

**OCCURRENCE OF *Anguillicola crassus* IN THE WATER RESERVOIR  
KORYČANY (CZECH REPUBLIC) AND ITS INFLUENCE ON THE HEALTH  
CONDITION AND HAEMATOLOGICAL INDICES OF EELS**

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**Abstract**

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The occurrence of the nematode *Anguillicola crassus* in eels in the Water Reservoir Koryčany was studied from 1994 to 2000. In 1998 and 1999 the study was performed at more frequent intervals in order to evaluate possible seasonal changes of the disease and the influence of the parasite on the health condition of eels in this water reservoir.

In all, 143 eels were sampled. Haematological examinations were performed using 46 specimens in 1999. Prevalence of nematodes showed seasonal dynamics and varied from 43 to 100%. Intensity of infection by young and adult nematodes in the swim bladder of eels varied from 1 to 66 specimens, with the mean being 6.6 nematodes per individual. Prevalence of larval stages in the swim bladder amounted from 38.5 to 100%. There were patho-anatomical changes in the swim bladder as well as in the *ductus pneumaticus* found. Severe damage to the swim bladder was observed especially during autumn months. No significant differences were found between infected and non-infected eels, or in dependence on the numbers of nematodes in the swim bladder with regards to the haematological parameters examined (i.e., hematocrit, red and white blood cell counts). The obtained values are also mentioned in relation to the date of sampling. There was a rise ( $p < 0.05$  and  $p < 0.01$ ) in the hematocrit value sampled on October 10, 1999 ( $0.29 \text{ L}\cdot\text{L}^{-1}$ ) in comparison to sampling on June 7, 1999 ( $0.25 \text{ L}\cdot\text{L}^{-1}$ ), September 16, 1999 ( $0.25 \text{ L}\cdot\text{L}^{-1}$ ) and June 30, 1999 ( $0.27 \text{ L}\cdot\text{L}^{-1}$ ), respectively. Red blood cells were significantly ( $p < 0.05$  and  $p < 0.01$ ) lowest on September 16, 1999 ( $1.04 \text{ T}\cdot\text{L}^{-1}$ ) as compared to sampling on June 7, 1999 ( $1.28 \text{ T}\cdot\text{L}^{-1}$ ), October 6, 1999 ( $1.24 \text{ T}\cdot\text{L}^{-1}$ ) and June 30, 1999 ( $1.41 \text{ T}\cdot\text{L}^{-1}$ ), respectively. White blood cell counts were lower ( $p < 0.05$  and  $p < 0.01$ ) on October 6, 1999 ( $27.6 \text{ G}\cdot\text{L}^{-1}$ ) as compared to values obtained on June 7, 1999 ( $56.2 \text{ G}\cdot\text{L}^{-1}$ ), and September 16, 1999 ( $41.2 \text{ G}\cdot\text{L}^{-1}$ ) and June 30, 1999 ( $39.0 \text{ G}\cdot\text{L}^{-1}$ ), respectively. Differential counts of white blood cells showed no significant changes. Infected as well as non-infected eels were characterised by lymphocytic type of blood (88.02 and 90.34 % of lymphocytes, 10.27 and 7.38 % of granulocytes, 0.52 and 0.94 % of monocytes, 1.17 and 1.34 % of blasts, respectively).

Anguillicolosis under conditions of the Koryčany Water Reservoir did not cause mortality and clinical signs of the disease in the population of eels.

*Prevalence, intensity of infection, hematocrit, erythrocytes, leukocytes, differential leukocyte count*

Anguillicolosis of eels is a parasitic disease of the swim bladder caused by the nematode *Anguillicola crassus* Kuwahama, Niimi and Itagaki, 1974. The cycle of development of the parasite includes one inter-mediate host, i.e., copepods (*Copepoda*) (Hartmann and Nellen 1994). Larvae released from eels and devoured by the inter-mediate host become infectious. The next development is either direct or includes parathenic hosts such as fish species feeding on plankton, amphibians, slugs and larvae of aquatic insects (Moravec 1996; Moravec and Škoríková 1996; Baruš et al. 1999b). In the Czech Republic, anguillicolosis was first found in eels in the Elbe River near Hřensko in 1991 (Moravec

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1992). The nematode significantly participated in mass mortality of eels in the Vranov Dam Lake in 1994 (Baruš 1994; Baruš and Kotulán 1997).

