

Efficacy Testing of Orally Administered Praziquantel to Common Carp Naturally Infected by Caryophyllidean Tapeworms (Platyhelminthes: Eucestoda)

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Abstract

The aim of the study was to assess the effects of orally applied praziquantel on the treatment efficacy against most abundant tapeworms (*Atractolytocestus huronensis* Anthony, 1958 and *Khawia sinensis* Hsü, 1935) of common carp (*Cyprinus carpio* Linnaeus). Naturally infected fish (1.5-year-old; K₁₋₂) were divided into 6 groups. Three trial groups were administered praziquantel mixed in heat-treated amyloid vehicle (at doses of 50 mg·kg⁻¹ body weight). In 3 groups of control, the fish were administered amyloid vehicle only. Fish were examined using gut dissection 2, 4 and 6 days after the administration. The location, intensity and prevalence of individual species of tapeworms were recorded. *A. huronensis* and *K. sinensis* were found in the gut of carp together. *A. huronensis* was situated in the oesophageal part of foregut, and *K. sinensis* came after – immediately before the first intestine loop, of the intestines of carp. The invasion of *A. huronensis* was fully eliminated in all trial groups after 4 days whereas some specimens of *K. sinensis* still persisted in the gut of few fish. The full elimination of both parasites was observed 6 days after administration. Our results showed that elimination of *K. sinensis* takes longer than elimination of *A. huronensis*. The dose of 50 mg·kg⁻¹ of orally administered praziquantel is effectual to control *A. huronensis* and *K. sinensis* infections in common carp successfully, however, a significant decrease of the number of parasites was also caused by a single oral administration of big bolus vehicle and leaving unfed fish in the clean water.

Khawia sinensis, *Atractolytocestus huronensis*, *Cyprinus carpio*, treatment, anthelmintic

Fish tapeworms are widespread throughout all major water systems of Europe and demonstrate a high degree of host specificity. Some of these parasites could cause disease and death in carp in aquaculture settings and may retard growth in hatchery-reared common carp (*Cyprinus carpio* L.). Other consequences include dysfunction or destruction of the intestinal lining and adverse changes to certain blood indicators (Niemczuk 1991; Molnár et al. 2003). There are two species of tapeworms from the order Caryophyllidea frequently found in the intestine of South Bohemian carp.

The older known species is *Khawia sinensis* Hsü, 1935. It is the easily identified carp tapeworm whose biology and developmental cycle is described in detail by various authors (Kulakovskaya 1963; Demshin and Dvoryadkin 1980; Scholz 1991). The range of the parasite has extended greatly, despite legal prohibition of the introduction and movement of infected fish in the end of 20th century (Yeomans et al. 1997).

The second relatively new species is *Atractolytocestus huronensis*. This parasite was first described by Anthony (1958) in North America. Now, it is an important endoparasite in the intestine of common carp, *Cyprinus carpio* L. reared in ponds in the Czech Republic. During the last decades, this parasite has spread across Europe. Occurrence of this tapeworm was recorded in England (Chubb et al. 1996), Czech Republic and Hungary (Molnár et al. 2003; Majoros et al. 2003), Slovakia (Oros et al. 2004) and Germany (Kappe et al. 2006). Scholz et al. (2001) noted the occurrence of *A. sagittata*, very close species, in the intestine of *C. carpio* from Japan. Other dispersion could be assumed with regard to progressive export of parasitized carp to many other countries. The prevalence of infection

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may reach 100% in intensive and semi-intensive pond culture systems, where the parasite is a particular threat to underyearling carp. Pathology caused by *A. huronensis* is described in detail by Molnár et al. (2003), who pointed out the potential of higher pathogenicity of *A. huronensis* in comparison with *Bothriocephalus acheilognathi*.

Eradication of fish tapeworms is problematic. Intensive production of fish has inevitably created a requirement for medicines, but the legislative framework in the European Union (EU) has made it uneconomic for pharmaceutical companies to develop medicines for a small market (Treves-Brown 1999). Therefore, the limited possibility of using chemicals and drugs for treatment makes the situation even more difficult to handle. Many veterinarians try to use chemicals with a potential effect on fish tapeworm, but hardly anybody knows exactly the consequent effect. Various chemicals including praziquantel, benzimidazoles and niclosamides are recommended for oral treatment of fish tapeworms (Treves-Brown 2000), however, there are no determined maximum residue limits (MRL) in fish for these substances, which means the maximum level of a chemical which is permitted to be present in a human food, expressed in milligrams of the chemical per kilogram of the food ($\text{mg}\cdot\text{kg}^{-1}$) unless otherwise stated. That is why, the most perspective praziquantel is allowed to use only for ornamental and non-food fish, and for food fish treatment it is still under experimental studies such as this one. Nevertheless, the development of a tapeworm eradication procedure is necessary for continued interstate shipment of tapeworm-free fish (Mitchell 2004) and economical profitable of fish growth rate. The aim of the present study was to determine location, intensity and prevalence of individual species of fish tapeworms before and after praziquantel treatment.

