VETERINARSKI ARHIV 82 (3), 273-282, 2012

Pathogenicity of *Atractolytocestus huronensis* (Cestoda) for cultured common carp (*Cyprinus carpio* L.)

Emil Gjurčević[1]*, Ana Beck[2], Krešimir Drašner[3], Damir Stanin[4], and Snježana Kužir[5]

[1]*Department for Biology and Pathology of Fish and Bees, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[2]*Department of General Pathology and Pathological Morphology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[3]*Fish Farm Poljana, Kaniška Iva, Croatia*

[4]*Department of Radiology, Ultrasound Diagnostics and Physical Therapy, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[5]*Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

GJURČEVIĆ, E., A. BECK, K. DRAŠNER, D. STANIN, S. KUŽIR: Pathogenicity of *Atractolytocestus huronensis* (Cestoda) for cultured common carp (*Cyprinus carpio* L.). Vet. arhiv 82, 273-282, 2012.

ABSTRACT

This study presents 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 1 to 183 parasites per fish (mostly 4 to 9). 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).

Key words: *Atractolytocestus huronensis*, common carp, histopathology, pathogenicity

Introduction

Over the past two decades, the non-native monozoic cestode *Atractolytocestus huronensis* (Caryophyllidea), originally described in the USA (ANTHONY, 1958), has

*Corresponding author:

Emil Gjurčević PhD. Department for Biology and Pathology of Fish and Bees, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10 000 Zagreb, Croatia, Phone: +385 1 2390 152; Fax: +385 1 2441 390; E-mail: egjurcevic@vef.hr

ISSN 0372-5480
Printed in Croatia

spread throughout Europe. Following the first report in Great Britain (CHUBB et al., 1996), *A. huronensis* has been reported in several other European countries, i.e. Hungary, Czech Republic, Slovakia, Germany and Romania (MAJOROS et al., 2003; OROS et al., 2004; KAPPE et al., 2006; BAZSALOVICSOVÁ et al., 2011).

Until recently, only two cestodes, namely *Khawia sinensis* (Caryophyllidea) and *Bothriocephalus acheilognathi* (Bothriocephalidea), were reported more frequently from cultured common carp in Croatia. *A. huronensis* was first detected in 2005 (GJURČEVIĆ et al., 2009). This parasite was most likely introduced to Croatia via import of infected carp from Hungary. Today, *A. huronensis* is widely distributed among fish farms throughout the country (personal communication).

Although the histopathological changes caused by this caryophyllidean cestode were studied by MACKIEWICZ et al. (1972), MOLNÁR et al. (2003) and WILLIAMS (2007), the impact of this parasite on the carp cultures is still not fully known. Therefore, the aim of this study was to investigate its implications on fish health. Furthermore, this paper presents the morphological characteristics of *Atractolytocestus* specimens collected and data on the prevalence of infection in different age classes of cultured common carp during the year in a fish farm in Croatia.

Materials and methods

This study involved different age classes of common carp (*Cyprinus carpio* L.) collected from a fish farm in Croatia from April to September. In addition, several one- and two year old carp were collected during the winter (at the end of December). A total of 730 fish, including carp fry (n = 141), one-year old carp (n = 471) and two-year old carp (n = 118) were examined. Following dissection, the intensity of infection was evaluated and its prevalence was calculated.

For the purpose of morphological observation, cestodes were isolated, fixed in hot 4% formaldehyde solution, stained with alum carmine or borax carmine, dehydrated in ethanol series and mounted in Canada balsam or glycerin jelly. A total of 15 specimens were measured using an Olympus DP 12 digital camera and Cell B software (Soft Imaging System). Some specimens were also routinely processed for histology, cut longitudinally and stained with haematoxylin and eosin (H&E).

