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In Vitro and *In Vivo* Antiparasitic Efficacy of Praziquantel against Monogenean *Ligictaluridus floridanus* in Channel Catfish (*Ictalurus punctatus*)

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Abstract

Gill monogenean *Ligictaluridus floridanus* is the most common parasite on cultured channel catfish (*Ictalurus punctatus*) in Tamaulipas, Mexico, affecting fish growth and possibly promoting secondary infections. In aquaculture, treatments against fish parasites include the use of chemotherapeutic agents to control and prevent diseases. The aim of this study was to evaluate the *in vitro* and *in vivo* efficacy of praziquantel baths against *L. floridanus* on *I. punctatus*. *In vitro* experiments evaluated *L. floridanus* attached and unattached to gills; for this, parasites were exposed to 2, 5, and 10 mg L⁻¹ of praziquantel in distilled or fish aquarium water, and saline solution. For the *in vivo* experiments, channel catfish infected with *L. floridanus* were separated into two groups; fish of the treatment group received three 90-min baths with praziquantel (10 mg L⁻¹) at 72 h intervals. Parasite prevalence, mean intensity, and abundance were assessed by gill examination. *In vitro* results of the praziquantel in saline and distilled water showed that it reduced the time needed for parasite elimination ($p < 0.05$); whereas no difference was observed in the aquarium water, as compared with the control group. *In vivo* results showed a reduction in the prevalence and mean intensity of the parasite, and a significant reduction ($p < 0.05$) in the parasite abundance in the treated fish. Consequently, the present study shows that praziquantel is an effective chemotherapeutant against the ectoparasite *L. floridanus*, significantly reducing its abundance.

Keywords: anthelmintics, catfish, monogenean, trematode

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Introduction

Channel catfish, *Ictalurus punctatus*, is an important aquaculture species in some regions of the world. In Mexico, intensive cage culture system production promotes crowd and in consequence transmission of ectoparasites. *Ligictalurus floridanus* is a monogenean, distributed in wild fish in North America (Rosas-Valdez and Pérez-Ponce de León, 2005). In Tamaulipas, this parasite shows the highest prevalence in cage-cultured catfish (Rábago-Castro et al., 2011). Praziquantel (Pzq) is a wide-spectrum isoquinolone that causes paralysis in flukes and integument damage; it is used against parasites, and is effective against monogeneans in immersion baths (KiHong and JaeBum 2000; Del Rio-Zaragoza et al., 2011). Studies *in vitro* have shown that Pzq has advantages compared with other anthelmintics (Hardy-Smith et al., 2012), when it has been used in different dosages (2-20 mg L⁻¹) according to the species and parasite infection (Buchmann 1988^a; KiHong and JaeBum, 2000; Stephens et al., 2003; Buchmann et al., 2011). However, there are few *in vivo* studies of this product on fish ectoparasites. The present study was undertaken to evaluate the anthelmintic effect of Pzq against *L. floridanus* in channel catfish, *I. punctatus*, juveniles under laboratory conditions on *in vitro* and *in vivo* experiments.

Materials and Methods

The *in vitro* experiment was done according to Hirazawa et al. (2000), with slight modifications. Three Pzq (Cisticid[®], Merck SA de CV, Mex.) solutions were prepared at 2, 5 and 10 mg L⁻¹. Each Cisticid[®] tablet and powder was weighed to ensure that correct Pzq dosage was used. Saline solution (0.65%) (SS) (Jiménez-Guzmán et al., 1988), aquarium water (AW) and distilled water (DW) were used as diluents. Effect of each solution against parasite was compared in triplicate. Parasites were obtained from naturally infected *I. punctatus* from a local hatchery (Abasolo, México). Mean weight and mean fork length of the fish were 31.87 ± 3.98 g and 14.40 ± 0.50 cm, respectively. The fish were euthanized by cranial puncture and excised gill archs infected with more than five *L. floridanus* per gill arch were immersed in 50 ml each of the Pzq solutions and the control solutions at 22°C. Effectiveness of the Pzq and control solutions was

determined by the time taken to kill the parasites observed under a stereomicroscope (Carl Zeiss[®], Stemi 2000-C; Germany).

