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Contents of several inorganic substances in European eel infected and uninfected by *Anguillicola crassus* (Nematoda)

Vlastimil Baruš[1,*], František Tenora[2], Stanislav Kráčmar[2], Jan Dvořáček[3]

[1]Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, CZ-603 65 Brno, Czech Republic
[2]Mendel University of Agriculture and Forestry Brno, Zemědělská 1, CZ-613 00 Brno, Czech Republic
[3]Institute for Service and Business, Department of Ecology and Chemistry, CZ-679 01 Skalice nad Svitavou, Czech Republic

ABSTRACT: The content of 5 macroelements and 5 microelements were analyzed using the atomic absorption method in muscle samples of European eels infected and uninfected by *Anguillicola crassus*. The mean contents of these substances in infected eels were statistically highly significantly lower in Ca, P, Fe, Mn, but only statistically significantly lower in Na, Mg, Zn and Cu as compared to uninfected fishes. These differences are discussed in relation to hematophagus feeding and pathogenity of the parasite.

KEY WORDS: Eel muscle · *Anguillicola crassus* · Inorganic substances · Atomic absorption spectrometry

INTRODUCTION

The swimbladder nematode *Anguillicola crassus* Kuwahara, Niimi et Itagaki, 1974 (Dracunculoidea, Anguillicolidae) is an eel-specific parasite, recently introduced into Europe (Taraschewski et al. 1987, Kennedy & Fitch 1990, Moravec 1994). A high mortality of European eel *Anguilla anguilla* (L.) due to *A. crassus* infection was previously observed (in 1991 and 1992) and described from the Balaton Lake, Hungary (Molnár et al. 1991, 1994, Bíró 1992); later on in 1994 it was also reported in the Vranov Valley Reservoir, Czech Republic (Baruš 1995, Baruš & Prokeš 1997). Various aspects of pathological changes in eels infected (mainly in the fish swimbladder and adjacent tissues) were evaluated by Banning & Haenen (1990), Haenen et al. (1989), and Molnár et al. (1993). The high pathogenity of this parasite is manifested, first of all, in its influence on the respiration process physiology in the definitive host, which was confirmed significantly by Molnár (1993) and Würtz et al. (1996).

Disturbed absorption of inorganic substances in the host has been described occasionally from various parasitic infections (review in Brand 1973). With respect to *Anguillicola crassus* infection, only Sures et al. (1994) and Sures & Taraschewski (1995a, b) have studied as yet the content of heavy metals (mainly lead) in parasite and eel tissues (liver, bile-bladder and intestine). We assume that blood loss due to the ingestion of blood by this specialized hematophagous parasite (Polzer & Taraschewski 1993) can also have a pathogenic effect on the content of several inorganic substances in comestible fish body muscles. Our findings are presented in this communication.

MATERIAL AND METHODS

The infected (IN) eels used in the present study were caught with a dip net in the shallow water close to banks in the Vranov Valley Reservoir (South Moravia) in September 1994, during mass mortality caused by anguillicolosis. Surviving specimens (n = 19) with pronounced abnormal behaviour were caught and immediately examined parasitologically in the laboratory. The total length (TL) of fish was measured to the nearest mm; weight was determined to the nearest g. The intensity of infection (in 100% prevalence) was calculated as the range and mean value (Table 1). Only adult parasite specimens were included.

*E-mail: kozena@brno.cas.cz

Table 1 *Anguillicola crassus* infecting *Anguilla anguilla*. Total length (TL, in mm) and weight (W, in g) of uninfected (UN) and infected (IN) European eels, and intensity of infection (Ii, 100% prevalence) with the nematode

	TL		W		Ii	
	Range	Mean	Range	Mean	Range	Mean
UN	415–962	685	345–1150	635	–	–
IN	705–890	793	589–1020	877	3–45	16.2

The uninfected (UN) eels were caught by electrofishing in the Mostiště Valley reservoir (Western Moravia) in May 1994, and the specimens (n = 10) were examined immediately in the same way as the previous group. The eel populations of this locality were monitored parasitologically from 1992 to 1997.

After dissection of eels, a muscle sample (without skin) of 80 to 100 g was taken from the middle body part of each specimen, and rinsed in double-distilled water for 1 min. Inorganic element determination was performed in 19 muscle samples of IN and 10 samples of UN eels. After dry mineralization and dissolution of samples, atomic absorption spectrometry (AAS) was performed using a GBC-932 AA (USA) apparatus with a 1 M HNO_3 flame environment. The dry samples were burnt for 1½ h at 200°C, for 1 h at 350°C and for 6 h at 480°C. Ash was transferred into a solution with a final concentration of 1 M HNO_3. The content of selected inorganic substances was examined for macroelements (g/1000 g of 100% dry matter) and for microelements (mg/1000 g of 100% dry matter). The results were assessed using variation statistics (Snedecor & Cochran 1967).