Up to ten heavily infected fish (more than 12 parasites per fish) of each age class were processed for histopathology. Prior to processing, all fish were examined for gross pathological changes. For histopathology, carp fry were collected in June, one-year old carp in July and two-year old carp in August. Samples of the intestine, kidney, spleen, swim-bladder and liver (hepatopancreas) of each fish were removed and fixed in 10% neutral buffered formalin. The fixed material was embedded in paraffin and serially sectioned at 5 μm. Sections were stained with H&E, periodic acid-Schiff (PAS), toluidine blue, Gram, Ziehl-Neelsen and van Gieson stain.

Results

The morphological characteristics and measurements of the caryophyllidean cestode *A. huronensis* (Fig. 1) observed in this study are presented in Table 1.

Table 1. Comparison of morphological characteristics of *A. huronensis* found in cultured common carp from different European countries; adapted from OROS et al. (2004)

	Reference (country)					
	Present study (Croatia) n = 15	Majoros et al. (2003) (Hungary)	Oros et al. (2004)			Kappe et al. (2006) (Germany)
			(Czech Republic)	(Slovakia)	(Hungary)	
Body length (mm)	4.3-7.4	3.0-9.0	4.0-9.0	5.0-8.0	3.0-7.5	5.1-7.9
Body width (mm)	0.6-0.9	0.5-1.2	0.5-0.9	0.5-0.8	0.4-0.6	0.9-1.3
Scolex width (µm)	374-714	-	415-938	516-741	342-497	500-900
Distance between first vitelline follicles to anterior extemity (mm)	0.9-1.6	-	0.9-1.9	0.8-1.1	0.8-1.8	-
Distance between first testes to anterior extremity (mm)	1.2-1.7	-	1.0-2.6	1.1-1.5	1.0-2.2	-
Distance between first vitelline follicles and first testes (µm)	169-390	-	213-703	250-384	156-407	-
Testis number	7-20	3-5	9-14	16-20	6-10	2-14
Cirrus-sac length (µm)	265-504	350-450	350-555	506-617	232-417	325-575
Cirrus-sac width (µm)	229-329	-	214-347	299-374	210-276	275-375
Ovary width (µm)	481-589	600-900	297-482	421-574	237-440	500-850
Ovarian arm length (µm)	433-535	800-1100	365-691	446-703	203-466	-
Ovarian arm width (µm)	132-182	-	117-172	134-210	83-147	-
Extent of uterus in relation to length of testicular area	1/2	-	1/3-1/2	1/3-1/2	1/4-1/3	-
Egg length (µm)	47-51	-	46-67	48-52	47-50	-
Egg width (µm)	30-35	-	30-39	35-36	28-31	-

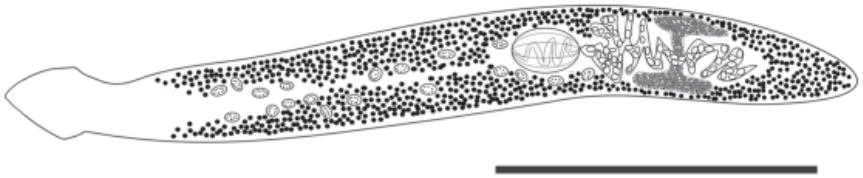

Fig. 1. *Atractolytocestus huronensis* from cultured common carp (*Cyprinus carpio* L.); line drawing. Scale bar = 2 mm.

Cestodes were found in all examined age classes of common carp, and were always localized in the anterior part of the intestine. The overall prevalence of infection was 3.2% in carp fry, 7.8% in one-year old carp, and 3.8% in two-year old carp. The intensity of infection ranged from 1 to 183 parasites per fish (mean intensity = 10.6 parasites). Throughout the sampling period, the prevalence in one- and two-year old carp showed fluctuations. However, in both one- and two-year old carp, the highest prevalence was observed during August, whereas no *A. huronensis* was found in April or during the winter period (end of December). No comparison could be made for carp fry since fry were only available in June. The prevalence of infection data are summarized in Table 2.

