testing the effectiveness of praziquantel on other life stages of Asian tapeworm. Moser et al. (1986) found that the activity of procercoids of the parasitic trematode *Nybelinia* sp. in its intermediate host, the marine copepod *Tigriopus californicus*, was not affected by praziquantel. This is important because copepods can go into diapause during unfavorable conditions, but still remain infected by Asian tapeworm procercoids (Riggs and Esch 1987). If the procercoid stage of Asian tapeworm is not susceptible to praziquantel, it may persist through the treatment period and infect fish after copepods emerge from diapause. We avoided this in our experiment by conducting treatments at warm temperatures when copepods were not likely to be in diapause.

Minimal effective dosages for field use would need to be investigated. Our concentration of praziquantel (2.5 mg/L in water), although applied at a rate recommended by the manufacturer, was higher than that (0.75 mg/L in water) used by Mitchell and Darwish (2009), which proved effective in single 24-h bath treatments of fish held at a densities up to 60 g/L in 22-L containers. Effects of sediment, longer time exposures of bath treatments, fish densities, and other factors important in field situations would have to be examined to estimate a minimal effective dosage.

Finally, regulatory restrictions must be considered before praziquantel is used in field situations. For example, to date praziquantel is not approved for use in food fish in the United States nor in waters where food fish can exist. However, there are some case-by-case exceptions for the use of nonapproved drugs for treatment of U.S. federally listed threatened and endangered fishes. Further information can be obtained from the U.S. Fish and Wildlife Service's Aquatic Animal Drug Approval Partnership (AADAP) Program (USFWS 2011).

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