The *in vivo* challenge was performed according to Silveira-Coffigny (2006), with slight modifications. Twenty-three juvenile catfish, clinically healthy, but naturally infected with *L. floridanus*, were obtained from the same hatchery and transported to the laboratory. The fish had a mean weight of 27.24 ± 2.04 g and a mean fork length of 13.30 ± 0.86 cm. They were stocked in an 80 L glass fiber tank, with aeration supplied by a blower and a continuous flow of water supplied from a ground well. Five fish samples were taken to confirm the parasite infection. The fish were anesthetized with benzocaine (40 mg L⁻¹) and were euthanized by cranial puncture. Infection with the parasite and identification were confirmed according to Hoffman (1985); results showed a mean of 2.5 ± 1.0 parasites per gill arch. The remaining catfish were randomly divided into six aquaria (40 L volume) for control and treated groups, each with three replicates containing three fish in each. The aquaria were filled with 37 L of water and a constant flow (18 L h⁻¹) was maintained.

The fish were acclimated for 4 days. At the beginning of the treatment, the water flow in the control and treated aquaria was interrupted, reducing the volume of water to 10 L in the aquaria; 100 mg of Pzq diluted in water (100 ml) were added to the water in the treated aquaria to reach a concentration of 10 mg L⁻¹. Three bath treatments for 3 h each were carried out with 72 h intervals in between. During treatments the internal walls of treated aquaria were cleaned with paper towels to ensure that no *L. floridanus* eggs remained, and the aeration was diminished to avoid eggs sticking to the walls. Additionally the walls were washed with the Pzq solution (10 mg L⁻¹). The same management of reduction in water level and wall cleaning was performed with the water in control aquaria. After 3 h of treatment, water levels were returned to the initial volume (37 L). The fish were fed twice a day *ad libitum* with 3 mm commercial catfish food (32% protein). Twenty-four hours after the last bath treatment, the fish were anesthetized and euthanized as described above. Four gill arches of the left side of each fish were examined for prevalence, mean intensity and abundance of *L. floridanus* under a stereomicroscope by placing them on Petri dishes containing 0.65% saline solution.

Table 1 Mean of total number of *Ligictalurus floridanus* per gill arch counted 24 h after the third praziquantel bath in control and treated groups.

Aquarium	Group	Fish number			Aquarium mean*
		1	2	3	
A	Treated	0	0	0	0 ^a
B	Control	3 ± 0.81	4.5 ± 1.91	0.75 ± 0.95	2.75 ± 1.89 ^b
C	Treated	1 ± 0.81	0.5 ± 0.57	0.5 ± 0.57	0.67 ± 0.29 ^a
D	Control	6.25 ± 3.59	33.25 ± 19.70	21.25 ± 8.13	20.25 ± 13.50 ^b
E	Control	4.25 ± 2.63	8.5 ± 3.69	4 ± 2.30	5.58 ± 2.53 ^b
F	Treated	0.25 ± 0.50	0.25 ± 0.50	1.25 ± 1.89	0.58 ± 0.58 ^a

*Differences between groups in the column are indicated by different letters ($p < 0.05$)

Table 2 Mean \pm SD of survival time expressed in seconds of *Ligictaluridus floridanus* on gill arch immersed in praziquantel (Pzq) prepared solutions using saline solution (0.65%), distilled water and aquarium water as diluents.

Group	Gill arch 1	Gill arch 2	Gill arch 3
Saline Sol. (SS) (0.65%)	661.88 \pm 169.47	675.55 \pm 19.17	864.29 \pm 144.75
2 mgL ⁻¹ Pzq (SS)	549.33 \pm 93.96	645.22 \pm 115.99	574.00 \pm 152.78
5 mgL ⁻¹ Pzq (SS)	502.40 \pm 229.32	448.36 \pm 136.45	353.09 \pm 102.39
10 mgL ⁻¹ Pzq (SS)	244.00 \pm 198.67	328.17 \pm 116.82	381.38 \pm 107.26
Distilled Water (DW)	3000.50 \pm 833.97	3419.00 \pm 859.55	3071.17 \pm 1198.37
2 mgL ⁻¹ Pzq (DW)	2389.00 \pm 183.21	2387.00 \pm 696.58	1880.50 \pm 232.76
5 mgL ⁻¹ Pzq (DW)	890.50 \pm 243.02	1273.63 \pm 414.22	692.50 \pm 116.61
10 mgL ⁻¹ Pzq (DW)	1370.20 \pm 627.48	923.00 \pm 854.17	1338.80 \pm 596.07
Aquarium Water (AW)	6039.00 \pm 1034.95	5390.82 \pm 907.72	5742.29 \pm 182.87
2 mgL ⁻¹ Pzq (AW)	3295.11 \pm 818.16	3214.86 \pm 849.32	6061.50 \pm 439.75
5 mgL ⁻¹ Pzq (AW)	2413.43 \pm 1244.07	2099.89 \pm 835.99	2674.86 \pm 1544.68
10 mgL ⁻¹ Pzq (AW)	1408.60 \pm 1422.13	3463.43 \pm 1941.51	3466.60 \pm 852.38