Table 2. Numbers of fish examined (n) and prevalence (P) of infection in different age classes of common carp throughout the sampling period

Sampling date	Carp fry		One-year old carp		Two-year old carp	
	n	P (%)	n	P (%)	n	P (%)
April	-	-	20	0.0	20	0.0
May	-	-	62	12.9	22	9.1
June	141	16.3	30	6.7	7	14.3
July	-	-	71	15.5	31	25.8
August	-	-	117	16.2	16	87.5
September	-	-	166	10.2	15	20.0
December	-	-	5	0.0	7	0.0

During the survey, there were no mortalities of carp or increased losses during over-wintering.

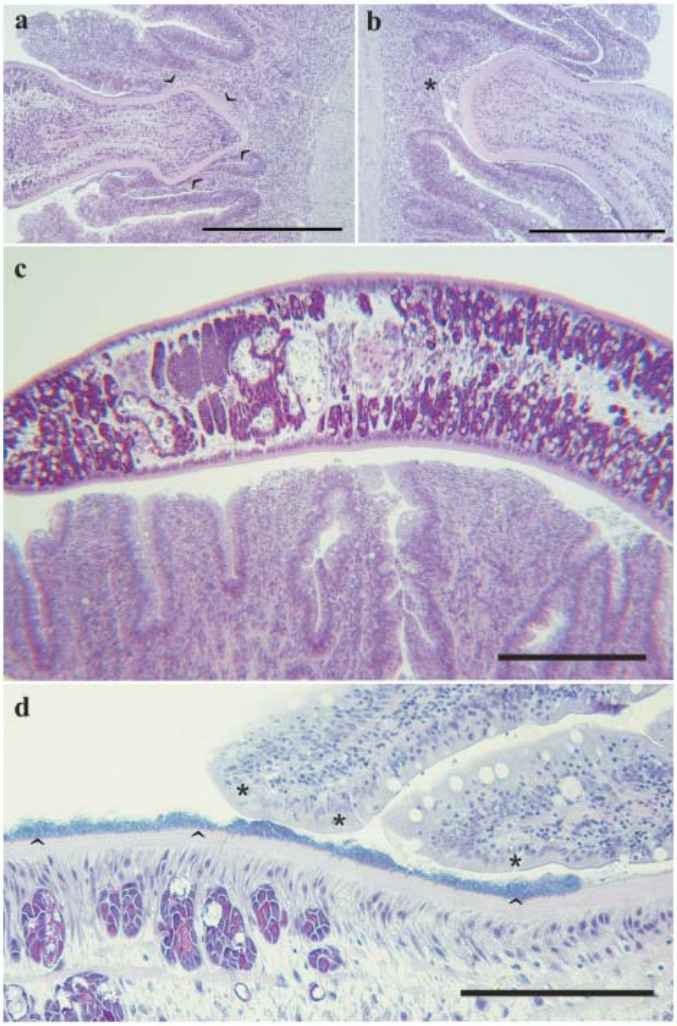

Fig. 2. Common carp intestine infected with *Atractolytocestus huronensis*; histological sections. (a) Attachment site of *A. huronensis* with atrophy and destruction of mucosal epithelium (arrowheads). H&E, scale bar = 500 µm. (b) Within the attachment site, loss of basement membrane leads to shallow ulceration (✶). H&E, scale bar = 500 µm. (c) Erosion of the apical part of intestinal folds adjacent to the parasite body. H&E, scale bar = 500 µm. (d) Masses of bacteria on the tegument of the neck and body (arrowheads). Note intact mucosal epithelium (✶). PAS, scale bar = 200 µm.