Materials and Methods

Individuals of 1.5-year-old carp ($K_{1,2}$) weighing 272 ± 50 g naturally infected by tapeworms were obtained from commercial producer. The fish were acclimated to $20 \pm 2^\circ\text{C}$ for 3 days prior to the experiment and were not fed throughout the trial. The presence and numbers of tapeworms in the intestine were confirmed by examination of 15 fish and the results from this initial examination were used as starting values for final evaluation. A total of 108 carp intended for test were divided into 6 groups consisting of 6 fish per tank and each group was replicated three times. The volume of each experimental tank was 250 l and water from each tank was changed daily. All used tanks were aerated with an air pump attached to aeration stones and oxygen saturation and pH were measured every day. Oxygen saturation of water was above 80% (ranging from 72% to 91%) and pH ranged from 6.28 to 7.30.

Trial

Fish from 3 trial groups were intubated directly into the oesophagus with the drug mixed into a heat-treated amyloid vehicle at a dose of $5 \text{ ml}\cdot\text{kg}^{-1}$ body weight. The concentration of praziquantel was $50 \text{ mg}\cdot\text{kg}^{-1}$ bw. The vehicle was prepared by heating 60 g food starch (Solamyl) in 1 l water to 100°C temperature. In 3 control groups, the fish were administered amyloid vehicle in the same dose only. The fish were subsequently observed in the recovery tanks for any signs of regurgitation of anthelmintics. Tapeworm survival in common carp was determined 2, 4 and 6 days after administration (control and trial group in each time together). Fish were killed with a blow on the head, the visceral cavity was cut open and intact intestinal tracts were removed and examined for tapeworms. The effectiveness of treatment for both species of tapeworms was confirmed by comparison of the number of parasites with those in the control at the same examination time. The location, intensity, prevalence and the time of elimination of tapeworms were recorded.

Statistical analysis

Statistical analysis was carried out using STATISTICA software 8.0 for Windows (StatSoft, Prague, Czech Republic).

The prevalence of tapeworms was represented in percentage and thus had to be subjected to arcsin transformation prior to any further processing. Data were first tested for normality (Kolmogorov Smirnov test) and homoscedasticity of variance (Bartlett's test). If those conditions were satisfied, two-way analysis of variance (ANOVA) was used to determine if there were any significant differences in measured variables among control and trial groups.

Results and Discussion

No clinical abnormalities were observed in the fish treated with praziquantel. Praziquantel and heat-treated amyloid vehicle were well tolerated by the fish and no fish were lost during the experiment.

Two species of tapeworms, *Atractolytocestus huronensis* and *Khawia sinensis*, were found in the gut of examined carp concurrently. Both species of tapeworms have their specific position of occurrence in the intestine. *A. huronensis* was situated in the oesophageal part of foregut. This part of the intestine is characterised by the presence of numerous intestinal crypts located close to one another, in addition to intestinal folds generally typical of the gut of cyprinids. *K. sinensis* came after – immediately before the first intestine loop, of the gut of carp (Plate I, Fig. 1). Scholz et al. (1990) described the location of *K. sinensis* and found the parasite in all parts of the gut; however, the position of the majority of parasites (73%) from their study corresponded with our findings. The location of attachment of *A. huronensis* is described by various authors, who pointed out the presence of tapeworms in the foregut, often in the most proximal 3 to 5 cm segment thereof (Moľnár et al. 2003; Majoros et al. 2003; Williams 2008). There are no data available on the presence of both tapeworms together in the gut of fish.

The intensity of infestation of *A. huronensis* and *K. sinensis* was very variable depending on individuality of each fish, however, the mean intensity of both tapeworms continually decreased in control and trial groups during the trial time. The number of *A. huronensis* decreased more rapidly than the number of *K. sinensis* in praziquantel-treated groups. There were no tapeworms *A. huronensis* found in the intestine of praziquantel-treated carp four days after application while the decreasing trend of *K. sinensis* was still continuing. The intestines of treated fish were free from both tapeworms 6 days after treatment (Table 1). Moľnár et al. (2003) observed that species *A. huronensis* may have a more important pathological role than *K. sinensis* because of *A. huronensis* rather securely attaches itself to the intestinal epithelium and can be removed from there only with difficulty. This is in contrast with our findings. We observed easy and more rapid elimination of *A. huronensis* than *K. sinensis* in control and praziquantel-treated groups.