Data for *in vitro* trials were analyzed with a non-parametric Kaplan-Meier cumulative proportion surviving test, grouped by control and treatment Pzq dosages in different trials. In addition, data obtained from each challenge of survival of each parasite were analyzed under ANOVA test. Data where DW and AW were used needed Sin transformation to meet ANOVA assumptions, whereas SS data did not need transformation. For the *in vivo* experiment, prevalence, mean intensity and abundance of *L. floridanus* were obtained according to Bush et al. (1997) and Mladineo (2005), where the mean number of parasites per gill arch was obtained, and data were evaluated by ANOVA. Additionally, data were transformed to Log¹⁰ to meet ANOVA assumptions. All data were analyzed using the commercial software Statistica® v6.1 (StatSoft®, Inc., USA) using $\alpha=0.05$ and $\alpha=0.02$, as levels of significance.

Results and Discussion

Praziquantel seems to be an effective anthelmintic drug suitable to be used as a control agent (Martin et al., 1997). A noticeable effect of Pzq on survival time of parasites was observed during the *in vitro* trial (Table 2, Fig 2); parasites were commonly released from the gills, before they stopped moving. Parasites which received 10 mg L⁻¹ of Pzq diluted in SS, contracted (curved shape) almost immediately; this was not observed during the DW and AW treatments. Hirazawa et al. (2000) also reported helminth contraction on *in vitro* trials with 20 mg L⁻¹ of Pzq, using filtered sea water as diluent. Schelkle et al. (2011) also

reported salt parasite treatments affecting monogeneans in guppies (*Poecilia reticulata*), using up to 33 g L⁻¹ and decreasing parasite survival time to less than 1 h causing osmoregulation disruption.

Survival time of *L. floridanus* on SS (Table 2) showed a significant difference ($p<0.05$) between the groups (Fig 1a); the control and 2 mgL⁻¹ groups had a higher difference ($p<0.02$) compared with the other groups. Death of parasite gill arch populations in the control groups and in the groups treated with Pzq fit into a normal distribution, in which almost 40% of total parasite counted died in a short period of time close to survival time mean. Parasites exposed to AW had a longer survival time than parasites exposed to the other solutions (Figs 2, 3). The Kaplan-Meier analysis showed differences among the treatment and control groups (Fig 3). Parasites in the control and 2 mg L⁻¹ groups began to die at very different times, whereas in the 5 and 10 mg L⁻¹ groups, almost 20% of all parasites died in less than 100 sec.

The parasite prevalence of the control and treated groups on *in vivo* trial was 100% and 66.6%, respectively, whereas the parasite mean intensity among the control and treated groups were 76.22 and 5.0, respectively. Moreover, the parasite abundance in the control group was higher than the Pzq group, 76.22 and 3.33, respectively. The mean of parasites per gill arch showed significant differences ($p<0.05$) between the control and treated groups (Table 1). Log₁₀ data also showed significant differences ($p=0.02$) between the control and treated groups (Fig 1b). Advantages of Pzq include its use in the control of internal parasites in channel catfish because it has good absorption

through epitheliums, evidenced by the reduction in the trematode larvae after bath treatments (Plumb and Rogers, 1990). However, establishing a proper lethal dose is also required to minimize potential development of Pzq resistance caused by exposure of parasite to sub-lethal doses. Future research of treatments on different *L. floridanus* stages is also needed due to the fact that certain kinds of immature monogeneans show resistance to Pzq, such as *Pseudodactylogyryus bini* (Buchmann, 1988^b). In

conclusion, Pzq showed anthelmintic action, being able to kill *L. floridanus* on catfish gills and increasing its killing potential when mixed with SS. However, DW and AW could decrease its potential. Therefore, Pzq is recommended for the control or treatment of ectoparasites. Further studies will focused on timing of Pzq administration and water quality effects on its efficacy.