Gross pathology and histopathology. No gross pathological changes were found in any of the examined carp fry or two-year old carp. In contrast, two of the ten one-year old carp collected in July showed swim-bladder inflammation and slight enlargement of the kidney. Histological examination of the swim-bladder and trunk kidney of these two fish revealed different developmental stages of *Sphaerospora* sp. Histopathological changes associated with *Sphaerospora* infection were predominantly found in the swim-bladder submucosa and were characterized by edema, vascular congestion, disseminated focal hemorrhages and a massive infiltration of mononuclear cells, leading to a thickening of the swim-bladder wall. Slight fibrin exudation and small foci of necrosis were also evident in the submucosal loose connective tissue. In the trunk kidney, changes were characterized by the dilatation of tubules, accompanied by atrophy, hyperplasia, vacuolar degeneration and necrosis of tubular epithelial cells. Occasionally, small areas of necrosis were observed within the interstitial haematopoietic tissue.

All age classes of carp harboring *Atractolytocestus* infection showed numerous histopathological changes within the infected part of the intestine (Fig. 2). There were no differences in histopathological findings in the intestine between the examined age classes. In general, cestodes were attached to the intestinal mucosa. At the site of attachment, atrophy and destruction of the mucosal epithelium was evident, and the basement membrane was usually intact. However, necrosis of epithelial cells was commonly observed in the basal part of the intestinal folds. Parasites only occasionally penetrated through the lamina propria into the submucosa and caused shallow ulceration. In some areas, the normal architecture of the neighboring crypt was destroyed at the site of penetration. On the surface of the intestine, erosion of the apical part of the intestinal folds was observed adjacent to the parasite body. Occasionally, masses of bacteria were evident on the surface of the parasite body. A moderate infiltration of lymphocytes and eosinophilic granular cells, and a mild infiltration of macrophages were recorded in the deeper intestinal layers (i.e. lamina propria and submucosa).

In addition to these histopathological changes in the intestine, changes of unknown etiology were also observed in the kidney, spleen and liver of several heavily infected fish. Formation of multiple granulomas was evident in the kidney and spleen in two of the ten one-year old carp, and degenerative changes of hepatocytes (hydropic degeneration) were observed in the liver tissue in three of the nine two-year old carp.

Discussion

One of the purposes of this study was to determine the prevalence of *A. huronensis* in different age classes of common carp during the year and to provide detailed morphological characteristics of specimens from Croatia. Morphologically, Croatian *A. huronensis* mainly correspond to those found in cultured common carp from Continental

Europe (Table 1). The genetic identity of *A. huronensis* specimens of the Hungarian, Slovakian, Croatian and Romanian populations confirms that they form a single genetic pool (BAZSALOVICSOVÁ et al., 2011). This molecular finding supports the assumption that *A. huronensis* was introduced to Croatia through the import of infected carp from Hungary. Such uncontrolled imports of common carp brood-stock from Hungary also resulted previously in the introduction of *Dermocystidium koi* (GJURČEVIĆ et al., 2008). It is essential to subject carp importation to regular health monitoring and quarantine, but these measures were apparently not sufficient during previous imports of carp to Croatia.

Both a previous study (MAJOROS et al., 2003) and our present study indicate that *A. huronensis* can infect common carp of all ages (both young fish and adults). In the earlier study, the prevalence differed between age classes, i.e. it was highest in fry and lowest in two-year old carp. In contrast, observations from a fishery in England indicated a general trend of increasing parasite prevalence with host size, peaking at the host range of 32.3−36.8 cm (WILLIAMS, 2007). In this study, however, only common carp ranging from 11.3 to 70.6 cm in length were examined and data on smaller fish or fry were not available.

In the present study, the prevalence was highest in one-year old carp and lowest in fry, but fry were available only in June, when the prevalence may have been at its minimum, as indicated by its low values in late spring in older fish (Table 2). The prevalence of infection depends on many factors, but in this case, the lower prevalence in fry is likely a consequence of the regular maintenance (drying, cleaning of the pond bottom and liming) of fry rearing ponds.

