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The efficacy of fenbendazole against tapeworm infections in the common carp (*Cyprinus carpio L.*) in Veľké Blahovo ponds

Michal Madzunkov, Stanislav Navratil*

University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

**Corresponding author: dzunko@post.sk*

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Abstract: A survey of the pond system in Veľké Blahovo, Dunajská Streda district, Slovak Republic, confirmed the occurrence of tapeworms in carp with a prevalence of 63.5% and mean intensity of 1.7 ± 2.72. The species *Khawia sinensis* and *Atractolytocestus huronensis* were identified. We attempted to reduce the occurrence of tapeworms using a medicated feed mixture (granules) with 1% fenbendazole as active substance under controlled conditions. Faeces collected from carp (K2) were examined and the presence of tapeworm eggs was confirmed by the flotation method. Positive carp were divided into three groups; each group, labelled F1 to F3, included 15 individuals. The carp were placed in concrete pools (storage ponds) nourished by fish pond water at the peak of the vegetation period from August 7–17, 2015. The medicated feed mixture was administered to the first two groups (F1 and F2), while the third group was fed with a non-medicated feed mixture and served as the control (F3). A single dose was administered to the F1 group; tapeworm prevalence 24 hours after administration in the F1 group was 26.66% with a mean intensity of 1.3 ± 0.57. The F2 group was fed three times in total with 24-hour intervals between feeding. Tapeworm prevalence 24 hours after the last administration in the F2 group was 13.33% with a mean intensity of 2.0 ± 0.77. The prevalence in the control group was 66.66% with a mean intensity of 2.2 ± 1.62. *Khawia sinensis* tapeworms responded well to fenbendazole, while *A. huronensis* tapeworms responded poorly and, despite repeated administration of the active substance, this species remained attached to the intestinal mucosa. When compared with the control group, the reduction in the number of tapeworms was statistically significant ($P \leq 0.05$) in the F2 group; however, it was not statistically significant in the F1 group.

Keywords: *Atractolytocestus huronensis*; caryophyllidea; *Khawia sinensis*; treatment; fish; Slovakia

Several species of tapeworms from the Caryophyllidea order are often found in carp intestines. The earliest described species is *Khawia sinensis*. It is an easily identifiable carp tapeworm and various authors have described its biology and life cycle (Kulakovskaya 1963; Demshin and Dvoryadkin 1980; Scholz 1991). The original distribution area of *K. sinensis* was limited to eastern Asia (China and the Russian Far East), but the tapeworm was imported with the grass carp *Ctenopharyngodon idella* and the common carp *Cyprinus carpio* to many European countries and North America (USA). The history of the introduction of *K. sinensis* to Europe and North America was reviewed by Oros et al. (2009). The *Atractolytocestus huronensis* parasite was first described by Anthony (1958) in North America. Over the last few decades, this tapeworm has spread throughout Europe. Its occurrence has been recorded in England (Chubb et al. 1996), Czech Republic, Hungary (Molnar et al. 2003; Majoros et al. 2003), Slovakia (Oros et al. 2004) and Germany (Kappe et al. 2006). The *Khawia japonensis* (Oros et al. 2015) invasive fish tapeworm, originally described in Japan (1934, Yamaguti),

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was recently reported for the first time in Slovakia. The tapeworm was found in farmed common carp (*Cyprinus carpio* L.) from a pond for fish breeding in eastern Slovakia. The invasive and pathogenic potential of *K. japonensis* in commercial breeding fisheries and its possible further spread in natural habitats is difficult to estimate for now. As yet, *K. japonensis* does not appear to exert any major impact on commercial breeding fisheries, but more attention should be paid to potential biological invasions (Oros et al. 2015). Fenbenzadole, as a wide-spectrum antiparasitic preparation, is probably effective against *K. japonensis*; its spectrum of action covers gastrointestinal nematodes, lung worms and some tapeworms. The preparation has an ovocidal effect on the eggs of roundworms. The transport function of a parasite's absorptive cells is impaired, and the preparation acts against immature adult forms of sensitive helminths (Noga 2010; Duchacek and Lamka 2014). Compared to praziquantel, which is not registered, fenbendazole is registered and approved in Slovakia and is used in practice. The aim of this study was to determine the intensity and prevalence of different fish tapeworms before and after fenbendazole treatment.