Table 1. Survey of carp (*Cyprinus carpio* – K_{1,2}) examined during the trial and their infestation with tapeworms

Group of fish	Trial time (day)	Name of group	No. of carp			No. of tapeworms <i>A. huronensis</i> / <i>K. sinensis</i>	Mean intensity of <i>A. huronensis</i> (min.-max.)	Mean intensity of <i>K. sinensis</i> (min.-max.)
			examined	infested by <i>A. huronensis</i>	infested by <i>K. sinensis</i>			
Pretrial	-	C 0	15	15	8	797/38	53 (1-184)	5 (2-18)
Control	2	C 2	18	14	6	122/25	9 (1-29)	4 (2-10)
Trial	2	T 2	18	6	5	10/8	2 (1-3)	2 (1-2)
Control	4	C 4	18	8	5	131/15	19 (2-83)	3 (2-5)
Trial	4	T 4	18	-	2	-/2	-	1 (1)
Control	6	C 6	18	4	7	27/20	7 (2-11)	3 (1-9)
Trial	6	T 6	18	-	-	-/-	-	-

Scholz et al. (1991) described the effect of starvation of the infected carp for survival of the parasite. The burden in fed fish, in his trial, was conspicuously higher than in carp without feed and the greatest number of tapeworms was expelled from fish between day 3 and 10 of the experiment. In our study, we observed the main marked elimination of tapeworms between days 3 and 5 of starvation in all groups. An apparently higher number of tapeworms and their remnants were recorded in the sediment of treated fish than in the sediment of control fish. However, the exact number of expelled tapeworms was not calculated due to probable decomposition of tapeworms in the gut and thus difficult detection in the sediment. Yellow coloured remains of tapeworms were infrequently observed in the caudal portion of intestine of fish in all groups.

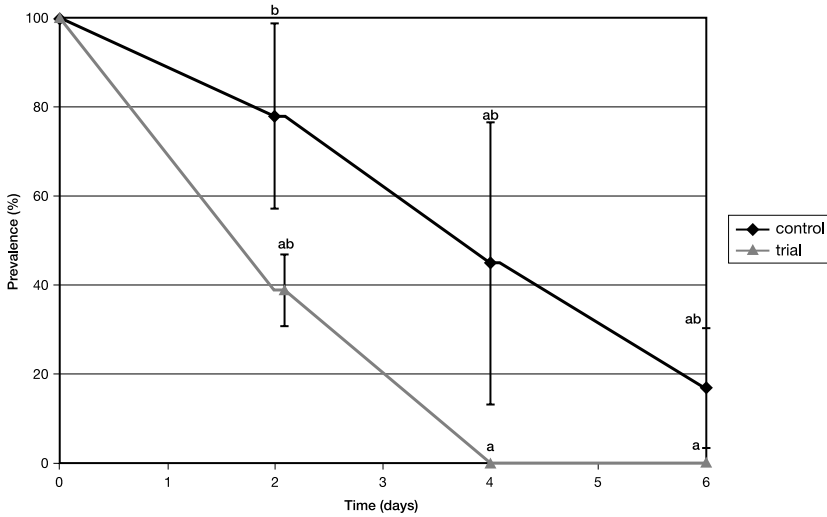


Fig. 2. Prevalence of *Atractolytocestus huronensis*
 Groups with different alphabetic superscripts differ significantly at $p < 0.05$ or $p < 0.01$

