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# Occurrence and intensity of hepatic capillariasis in the barbel (*Barbus rebeli*) from the river Crn Drim in the Republic of Macedonia

## Присуство и интензитет на хепаталната капиларијаза кај црната мрена (*Barbus rebeli*) од реката Црн Дрим во Република Македонија

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**Abstract**

Capillariasis is one of the many parasites which can infect different fish tissues including the liver. The aim of our study is to demonstrate incidence of hepatic capillariasis in the population of barbel (*Barbus rebeli*) from the river Crni Drim, histopathological features of the liver of infected barbels, as well as associations of the infection with macrophage cells, involved in the non-specific immune response in fish. We use stereological methods to document the percentage of liver that is occupied by parasitic eggs and macrophages. Capillariid eggs were found in the liver of 68% of examined barbells. Investigations of histological slides showed that the intensity of infection varies, from one to 150 eggs per slide of liver tissue. They occupied 0.01% to 24.3% of the liver parenchyma volume. Necrosis, lymphocyte infiltration and fibrosis were weakly associated with capillariasis infection. Pigmented macrophages were found often in association with the parasite eggs. Significant positive correlation was found between the presence of capillariasis eggs and the macrophage amount. Such findings were not recorded for any fish species infected with capillariasis according to our knowledge. Moreover results of parasitic infections in barbel fish in the river Crn Drim can serve to warn fishermen and local citizens of potential risk to their health.

**Keywords:** parasites, fish, liver, pigmented macrophages

Капиларијазата е еден од многуте паразити кои можат да инфицираат различни ткива кај рибите, вклучувајќи го и црниот дроб. Цел на нашето истражување беше да ја определиме застапеноста на хепатичната капиларијаза кај популацијата на црна мрена (*Barbus rebeli*) од реката Црн Дрим, хистопатолошките промени во црниот дроб кај инфицираните риби, како и асоцијацијата помеѓу паразитската инфекција и макрофагните клетки инволвирани во неспецифичниот имун одговор кај рибите. Со примена на стереолошки методи ја

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документираме процентуалната застапеност на паразитските јајца и макрофагите во црниот дроб. Јајца од капиларијаза се пронајдени во црниот дроб кај 68% од испитуваните црни мрени. Испитувањето на хистолошките препарати покажа дека интензитетот на паразитската инфекција варира од едно до 150 јајца на пресек. Јајцата окупираат од 0.01% до 24.3% од волуменот на црниот дроб. Некрозата, лимфоцитната инфилтрација и фибриозата се слабо присутни кај инфицираните единки. Меѓутоа, пронајдовме дека пигментираните макрофаги се често во асоцијација со паразитските јајца. Значителна позитивна корелација постои помеѓу присуството на јајца од капиларијазата и количеството на макрофаги. Според наши сознанија ваквата појава досега не е евидентирана кај ниеден вид риба инфицирана со капиларијаза. Покрај ова, добиените резултати за паразитската инфекција кај црните мрени од Црн Дрим имаат за цел да ги предупредат риболовците и локалното население за опасноста која постои по нивното здравје.

**Клучни зборови:** паразити, риби, црн дроб, пигментирани макрофаги

## Introduction

The primary host of hepatic capillariasis are rodents although it can be found in all mammal species including humans (Fuehrer et al. 2011). Capillariasis can also infect fish species. In fish parasitic infection with nematodes, such as capillariasis are an important issue (Moravec 1994; Thilakaratne et al. 2003; Sattary et al. 2005) because in some regions they can represent a serious health problem for humans including mortality (Fuehrer et al. 2011). Parasites in fish can be transmitted from fish to humans by eating uncooked infected fish (Yong-Yill 2010).

In general, knowledge of capillariid nematodes in fish species is scarce and often they remain unidentified. However limited information for capillariasis has been recorded in the literature for some fish species (Simá et al. 1996; Roganovic-Zafirova et al. 2003; Moravec & Justine 2014).

In our previous research we found hepatic capillariasis in barbel from the Lake Ohrid for which we assumed that it is probably *Schulmanela petruschewskii* species (Roganovic-Zafirova et al. 2003). When we examined the liver structure in barbel (*Barbus rebeli*) from the river Crn Drim, which outflows from the Ohrid Lake, we discovered the same capillariasis eggs on light microscopy level. The aim of this paper is to demonstrate that the examined barbel species are frequently infected by capillariasis eggs. We will document their prevalence resulted by parasite infections and hepatic pathology as well as associations of the infections with non specific immune response. Particular attention was paid to the pigmented macrophages in association with capillariasis eggs, which was observed for the first time in the fish infected with hepatic capillariasis.