MATERIAL AND METHODS

The occurrence of tapeworms in common carp aged 3–4 years with an average weight of 2408.10 ± 679.94 g was examined from 2012 up to the end of 2016. Overall, we examined 200 carp to determine the prevalence and species composition of tapeworms in three ponds at Veľké Blahovo in Slovakia. The fish were caught with nets and fishing rods. Dissection of the fish confirmed the presence of tapeworms from the Caryophyllaeidae family. Tapeworms in the intestine were removed and preserved. A 4% formaldehyde solution was used for fixation. A solution consisting of 20 ml potassium acetate, 300 ml glycerine and 1000 ml distilled water was used for illumination of tapeworm samples. The samples were kept in this solution for five to seven days. The tapeworm samples were examined using light microscopy (Olympus Delta Optical Genetic Pro with 1.3Mp digital camera (1280 × 1024) connected by a USB cable to a computer) and identified by an identification key (Lucky 1966; Oros et al. 2010; Scholz et al. 2011). The degree of fish infestation was characterised according to Margolis et al. (1982) in the following terms: prevalence (percentage of infected fish from examined fish), mean intensity of infection (average number of parasites per infected fish) and minimum and maximum intensity of infection (minimum and maximum number of parasites in the host). The medicated feed was prepared according to veterinary prescription at the firm Tomáš, s.r.o., certified by the Central Agricultural Inspection and Testing Institute in Bratislava. The composition of the granules was 33% maize, 33% wheat, 33% wheat bran and 1% fenbendazole by weight. The source of fenbendazole was the Fenbion® powder form that is used in research. Two-year old common carp (K2) weighing 450 to 1060 ± 159.72 grams were included in the research. The faeces were collected from 80 fish using plastic tubes and syringes; 45 of them were positive for tapeworm eggs. The faeces were examined by a flotation method using BAF-Sheather's solution with the addition of sodium benzoate. In total, 45 positive common carp were divided into three groups labelled F1 to F3, with 15 fish in each group; the F3 group was used as a control. The average weight of the fish was 692.86 ± 169.66, 731.33 ± 167.92 and 726.66 ± 130.72 grams in groups F1, F2 and F3, respectively. One feed dose represented 3% of the fish population's weight in the group. In the F1 group, the medicated feed mixture was administered only once in a total amount of 312 grams (Fenbendazole 75.01 mg/kg fish) and the fish were dissected 24 hours later. In the F2 group, the medicated feed mixture was administered in three doses, in a total amount of 3 × 327 g = 981 g (final concentration of fenbendazole of 223.34 mg/kg fish). The time interval between feed doses and subsequent dissection was always 48 hours. F3, as a control group, was fed with a non-medicated mixture three times. The efficacy of fenbendazole against carp tapeworms in practice was tested on carp placed in storage ponds filled with fish pond water at a temperature ranging from 26.3 °C to 27.5 °C and an oxygen content ranging from 4.9 mg/l to 6.1 mg/l at the peak of the vegetation period from August 7–17, 2015.

The reduction in the prevalence of tapeworm-infested fish was compared using Pearson's Chi-squared test with Yates' continuity correction, calculated in Excel, taking $P \leq 0.05$ to be statistically significant. Comparison with the control group showed the reduction in prevalence in the F2 group to be statistically significant, but in the F1 group the reduction was not statistically significant.

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Table 1. Number of carp examined for the presence of tapeworms

Date	Category	Number of carp		Prevalence (%)	Number of tapeworms/group	Mean intensity of infection (min–max)
		examined	infected			
29.12.2012	K3–K4	30	12	40.00	34	2 (1–4)
24.10.2012	K3–K4	20	11	55.00	58	9 (1–35)
27.03.2013	K3–K4	15	5	33.30	14	2 (1–4)
04.04.2014	K3–K4	20	9	45.00	24	3 (1–6)
09.11.2014	K3–K4	55	20	36.36	35	2 (1–4)
15.11.2014	K3–K4	26	11	42.30	15	1 (1–2)
18.11.2015	K3–K4	8	4	50.00	6	2 (1–4)
20.12.2016	K3–K4	26	10	38.46	24	2 (1–4)
Total		200	127	63.50	210	1.7 (1–35)
Tapeworm species						
K. sinensis				78.57	165	
A. huronensis				21.42	45	

K3 = three-year old common carp; K4 = four-year old common carp

RESULTS

The presence of two tapeworm species (*Atractolytocestus huronensis* and *Khawia sinenis*) with a prevalence of 63.5% was found in the Veľké Blahovo pond system in the period 2012–2016 (Table 1). Fish to which fenbendazole was administered in one dose (F1) were infected with a total number of five individual tapeworms. These five individuals (*K. sinensis)* were attached to the mucosa in the first third of the intestine. In the F2 group, two individuals of the *Atractolytocestus huronensis* tapeworm were firmly attached to the wall of the small intestine. Fish in the F1 and F2 groups exhibited high activity and voracious appetites, while fish in the F3 control group, being the most infested, exhibited normal behaviour. In the F3 group, all tapeworms were firmly attached to the intestinal wall in every part. The proportion of tapeworm species was as follows: *Khawia sinenis*, 27 individuals (93.10%); *Atractolytocestus huronesis*, 2 individuals (6.89%). When comparing fenbendazole-administered groups with the control, the reduction in the prevalence of infection was statistically significant ($P \leq 0.05$) in the F2 group; however, it was not statistically significant in the F1 group (Table 2).