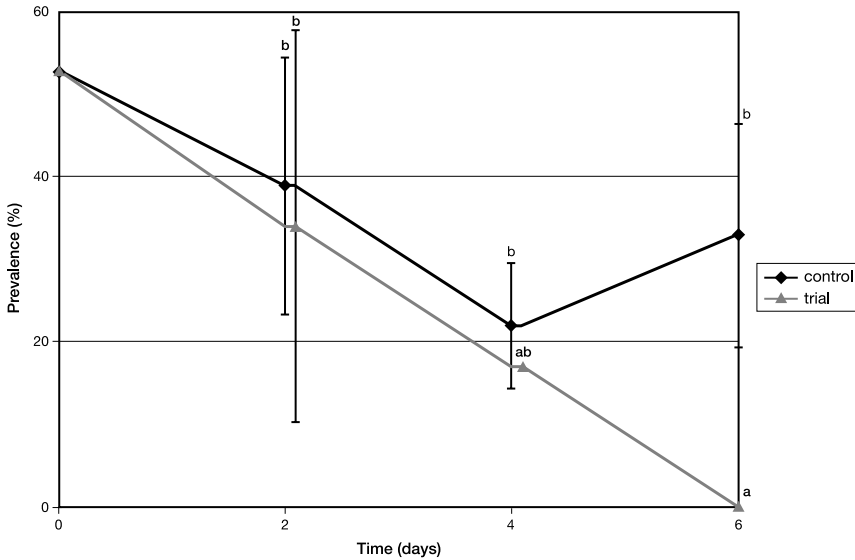


Fig. 3. Prevalence of *Khawia sinensis*
 Groups with different alphabetic superscripts differ significantly at $p < 0.05$ or $p < 0.01$

The prevalence of *A. huronensis* and *K. sinensis* was 100% and 60%, respectively, in fish checked before the experiment began (C0). The statistical evaluation was made with the results obtained after application of praziquantel and initial values were used only in figures in time zero. We observed a mild effect of praziquantel on the prevalence of *A. huronensis*. The trial groups did not differ from control groups in the same time of examination, whereas a significantly lower prevalence of *A. huronensis* ($p < 0.01$) was

observed in trial groups T4 and T6 compared to control group C2 (Fig. 2). We assume that the small differences between the control and trial groups could be caused by the high willingness of *A. huronensis* to be expelled due to starvation.

The substantial effect of praziquantel was observed on the tapeworm *K. sinensis* (Fig. 3). The prevalence in trial group (T6) was significantly lower ($p < 0.05$) compared to control group (C6). Decreasing values of prevalence ($p < 0.05$) were also noticed in the trial groups T2 and T6. These results point out the potential of praziquantel to eliminate or completely exterminate the tapeworms *K. sinensis*.

There are no data available for the effectual doses of orally administered praziquantel. Our results indicate that the dose of $50 \text{ mg} \cdot \text{kg}^{-1}$ of orally administered praziquantel is effectual to control *K. sinensis* and *A. huronensis* infections in common carp successfully, however, a significant decrease of the number of parasites was also caused by a single oral administration of big bolus vehicle and leaving unfed fish in the clean water.

Účinek perorálně podávaného praziquantelu na kapra obecného infikovaného tasemnicemi z řádu Caryophyllidea (Platyhelminthes: Eucestoda)

Cílem práce bylo posoudit účinnost orálně podávaného praziquantelu na nejčastěji se vyskytující tasemnice (*Atractolytocestus huronensis* Anthony, 1958 a *Khawia sinensis* Hsü, 1935) kapra obecného (*Cyprinus carpio* L.). Rok a půl staré infikované ryby ($K_{1,2}$) byly rozděleny do 6 skupin. Pokusným skupinám (3 skupiny) byl jícnovou sondou aplikován praziquantel rozpuštěný ve škrobovém vehikulu v dávce $50 \text{ mg} \cdot \text{kg}^{-1}$ živé váhy. Kontrolním skupinám ryb (3 skupiny) bylo aplikováno jen čisté škrobové vehikulum. Ryby byly vyšetřovány 2, 4 a 6 dní po aplikaci a byla zaznamenávána intenzita, prevalence a lokalizace jednotlivých druhů tasemnic. Ve střevech byly nalezeny současně tasemnice *A. huronensis* a *K. sinensis*. Tasemnice *A. huronensis* se nacházely v jícnové části předního střeva a tasemnice *K. sinensis* byly lokalizovány více kaudálně – bezprostředně před první střevní kličkou. Infekce *A. huronensis* byla 4. den u všech ryb z pokusných skupin plně eliminována, zatímco invaze tasemnic *K. sinensis* stále přetrvávala. K úplné eliminaci obou druhů tasemnic došlo 6 dní po aplikaci praziquantelu. Podle výsledků je zřejmé, že eliminace *K. sinensis* trvá delší dobu než eliminace *A. huronensis*. Dávka $50 \text{ mg} \cdot \text{kg}^{-1}$ orálně podávaného praziquantelu je účinná proti tasemnicím *A. huronensis* a *K. sinensis*, avšak je prokazatelné, že významný pokles počtu parazitů byl způsoben také jednorázovou aplikací velkého sousta škrobu do jícnu a ponecháním nekrmených ryb v čisté vodě.

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Fig. 1. Occurrence and position of tapeworms in the intestine of carp (*Cyprinus carpio* L.)
Ah - *Atractolytocestus huronensis*, Ks - *Khawia sinensis*