This nematode was found in eels from the Koryčany Water Reservoir during studies into the health condition of fish in water reservoirs in 1994. Repeated findings of the nematode in eels in the next years (1995 and 1997) initiated a systematic study. The occurrence of this nematode in the Koryčany Water Reservoir has been studied at regular intervals with the intention of evaluating possible seasonal changes, influence on the health condition of eels and selected haematological indices in relation to the disease (Boon et al. 1989, 1990; Van der Heijden et al. 1996; Baruš et al. 1999cd) and some other factors of the environment influencing the development of anguillicolosis since 1998.

#### Materials and Methods

The Koryčany Water Reservoir lies in south Moravia on the 74.5 km of the Kyjovka River, at the elevation of approximately 290 metres. It is a part of the Morava River catchment area. Its area is 35.15 hectares and the maximum depth of 20 metres. Water temperature (measured at the dam) varies from 0 to 24 °C with maximum values in July and August. Dissolved oxygen concentration varies from 6.0 to 16.0 mg/L with minimum values from August to October.

Using an electric aggregate machine, 5, 4, and 7 specimens of eels (*Anguilla anguilla* Linnaeus, 1758) were captured in 1994, 1995 and 1997, respectively. In 1998, eels were caught on 5 occasions from June to November. A total of 58 eels were obtained for the examination. In 1999, during 5 capturing occasions using the electric aggregate machine in the period from April to October we obtained 64 specimens of the eel. In all, 5 specimens were examined in 2000. A total of 143 eel specimens were examined. Their the mean body mass was of 318.7 g (min. 96 g, max. 650 g, SD 34.6) and the mean body length of 586.4 mm (min. 225 mm, max. 840 mm, SD 63.6).

Eels were transported to the laboratory, stunned and then killed by transection of the spinal cord. Weight and length were determined and individual specimens were inspected externally and submitted to patho-morphological and parasitological examination with special regards to anguillicolosis. Nematodes seen with the naked eye in the cavity of the swim bladder and *ductus pneumaticus* were counted. These nematodes were classified according to Baruš (1994) as young (from 1 to 14 mm of length) and adult ones (over 15 mm) in 1998 and 1999. Since 1998, scrapings of the swim bladder surface have been evaluated microscopically for the presence of 2<sup>nd</sup> stage larvae released by females of the nematode. The presence of 4<sup>th</sup> stage larvae within the swim bladder wall was not evaluated. Otoliths were sampled for the purpose of age determination of the eels examined (Peňáz and Tesch 1970). Only the intensity of infection and prevalence of the disease were evaluated in 1994, 1995 and 2000.

From June to October 1999 the above-mentioned procedures were modified. The collection of eels was kept for 2 to 3 days in laminate tanks and we blood-sampled the eels by cardiac puncture before killing. In these samples we determined hematocrit, red and white blood cell counts and the differential count. The parameters were determined in accordance with "Standard methods of haematology in fish" (Svobodová et al. 1986).

Data were statistically evaluated using hypothesis testing by Student's *t*-test. Software Stat-Plus (Matoušková et al. 1992) was used for this purpose. The statistical analysis of possible relations between haematological parameters and numbers of parasites in the swim bladder was done on groups of infected and non-infected eels and four groups of intensity of infection (0, 1-5, 6-10, >10).

#### Results

##### Prevalence of parasites, intensity of infection

Values of prevalence in individual collections of the eel varied from 43 to 100% (the total mean prevalence of 64.4%). The mean prevalence of nematodes showed seasonal dynamics with its maximum in July (82%) and minimum in April (50%), see Fig. 1. Evaluating the dependence on the age, the highest and lowest prevalence values were found in 10-year-old and 11-year-old eels (78.9%, *n* = 19; and 57.1%, *n* = 7), respectively. In seven-, eight- and nine-year-old eels it was 63.0, 60.1 and 52.2% (*n* = 27, 46, and 23), respectively.

Nematodes from 2 to 35 mm were found in the swim bladder of eels. The intensity of infection varied from 1 to 66 individuals. The total mean intensity of infection was 6.6 nematodes. The highest mean intensity of infection was found in October (14.7 nematodes) and the lowest in November (2.4 nematodes). The intensity of infection by young nematodes (1-14 mm long) varied from 1 to 15 individuals, while of adult ones (over 15 mm of length) from 1 to 20 individuals.