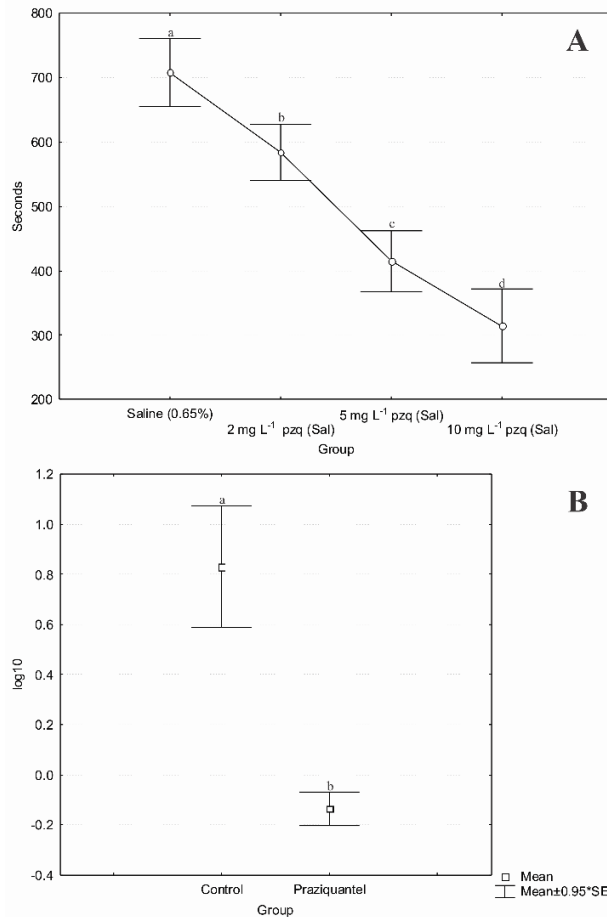


Figure 1 Differences ($p < 0.05$) of *L. floridanus*, indicated by different letters between groups, when 10 mg L⁻¹ of praziquantel were administered. (A) Mean of survival time expressed in seconds of *Ligictaluridus floridanus* per gill arch on saline and praziquantel groups on *in vitro* trial. Vertical bars denote 0.95 confidence. (B) Mean of total number of *Ligictaluridus floridanus* per gill arch of *in vivo* challenge, counted 24 h after treatments, in control and 10 mg L⁻¹ praziquantel-treated groups. Data are Log10 mean \pm SE. N=3 in each group.

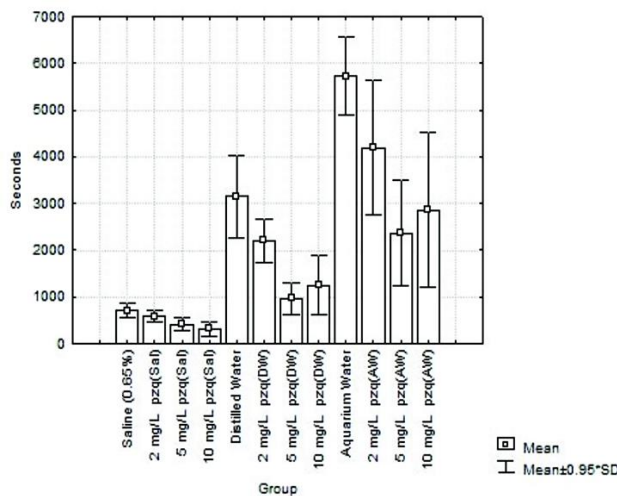


Figure 2 Comparison graph of survival time of *Ligictaluridus floridanus* on gill arch exposed to saline solution (0.65%), distilled water and aquarium water as diluents. Data are mean \pm SD. Pzq (Sal) represents praziquantel treatment diluted in saline solutions; pzq (DW) represents praziquantel treatment diluted in distilled water and pzq (AW) represents praziquantel treatment diluted in aquarium water.