## Material and methods

In total 279 specimens of barbel were collected along the river Crn Drim. The fish were captured with electrofishing and transported with an aerated container to the laboratory. In the laboratory fish were killed by severing the spinal cord. Weight (BW) and length (TL) were recorded for the estimation of condition factor (CF) according to the following formula $CF = BW \times 100/TL^3$. The livers were removed and weighed (LW) and immediately placed in a Bouin's fixative for 48 h. After fixation, they were embedded in paraffin, according to the standard histological protocol, sectioned in 5 μm thick sections and stained with Haematoxylin and Eosin (H&E). To estimate the amount (relative volume) of parasitic eggs and macrophages (MACs) within hepatic parenchyma standard point techniques were applied (Feere & Weibel 1967), according to following formula:

$$V_v(\text{structure, reference}) = [P(s) \times 100] \div P(r),$$

where $\mathbf{V_v}$ is the percentage of the total volume of a reference space occupied by one particular given structure within that space, **P(r)** is the number of points falling over a chosen structural component, and **P(s)** is the total number of test points lying over the reference space.

The $V_v$ of each structural element was estimated within a well-defined reference space, which in this case was the entire liver tissue. For manual point counting a square lattice grid with 180 points was inserted into the ocular of the Reichert microscope. From each animal in order to quantify the amount of parasites and macrophages at least 5 liver tissue pieces were used, per animal. From each block and from each section, 10-20 fields

sampled systematically were quantified. In total 50-100 fields per fish were point counting The first file was selected with a random number. Final magnification was 100x.

The obtained data was presented by the mean value, accompanied by the standard deviation. For statistical analyses Statistica 7 was used and with ANOVA we studied the effect of parasite infection on the CF and the LW of the fish. The ANOVA was followed by a post hoc Tukey test. Correlation analyses were used to find specific associations between the amount of capillariid eggs and the MACs amount. Differences were considered as significant when $p<0.05$.

## Results

The mean values obtained for BW, CF and LW of the examined fish without (WP) and with (P) hepatic capillariasis infection are presented in Table 1. Fish infected with parasites (capillariasis eggs) have higher statistically significant ($p<0.01$) LW compared with uninfected fish. Investigation of histological slides showed that intensity of infection varies from one egg to 150 eggs per liver tissue on a slide (Fig. 1). They occupied 0.01% to 24.3% of the liver parenchyma volume. Histopathological investigation of the barbel liver revealed a weak presence of pathological disorders in the liver parenchyma. Necrosis, lymphocyte infiltration, fibrosis and cholangipatic changes were the only lesions observed in the investigated fish. Necrosis was observed in 13%, lymphocyte infiltration in 9.7%, while fibrosis and cholangipatic changes were detected in 4.8% of infected fish. MACs were randomly distributed across the hepatic tissue or more often in association with eggs capsule (Fig. 2). Their $V_v$ ranged from 0.03% to 5.08%.

A linear correlation analysis was performed to check the associations between the presence of capillariasis and the average

**Table 1.** Body weight (BW)[1], condition factor (CF) and liver weight (LW) in the barbel without (WP) and with (P) hepatic capillariasis infection

| *Lesion Status* | *BW (g)* | *CF (%)* | *LW (g)* |
|---|---|---|---|
| WP | 107.41 (53.82) | 1.11 (0.152) | 0.98 (1.02) |
| P | 98.60 (52.72) | 1.09 (0.155) | 1.34 (1.11) * |

[1]Values are expressed as mean (standard deviation).

*Values are significantly different ($p<0.01$), according to the Tukey test.