Various numbers of 2<sup>nd</sup> stage larvae released by females, still in eggs or free, were found

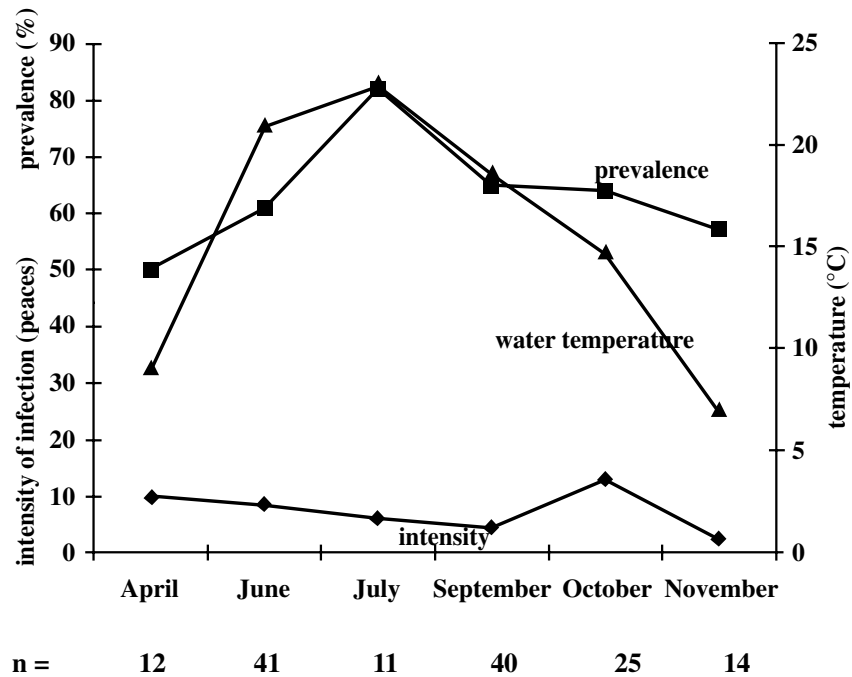


Fig. 1. Mean intensity and prevalence of *A. crassus* in eels in relation to water temperature in 1994-2000 (n = numbers of eels)

by microscopy of scrapings from the swim bladder wall. These developmental stages were found even in eels not harbouring young or adult nematodes. Their numbers amounted to a couple of dozens to thousands in individual swim bladders. Prevalence of the occurrence of the 2<sup>nd</sup> stage larvae varied from 38.5% (June 1999) to 100% (July 1998). The total mean prevalence of larval stages was 60.6% (n = 127). Table 1 summarises the prevalence of the parasite and the intensity of infection on individual sampling occasions.

Table 1  
Results of examination of eels for the *A. crassus* infection on individual sampling occasions

Date	n	Prevalence (%)	Intensity (pcs)	Water temperature (°C)
12 Sept 1994	5	100	1-7, x = 4	19
18 Sept 1995	4	75	8-11, x = 10	17
3 Sept 1997	7	43	1-3, x = 2.3	22
10 June 1998	11	63	1-34, x = 7	21
31 July 1998	11	82	2-29, x = 6	23
1 Sept 1998	12	58	1- 6, x = 2.6	16
16 Oct 1998	10	60	1-18, x = 8.5	12
12 Nov 1998	14	57	1- 5, x = 2.4	7
22 April 1999	12	50	3-27, x = 10	9
7 June 1999	13	46	1-12, x = 4	20
30 July 1999	17	71	1- 14, x = 5	21
16 Sept 1999	12	67	1-17, x = 5	19
6 Oct 1999	10	70	2-24, x = 10	17
3 Oct 2000	5	60	1-66, x = 29	5

### Patho-anatomical findings

At autopsy, patho-anatomical changes were found in the swim bladder and *ductus pneumaticus* that varied from petechiae to extensive damage including a reduction in the size of the bladder and thickening of its wall, obturation of *ductus pneumaticus* and presence of foamy or dark haemorrhagic fluid in the cavity of the swim bladder or *ductus pneumaticus*. Severe damage to the swim bladder was found mainly during autumn months.

### Haematological indices

No significant differences were in any of the haematological indices studied between the group of infected and non-infected eels or in dependence on the numbers of nematodes in the swim bladder. Table 2 presents values of hematocrit, red and white blood cell counts in infected and non-infected eels.