DISCUSSION

A. huronenis was confirmed in Slovakia in 2003 based on the examination of 16 carp from the Tisa River close to Veľké Trakany village and 13 carp from the Veľký Kamenec water basin in south-eastern Slovakia performed in the period

Table 2. Experimental groups of common carp receiving fenbendazole and control group

Group	Category	Number of carp	Prevalence (%)	Number of tapeworms	Number of tapeworms (min–max)	Average	± SD
F1	K2	15	26.66[a]	5	(1–2)	1.3	± 0.57
F2	K2	15	13.33[b]	4	(1–2)	2	± 0.77
F3	K2	15	66.66[a]	20	(1–5)	2.2	± 1.62
Tapeworm species							
K. sinensis			93.10	27			
A. huronensis			6.89	2			

Groups with different superscripts differ significantly at $P < 0.05$

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from May to October 2003 (Oros et al. 2004). *A. huronesis* tapeworms were detected in the intestines of farmed common carp (*Cyprinus carpio*) in several fish farms in Hungary (Majoros et al. 2003). Based on the results of this study, we can say that for use in aquaculture practice, it is necessary to apply the feed several times in repeated cycles, in order to minimise the risk of recurrent infection and transmission to other individuals or pond stocks. Administration of fenbendazole appears to be relatively less effective against *A. huronesis* than *K. sinensis*. This may be because the scolex of *A. huronesis* is more deeply embedded in the intestinal wall. The incidence of *A. huronensis* in the fish intestines was relatively low, compared to that of *K. sinensis.* Gjurcevic et al. (2012) presented data on the pathogenic effect of a non-native cestode, *Atractolytocestus huronensis* (Caryophyllidea), on common carp cultured at a fish farm in Croatia. Histopathological examination revealed that *A. huronensis* causes only local damage within the infected part of the intestine. No differences were observed in the pathological effect of the tapeworm on fish of different age classes. In the farm studied, no mortalities or increased losses during wintertime were observed. The cestode was found in all examined age classes (i.e. carp fry, one-year old and two-year old carp), and the intensity of infection ranged from one to 183 parasites per fish (mostly four to nine). The highest prevalence was observed during August in both one- and two-year old carp, with an absence of parasites in April and during the winter period (end of December). We did our research in August with the highest probability of tapeworm occurrence. Some studies have demonstrated that *K. sinensis* causes serious inflammation of the intestine of the fish host and mechanical destruction of its intestinal epithelium (Jara and Szerow 1981; Morley and Hoole 1995; Morley and Hoole 1997). Several cases of mortality of carp fry were also documented in Europe (Williams and Jones 1994), whereas other studies did not confirm a pathogenic effect of the tapeworm even on heavily infected fish hosts (Chubb and Yeomans 1995). The veterinary importance of *K. sinensis* seems to have decreased in recent times (Oros et al. 2009). The results showed a year-round occurrence of the parasite with the highest carp infestation in late spring (May) followed by the gradual exit of mainly gravid individuals from the host in late June and early July. Re-infection of fish occurred from mid-July. Tapeworms with eggs were found throughout the monitoring period. Although dehelminthisation reduced the number of infected fishes, their number increased again within a month due to the re-invasion of fish. In intensive aquaculture the risk of parasite transmission and consequent production losses is high. Therefore, for the profitability of carp production it is important to minimise pathogen impact. Larval stages of the parasite can survive in intermediate hosts also in the winter season when the pond is drained and the bottom is frozen (Scholz et al. 1990). Various chemicals including praziquantel, benzimidazoles and niclosamides are recommended for the oral treatment of fish tapeworms (Treves-Brown 2000); however, there are no determined maximum residue limits in fish for these substances, which means the maximum level of a chemical which is permitted in human food, expressed in milligrams of the chemical per kilogram of the food unless otherwise stated. A registered antiparasitic is not available, with parasitosis being the most common diagnosis in fish farming. The use of praziquanel is suitable for the control of helminthosis in fish (Sudova et al. 2010; Ishimaru et al. 2013). Pharmacokinetic studies, among others, indicate that praziquantel treatment is highly effective and relatively safe (Ishimaru et al. 2013), which holds the great benefit of allowing single-agent oral administration at a relatively low price (Cioli et al. 1995). Another option is to use the preparation of the active substance fenbendazole. This product is usually administered repeatedly, either as a bath or orally (Noga 2010). Nevertheless, the development of a tapeworm eradication procedure is necessary for continued international shipment of tapeworm-free fish (Mitchell 2004) and to ensure an economically profitable rate of fish growth. For fish farming production, fenbendazole can be used in practice in Slovakia unlike praziquantel, because fenbendazole is approved as an anthelmenthic agent in veterinary practice. A complex of multiple zoohygienic measures should be followed in order to reduce the occurrence and prevalence of fish tapeworm infections. The basic measures include wintering, liming of ponds together with administration of medicated feed during the vegetation season. A complex of measures is needed to reduce fish infections and the use of medicated feed is only one of these. More research on the use of medicated feed is warranted.

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