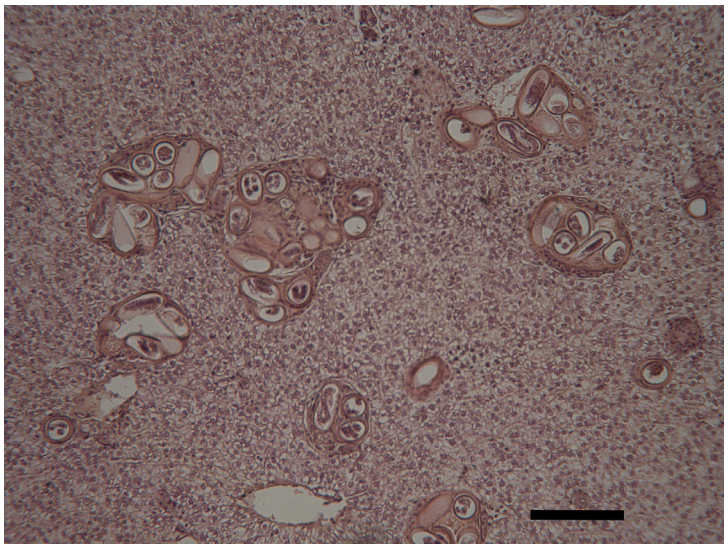

**Figure 1.** A tissue section of the liver showing numerous capillariasis eggs scattered through parenchyma. H&E, Bar line 100μm

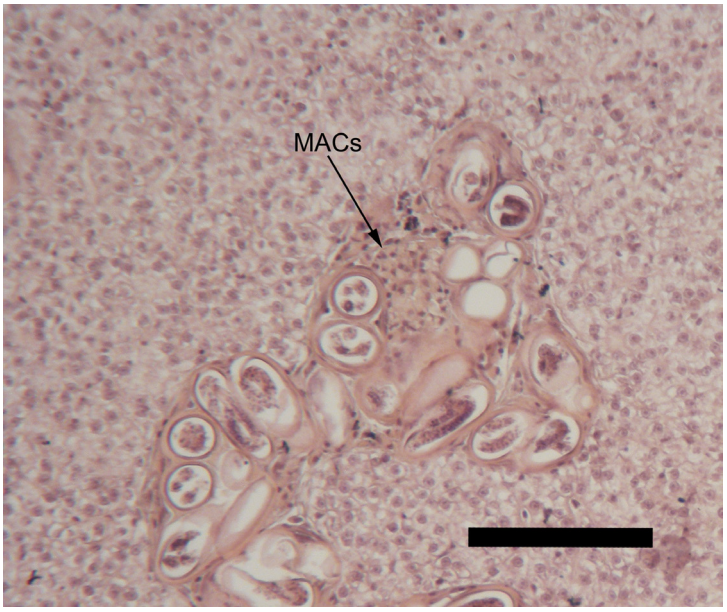


**Figure 2.** In association with cluster of eggs pale yellowish macrophage aggregates (MACs) can be seen. H&E, Bar line 100μm

values of the amount of MACs. This analysis revealed a significantly positive correlation between these two parameters (r=0.245; $P<0.05$).

## Discussion

An adult hepatic capillariasis takes residence in the liver tissue and there the female produces eggs, which can be found in the parenchyma and remain in the liver until death of the animals (Fuehrer et al. 2011). Capillariid eggs were found in the liver of 68% of the examined barbels. Parasitic infection has statistically significant ($p<0.01$) influence the LW. The parasitic eggs were found solitary, or more often several eggs surrounded by a capsule, as it is noted in other fish species with hepatic capillariasis (Simá et al. 1996; Roganovic-Zafirova et al. 2003). Contrary to the results of Roganovic-Zafirova et al. (2003) we did not find any adult parasitic form which could help us to make precise identification of the capillariid species. Roganovic-Zafirova et al. (2003) and Simá et al. (1996) observed necrosis, inflammatory response and fibrosis. Similar findings, fibrosis, necrosis and sometimes granulomatous infection were reported by Oliviera & Andrade (2001). In the present study, reaction of the capillariasis on the hepatic parenchyma was minimal. Necrosis, lymphocyte infiltration and fibrosis were weakly associated with parasite infection. Moreover cholangiopatic changes were extremely rare, only in 4.8% of infected fish, compared to data from Roganovic-Zafirova et al. (2003) where it is very often associated with hepatic capillariasis. On the other hand, compared to other authors we found MACs randomly present in the hepatic tissue, often in association with eggs capsules and they occupied from 0.03% to 5.08% of the liver tissue volume. Such findings were not recorded for any fish species infected with capillariasis according to our knowledge.

We found statistically significant increase in the LW of fish with capillariasis eggs. Investigation on MACs shows that significant positive correlation exists between the presence of capillariasis eggs and the MACs amount. Presence of capillariasis eggs can cause a loss of functional hepatic tissue (Simá et al. 1996). Having in mind the number of infected fish and amount of eggs within the parenchyma, hepatic capillariasis in the investigated barbel probably has an impact on the examined barbel health.

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