Table 2. Content of some inorganic substances in the muscle of infected and uninfected eels *Anguilla anguilla* in 100% dry matter. Mean values for uninfected eels that are significantly different from means for infected eels are indicated as follows: $^{*}p \leq 0.01$; $^{**}p \leq 0.05$

Substances	Infected n = 19		Uninfected n = 10	
	Mean ± SE	Range	Mean ± SE	Range
Macroelements (g)				
Ca	4.11 ± 0.04	3.72–4.28	11.02 ± 0.03**	9.82–12.13
P	2.11 ± 0.05	1.99–2.18	8.26 ± 0.08**	7.98–8.37
K	2.00 ± 0.04	1.92–2.13	2.12 ± 0.05	2.01–2.23
Na	0.53 ± 0.03	0.44–0.58	0.64 ± 0.04*	0.57–0.69
Mg	0.32 ± 0.04	0.25–0.35	0.42 ± 0.02*	0.39–0.44
Σ	9.07 ± 0.10	8.32–9.52	22.46 ± 0.11**	20.77–23.86
Microelements (mg)				
Zn	40.08 ± 0.01	36.11–43.19	45.55 ± 0.03*	42.03–48.22
Fe	5.91 ± 0.04	5.03–6.21	13.77 ± 0.04**	11.18–14.01
Cu	5.49 ± 0.03	5.31–5.79	7.42 ± 0.01*	6.99–7.52
Mn	1.05 ± 0.01	0.93–1.17	2.54 ± 0.04**	2.16–2.64
Co	0.19 ± 0.02	0.13–0.22	0.20 ± 0.02	0.14–0.23
Σ	52.72 ± 0.04	47.51–56.58	69.48 ± 0.06**	62.50–72.62

RESULTS

In muscle samples of IN and UN European eels, 5 macroelements (Ca, P, K, Na and Mg) and 5 microelements (Zn, Fe, Cu, Mn and Co) were determined. The mean and range weight contents are presented in Table 2. Of the macroelements, statistically highly significant differences between IN and UN specimens occurred in Ca and P mean content; only significant differences were found in Na and Mg. The lower mean content of macroelements in IN eel muscle samples and higher mean content in UN eel muscle samples indicates a general trend.

In the microelements evaluated, statistically highly significant differences occurred in Fe and Mn mean content; only significant differences were found in Zn and Cu. As in the case of the macroelements, a general trend is evident in inorganic substance content when the IN and UN muscle samples are compared. Of the 2 inorganic substance groups, only the mean content of K (macroelement) and Co (microelement) did not show statistically significant differences.

DISCUSSION

The lack of significant differences in the weights of eels UN and IN by *Anguillicola crassus* suggests a functional paradox in this parasite-host system, which, in our case, resulted in the death of the host (Baruš & Prokeš 1996a). In addition, significant quantitative differences in the values of 17 amino acids in UN and IN eel muscle were found only in methionine and aspartic acid (and in cystine + methionine sum), with lower values in parasitized specimens (Baruš et al. 1998).

As to the data on respiration physiology in eels, the swimbladder (in particular its anterior chamber) participates significantly in ensuring the general oxygen consumption balance (Krogh 1904). A reduction in or total elimination of the swimbladder function which is of a hydrostatic and partly respiratory nature, in the extreme situation, to the death of the parasitized fish. Molnár (1993) and Würtz et al. (1996) confirmed experimentally that major mortality factors in eel anguillicolosis are reduced oxygen content in the aquatic environment and changes in the chemical composition of the swimbladder gases in infected fish due to decay of parasite bodies.

The inorganic components of animal tissues may or may not be altered by parasitic infection (review in Brand 1973).

Abnormal fluctuations in phosphorus and calcium content occur, e.g. during trichinosis; a marked decrease in these elements takes place during the initial stages of infection. Also, in the case of anguillicolosis in the muscle of IN eels, we found a decrease in Ca content to 37.3% and in that of P to 25.5% from the normal (100%) values in UN fish.

In specialized hematophagous nematodes such as *Anguillicola crassus*, development of the nematode and its pathogenic influence on the host organism are expressed markedly by blood sucking (Polzer & Taraschewski 1993, Baruš & Prokeš 1996b). Blood loss in the host during the parasite's life cycle affects the content of several microelements in its tissues (review in Schulz & Gvozdev 1972, Brand 1973). Of these elements, iron, the fundamental structural part of hemoglobin (cytochroms), is affected the most. In addition, copper, manganese and cobalt participate in the blood production process in general (Schulz & Gvozdev 1972). In these inorganic substances, we found markedly lower values in the muscle of IN versus UN specimens (100% values). A decrease in Zn to 42.9%, in Cu to 73.9%, and in Mn to 41.3% of the values in UN specimens occurred.

We maintain that the significantly decreased values we found in 4 or 5 inorganic substances in IN eel muscle suggests the substantial pathogenic effect of infection by *Anguillicola crassus* (anguillicolosis disease symptom) on the quality and usable biomass of the host. This corresponds to the conclusions by Brand (1973) especially with respect to iron; this is true for the absorption process of hemoglobin during infection of the host by blood-sucking helminths. The proteolytic enzymes, namely aspartic proteinase, seem to degrade hemoglobin of ingested erythrocytes in adult *A. crassus* (see Polzer & Taraschewski 1993).

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