Regarding the seasonal occurrence of *A. huronensis*, MAJOROS et al. (2003) reported the highest prevalence in one-year old carp in Hungary in October, whereas the highest prevalence in two-year old carp was observed in July. At the same time, the data presented in this paper show fluctuations in prevalence throughout the year, with a maximum peak in both one- and two-year old carp in August and an absence of parasites in April and during the winter period (end of December). To some extent, this may be due to the small sample size (especially for the small number of fish examined during the winter period). However, it would be more reasonable to assume that the seasonal variation in the prevalence of *A. huronensis* may be related to the feeding habits of these two age classes of common carp and the availability of intermediate host(s). Further studies are necessary to understand better the seasonal occurrence of *A. huronensis*, which may aid in controlling the infection.

Histopathological findings in the intestines are in line with the findings described by MOLNÁR et al. (2003) and WILLIAMS (2007). In general, *A. huronensis* causes localized histopathological changes at the site of attachment. Observed shallow ulceration at

the attachment site and a moderate eosinophilic infiltration of the area adjacent to the scolex correspond to an earlier description by WILLIAMS (2007). Furthermore, erosion of the intestinal folds was observed adjacent to the body of the parasite. Similar but less pronounced changes were also recorded by MOLNÁR et al. (2003). It was suggested that this could be due to the proteolytic enzymes of *A. huronensis*. Although the results of our study revealed no evidence for the presence of secondary bacterial infections, coinfection with *Sphaerospora* sp. and some changes of unknown etiology (i.e. multiple granulomas in the kidney and spleen, and hydropic degeneration of hepatocytes) were observed in several heavily infected fish. However, this finding cannot be attributed to *A. huronensis* infection itself.

Despite the more or less obvious histopathology, the effect of the infection on common carp populations is still a matter of debate. According to MACKIEWICZ et al. (1972) and MOLNÁR et al. (2003), epithelial changes and tissue damage caused by *A. huronensis* are important pathological factors *per se*. Moreover, MOLNÁR et al. (2003) indicated that despite its smaller size, *A. huronensis* may play a more important pathological role than *K. sinensis*, and it can cause more severe histopathological changes than other tapeworms parasitizing carp (*C. fimbriceps*, *K. sinensis* or *B. acheilognathi*). However, these authors concluded that more data are necessary to evaluate the economic importance of *A. huronensis* and possible losses in pond farms.

In the present study, no mortalities due to infection or increased losses during overwintering were observed. Similarly, WILLIAMS (2007) reported that lesions caused by the parasite are generally mild and do not pose a serious threat to infected hosts. This view was supported by the fact that the infection does not have any adverse effect on the host's condition or blood composition. Despite this fact, it should be noted that the above study was not conducted in all age classes of common carp. On the other hand, our histopathological examination did not reveal differences between age classes. In conclusion, the results of our survey correspond to those reported by WILLIAMS (2007). Thus, we believe that *A. huronensis* should not be considered a serious pathogen of cultured common carp.

Acknowledgements

The authors would like to thank Tomáš Scholz for reading the manuscript and for his suggestions. This study was supported by the Ministry of Science, Education and Sport of the Republic of Croatia (project No. 053-0010501-1977).

References

ANTHONY, J. D. (1958): *Atractolytocestus huronensis* n. gen., n. sp. (Cestoda: Lytocestidae) with notes on its morphology. Trans. Am. Microsc. Soc. 77, 383-390.

BAZSALOVICSOVÁ, E., I. KRÁLOVÁ-HROMADOVÁ, J. ŠTEFKA, T. SCHOLZ, V. HANZELOVÁ, S. VÁVROVÁ, T. SZEMES, R. KIRK (2011): Population study of *Atractolytocestus huronensis* (Cestoda: Caryophyllidea), an invasive parasite of common carp introduced to Europe: mitochondrial *cox1* haplotypes and intragenomic ribosomal ITS2 variants. Parasitol. Res. 109, 125-131.