Table 2  
Haematological indices of eels uninfected and infected by *A. crassus*

	Hematocrit (L·L <sup>-1</sup> ) mean ± SD	Erythrocytes (T·L <sup>-1</sup> ) mean ± SD	Leukocytes (G·L <sup>-1</sup> ) mean ± SD	n
Uninfected	0.25 ± 0.35	1.23 ± 0.28	50.13 ± 26.00	16
Infected	0.27 ± 0.35	1.25 ± 0.26	37.00 ± 12.00	30

The obtained values are presented in relation to the date of sampling. There was a rise ( $p < 0.05$  and  $p < 0.01$ ) in the hematocrit value sampled on October 10, 1999 (0.29 L·L<sup>-1</sup>) in comparison to sampling on June 7, 1999 (0.25 L·L<sup>-1</sup>), September 16, 1999 (0.25 L·L<sup>-1</sup>) and June 30, 1999 (0.27 L·L<sup>-1</sup>), respectively. Red blood cells were significantly ( $p < 0.05$  and  $p < 0.01$ ) lowest on September 16, 1999 (1.04 T·L<sup>-1</sup>) as compared to sampling on June 7, 1999 (1.28 T·L<sup>-1</sup>), October 6, 1999 (1.24 T·L<sup>-1</sup>) and June 30, 1999 (1.41 T·L<sup>-1</sup>), respectively. White blood cell counts were lowest ( $p < 0.05$  and  $p < 0.01$ ) on October 6, 1999 (27.6 G·L<sup>-1</sup>) as compared to values obtained on June 7, 1999 (56.2 G·L<sup>-1</sup>), and September 16, 1999 (41.2 G·L<sup>-1</sup>) and June 30, 1999 (39.0 G·L<sup>-1</sup>), respectively. The above-mentioned data are summarised in Table 3.

Table 3  
Haematological indices of eels on individual sampling occasions

	Hematocrit (L·L <sup>-1</sup> ) mean ± SD	Erythrocytes (T·L <sup>-1</sup> ) mean ± SD	Leukocytes (G·L <sup>-1</sup> ) mean ± SD	n
Uninfected	0.25 ± 0.35	1.23 ± 0.28	50.13 ± 26.00	16
7 June 1999	0.25 ± 0.03 <sup>a</sup>	1.28 ± 0.29 <sup>a</sup>	56.17 ± 27.39 <sup>a</sup>	12
30 June 1999	0.27 ± 0.03 <sup>a</sup>	1.41 ± 0.23 <sup>a</sup>	39.00 ± 11.43 <sup>a</sup>	12
16 Sept 1999	0.25 ± 0.05 <sup>a</sup>	1.04 ± 0.16 <sup>b</sup>	41.17 ± 9.12 <sup>a</sup>	12
6 Oct 1999	0.29 ± 0.03 <sup>b</sup>	1.24 ± 0.23 <sup>a</sup>	27.60 ± 9.83 <sup>b</sup>	10

Indices a,b mean correspondence or significant differences of results

Differential counts of white blood cells showed no significant changes. Infected as well as non-infected eels were characterised by lymphocytic type of blood (88.02 and 90.34 % of lymphocytes, 10.27 and 7.38 % of granulocytes, 0.52 and 0.94 % of monocytes, 1.17 and 1.34 % of blasts, respectively). On individual sampling occasions, values of lymphocytes, granulocytes, monocytes and blasts amounted to 90.58, 7.19, 0.88 and 1.38 % (July 7, 1999), 86.67, 12.00, 0.42 and 0.92 % (July 30, 1999), 89.33, 9.08, 0.50 and 1.08 % (September 16, 1999), 89.20, 8.20, 0.90 and 1.60 % (October 6, 1999).

### Influence of the parasite on the health condition and body mass of eels

There were no cases of mortality reported during the period of the study. Eels showed no clinical signs of the disease. Two specimens had the body cavity enlarged.

The relation between the body weight ratio and the intensity of infection is presented in Fig. 2. It is clear from this graph that infected eels from a certain length and mass (600 mm, 330 g) have less body mass for their length and are longer for their body mass than the non-infected ones. In other words, infected eels grow more to the length, whereas the non-infected reach higher body mass.