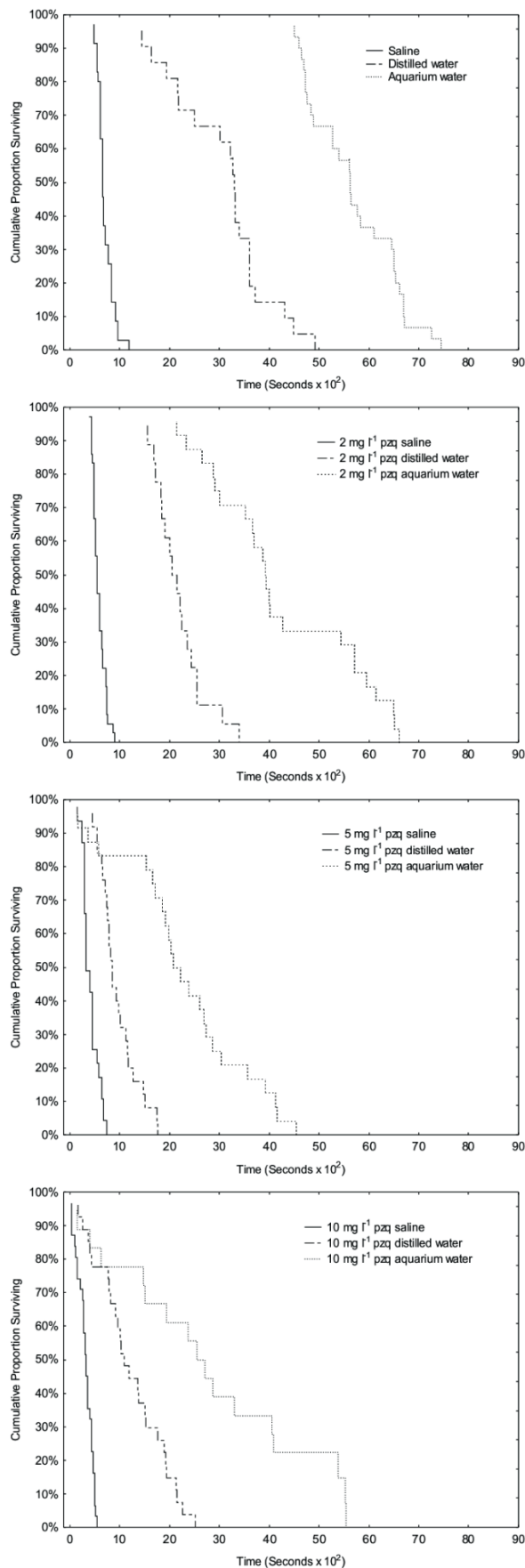


Figure 3 Comparison of survival time seconds of *Ligictaluridus floridanus* per gill arch, using Kaplan-Meier cumulative proportion surviving test *in vitro* experiment.

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บทคัดย่อ

ประสิทธิภาพในการต้านเชื้อปรสิตของพราซิควอนเทล *in vitro* และ *in vivo* ต่อพยาธิ monogenean ชนิด *Ligictaluridus floridanus* ในปลาดุกอเมริกัน (*Ictalurus punctatus*)

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พยาธิตัวแบน monogenean ชนิด *Ligictaluridus floridanus* เป็นปรสิตที่พบได้บ่อยที่สุดในปลาดุกอเมริกันในการเพาะเลี้ยง (*Ictalurus punctatus*) ในเมือง Tamaulipas ประเทศเม็กซิโก โดยมีผลกระทบต่ออาการเจริญเติบโตของปลา และอาจเสริมต่อการติดเชื้อแทรกซ้อน ในการเพาะเลี้ยงสัตว์น้ำ การรักษาปรสิตในปลามีการใช้ยาเคมีบำบัดในการควบคุมและป้องกันโรค วัตถุประสงค์ของการศึกษานี้ เพื่อประเมินประสิทธิภาพของการอาบพราซิควอนเทล *in vitro* และ *in vivo* ต้าน *L. floridanus* บนปลา *I. punctatus* ในการทดลอง *in vitro* ได้มีการประเมิน *L. floridanus* ที่เกาะอยู่บนเหงือกและที่หลุดจากเหงือก เชื้อปรสิตได้สัมผัสกับพราซิควอนเทล 2, 5 และ 10 มก./ลิตรของน้ำกลั่นหรือน้ำจากพิพิธภัณฑสถานปลาและน้ำเกลือ สำหรับการทดลอง *in vitro* ปลาดุกอเมริกันที่ติดเชื้อ *L. floridanus* ได้ถูกแยกเป็น 2 กลุ่ม ปลาในกลุ่มทดลองได้รับการอาบด้วยพราซิควอนเทล (10 มก./ลิตร) เป็นเวลา 90 นาที 3 ครั้ง โดยห่างกัน 72 ชั่วโมง ความชุก ความเข้มข้น และความมากมายของปรสิตถูกประเมินโดยการตรวจเหงือก ผลการทดลอง *in vitro* ของพราซิควอนเทลในน้ำเกลือและน้ำกลั่นแสดงให้เห็นว่ายาได้ลดเวลาที่ใช้ในการกำจัดเชื้อปรสิต ($p < 0.05$) ในขณะที่ไม่พบความแตกต่างในน้ำจากพิพิธภัณฑสถาน เมื่อเปรียบเทียบกับกลุ่มควบคุม ผลการทดลอง *in vivo* ได้แสดงให้เห็นว่ามีการลดลงของความชุกและความเข้มข้นของเชื้อปรสิต และการลดลงอย่างมีนัยสำคัญ ($p < 0.05$) ของความมากมายของเชื้อในปลาที่ได้รับการรักษา ดังนั้น การศึกษานี้แสดงว่าพราซิควอนเทลเป็นสารเคมีบำบัดที่มีประสิทธิภาพในการต้านปรสิตภายนอกชนิด *L. floridanus* โดยสามารถลดความอุดมของเชื้อลงได้

คำสำคัญ: ยาด้านพยาธิ ปลาดุก monogenean พยาธิใบไม้

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