CHUBB, J. C., R. KIRK, I. WELLBY (1996): Caryophyllaeid tapeworm *Atractolytocestus huronensis* Anthony, 1958 (= *Markevitschia sagittata* Kulakovskaya & Akhmerov, 1965) in carp *Cyprinus carpio* L. in British Isles - another translocation? Abstracts of the Spring Meeting of the British Society for Parasitology, 1-3 April. Bangor, Wales. pp. 66.

GJURČEVIĆ, E., S. BAMBIR, Z. KOZARIĆ, S. KUŽIR, A. GAVRILOVIĆ, I. PAŠALIĆ (2008): Dermocystidium infection in common carp broodstock (*Cyprinus carpio* L.) from Croatia. Bull. Eur. Ass. Fish Pathol. 28, 222-229.

GJURČEVIĆ, E., S. BAMBIR, A. BECK (2009): *Atractolytocestus huronensis* Anthony, 1985 from farmed common carp in Croatia. Abstract book of the 14th International Congress of the European Association of Fish Pathologists, 14-19 September. Prague, Czech Republic. pp. 235.

KAPPE, A., T. SEIFERT, G. EL-NOBI, G. BRÄUER (2006): Occurrence of *Atractolytocestus huronensis* (Cestoda, Caryophyllaeidae) in German pond-farmed common carp *Cyprinus carpio*. Dis. Aquat. Org. 70, 255-259.

MACKIEWICZ, J. S., G. E. COSGROVE, W. D. GUDE (1972): Relationship of pathology to scolex morphology among caryophyllid cestodes. Z. Parasitenk. 39, 233-246.

MAJOROS, G., Gy. CSABA, K. MOLNÁR (2003): Occurrence of *Atractolytocestus huronensis* Anthony, 1958 (Cestoda: Caryophyllaeidae), in Hungarian pond-farmed common carp. Bull. Eur. Ass. Fish Pathol. 23, 167-175.

MOLNÁR, K., G. MAJOROS, G. CSABA, C. SZÉKELY (2003): Pathology of *Atractolytocestus huronensis* Anthony, 1958 (Cestoda, Caryophyllaeidae) in Hungarian pond-farmed common carp. Acta Parasitol. 48, 222-228.

OROS, M., V. HANZELOVÁ, T. SCHOLZ (2004): The cestode *Atractolytocestus huronensis* (Caryophyllidea) continues to spread in Europe: new data on the helminth parasite of the common carp. Dis. Aquat. Org. 62, 115-119.

WILLIAMS, C. (2007): Impact assessment of non-native parasites in freshwater fisheries in England and Wales. PhD Thesis. Institute of Aquaculture, University of Stirling. Stirling, Scotland.

Received: 4 July 2011
Accepted: 4 November 2011

GJURČEVIĆ, E., A. BECK, K. DRAŠNER, D. STANIN, S. KUŽIR: Patogenost trakavice *Atractolytocestus huronensis* za šarana (*Cyprinus carpio* L.) iz uzgoja. Vet. arhiv 82, 273-282, 2012.

SAŽETAK

U radu je prikazan patološki učinak trakavice *Atractolytocestus huronensis* (Caryophyllidea) na šarana, podrijetlom s ribnjačarstva smještenog u Republici Hrvatskoj. Histopatološki nalaz pokazao je da, neovisno o uzrasnoj skupini šarana, *Atractolytocestus huronensis* uzrokuje samo ograničena oštećenja na mjestu prihvaćanja. Kao posljedica nametničke invazije, nisu utvrđena uginuća niti povećani gubitci tijekom zime. Prisutnost trakavice utvrđena je u svim pregledanim uzrasnim skupinama (mladunci, jednogodišnji i dvogodišnji mlađ) s jačinom invazije od 1 do 183 nametnika po domaćinu (najčešće od 4 do 9). Najviša prevalencija kod jednogodišnjega i dvogodišnjega mlađa utvrđena je u kolovozu, dok u travnju i tijekom zime (kraj prosinca) trakavica nije pronađena.

Ključne riječi: *Atractolytocestus huronensis*, šaran, histopatologija, patogenost