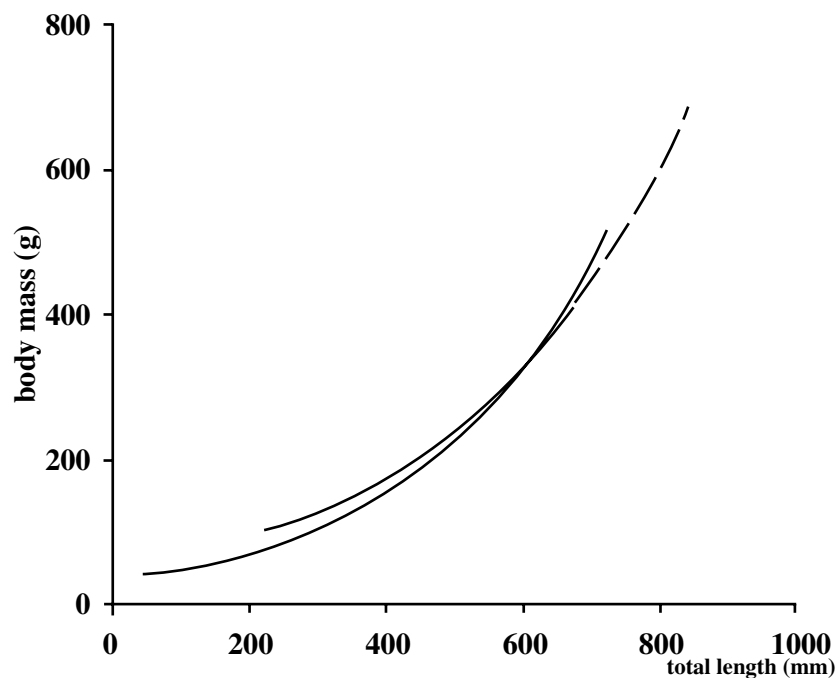


Fig. 2. Length-body mass relationships in groups of eels uninfected ( $n = 45$ ) and infected ( $n = 76$ ) by *A. crassus*; according to Baruš and Prokeš (1996)

### Discussion

Baruš (1994) and Baruš and Kotulán (1997) mention the prevalence of young and adult nematodes in the swim bladder amounting to 80% and the intensity of infection of 1 to 22 individuals during mass mortality of eels in the Vranov Dam Lake. Similar results were obtained by Molnár et al. (1994) in eels from the Balaton Lake, where the prevalence varied from 8 to 95% in dependence on the locality studied. Mass mortality of eels occurred in the Lake Balaton in 1991 and 1992. From our results it is clear that the nematode *A. crassus* reaches high prevalence and intensity of infection in the Koryčany Water Reservoir. The population of eels thus gets repeatedly re-infected. In spite of the high rate of infection intensity of the disease in eels from the Koryčany Water Reservoir, there was no mortality or clinical signs present, the fact probably being caused by the absence of other negative factors in this water reservoir (such as high water temperature and the concentration of dissolved oxygen in the water) which took part in the mortality cases of eels both in the Vranov Dam Lake and the Balaton Lake (Baruš 1994; Molnár 1993; Molnár et al.

1994). The dynamics of the disease and patho-anatomical lesions during the year are in close relation to the developmental cycle of the parasite and foraging activities of the eel (Baruš et al. 1999a). We have not found any significant difference in the total length and body mass of infected and non-infected eels, as well. The relation of the parasitic infection to the ratio of body length and mass in infected and non-infected eels is in accordance to the results by Baruš and Prokeš (1996).

It is especially the prevalence of young and adult nematodes in the swim bladder that is to be considered a very important factor of anguillicolosis of eels. The mean intensity of infection is influenced considerably by individuals suffering from a mass infestation and as such it serves only for orientation purposes. The mean prevalence amounted to the highest numbers in July, i.e., a summer month with the highest mean water temperature which may promote the development of the disease. Similar conclusions were also reached by Baruš (1994) and Baruš et al. (1999a).

The 2<sup>nd</sup> stage larvae of the nematode reach various values of prevalence in the swim bladder probably due to continuous maturation of nematodes and release of larvae-containing eggs.

Haematological indices are only slightly influenced by the disease. We found no significant changes in relation to the number of parasites in the swim bladder. Baruš et al. (1999c) described a significant change to lower levels of micro- and macro-elements in the muscle tissue of eels (Ca, P, Fe, Zn, Cu, Mn) due to the haematophagy of the parasite whose tissues contained high levels of Fe, Zn, Cu and other micro-elements (Baruš et al. 1999d). Differences in the values are also dependent on the sex of the parasite. Boon et al. (1990) described a drop in the hematocrit values in relation to the infection by *A. crassus*. The above-mentioned results, however, were obtained within the framework of experimental infections by the parasite. Boon et al. (1989) did not find any significant changes in the hematocrit of naturally infected eels. The significant changes of haematological parameters that we found are probably more in relation to the season of sampling than the parasitic infection. In other words, they present the possible seasonal dynamics of haematological parameters in the eel.

#### **Výskyt *Anguillicola crassus* ve vodárenské nádrži Koryčany (Česká republika) a její vliv na zdravotní stav a hematologické parametry úhořů**

V letech 1994 - 2000 byl sledován výskyt hlístice *A. crassus* u úhořů ve vodárenské nádrži Koryčany. V letech 1998 a 1999 byla prováděna sledování v častějších intervalech se snahou zachytit možnou sezónnost onemocnění a vliv parazita na zdravotní stav populace úhořů v této nádrži.

Celkem bylo získáno 143 ks úhořů. Pro hematologické vyšetření bylo v roce 1999 získáno 46 ks úhořů. Prevalence hlístic vykazovala sezónní dynamiku a pohybovala se v rozmezí od 43 do 100%. Intenzita invaze mladých a dospělých hlístic v plynových měchýřích byla 1 – 66 exemplářů, průměrná intenzita byla 6.6 hlístic v rybě. Prevalence larválních stádií v plynových měchýřích byla 38.5 – 100%. Byly zjišťovány patologicko-anatomické změny na plynových měchýřích a ve spojce plynového měchýře s trávicím traktem. Těžké poškození plynových měchýřů bylo zjišťováno zejména v podzimních měsících. V žádném ze zjišťovaných hematologických ukazatelů (hematokrit, počet červených a bílých krvinek) nebyly nalezeny signifikantní změny mezi infikovanými a neinfikovanými úhoři, ani v závislosti na množství hlístic v plynovém měchýři. Získané hodnoty jsou uváděny i v závislosti na době odběru. Zde byl nalezen statisticky významný vzestup hematokritu při odběru 6.10. 1999 ( $0.29 \text{ L}\cdot\text{L}^{-1}$ ), ( $p < 0.01$ ) oproti odběrům 7.6. 1999 ( $0.25 \text{ L}\cdot\text{L}^{-1}$ ) a 16.9. 1999 ( $0.25 \text{ L}\cdot\text{L}^{-1}$ ), ( $p < 0.05$ ) oproti odběru 30.6. 1999 ( $0.27 \text{ L}\cdot\text{L}^{-1}$ ). Počet erytrocytů byl nejnižší 16.9. 1999 ( $1.04 \text{ T}\cdot\text{L}^{-1}$ ) a to signifikantně oproti 7.6. 1999 ( $1.28 \text{ T}\cdot\text{L}^{-1}$ )

a 6.10. 1999 ( $1.24 \text{ T}\cdot\text{L}^{-1}$ ); ( $p < 0.05$ ) a oproti 30.6. 1999 ( $1.41 \text{ T}\cdot\text{L}^{-1}$ ), ( $p < 0.01$ ). Počet leukocytů byl nejnižší 6. 10. 1999 ( $27.6 \text{ G}\cdot\text{L}^{-1}$ ) a to signifikantně oproti 7. 6. 1999 ( $56.2 \text{ G}\cdot\text{L}^{-1}$ ) ( $p < 0.01$ ) a oproti 16. 9. 1999 ( $41.2 \text{ G}\cdot\text{L}^{-1}$ ) a 30. 6. 1999 ( $39.0 \text{ G}\cdot\text{L}^{-1}$ ),  $p < 0.05$ . V diferenciálním počtu leukocytů nebyly nalezeny signifikantní změny. Infikovaní i neinfikovaní úhoři měli lymfocytární charakter krve (88.02 resp. 90.34 % lymfocytů, 10.27 resp. 7.38 % neutrofilních granulocytů, 0.52 resp. 0.94 % monocytů a 1.17 resp. 1.34 % blastických buněk).

Anguilikolóza v podmínkách Koryčanské nádrže nevyvolává hynutí ani klinické příznaky onemocnění u populace úhořů.

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