Ecotoxicology (2010) 19:928–932
DOI 10.1007/s10646-010-0474-3

Experimental studies on the lead accumulation in the cestode *Moniezia expansa* (Cestoda: Anoplocephalidae) and its final host (*Ovis aries*)

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Accepted: 16 February 2010 / Published online: 6 March 2010

Abstract The tapeworm *Moniezia expansa* and naturally infected sheep were investigated with respect to their lead accumulation. Lead—Pb$(CH_3COO)_2$ was added to the distilled water and administered orally to the sheep every day for a period of 1 week. After the exposure period the sheep were killed and the metal levels were determined in the muscle, liver, kidney and blood of the sheep as well as in the cestode parasites (*Moniezia expansa*). The impact of an infection with the cestode *Moniezia expansa* and a simultaneous Pb exposure, on the concentrations of heavy metals in the host kidney, liver, muscle, blood and cestodes was studied. The concentration of lead in the cestodes was on average 458, 5 and 4-fold higher in the cestodes than in the muscle, liver and kidney of the host, respectively. Parasitised sheep accumulated significantly less lead in their tissues than their uninfected conspecifics (ANOVA test, $P \leq 0.05$). Also the differences between host′s tissues and tapeworms were found to be significant (ANOVA test, $P \leq 0.05$). Thus, this study reveals that lead accumulation also occurs in cestodes parasitizing mammals. The host-parasite-system sheep-*Moniezia expansa* appears to be a useful and promising bioindication system especially in farming (rural, agricultural) and the natural ecosystem.

Keywords *Moniezia expansa* · Cestodes, lead accumulation · Sheep · Bioindication

Introduction

Several helminths (above all acanthocephalants in fish) are able to accumulate considerable concentrations of heavy metals (Lafferty 1997; Sures et al. 1998; Sures 2001; Sures and Siddall 2001; Sures 2003; Thielen et al. 2004). Information on parasites of terrestrial vertebrates as sentinels for heavy metal environmental pollution and the benefit to their host is scarce (Sures et al. 2002a, b; Baruš et al. 2003; Torres et al. 2004, 2006; Jankovská et al. 2008, 2009, 2010). It remains unclear if the conspicuous metal accumulation of the parasitic worms affects the metal levels in the tissues of the definitive host as very few comparative studies on heavy metal concentrations in tissues of infected and uninfected host are available.

As cestodes are more abundant in terrestrial mammals than acanthocephalans and thus potentially more useful in attempts toward passive as well as active biomonitoring, a very common farm animal (*Ovis aries*) and their common tapeworm (*Moniezia expansa*) were selected for the present study.

The aim of the present study was to assess the concentration of Pb using the model *Ovis aries*/*Moniezia expansa* in order to test the potential suitability of *Moniezia expansa*

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as a sentinel organism and to determine whether Pb levels had been affected by the presence of helminth parasites.

Materials and methods

The concentrations of lead were determined in the tapeworm *Moniezia expansa* and in the kidney, liver, muscle and blood of an infected and uninfected sheep host (*Ovis aries*) from a small farm in the central region of the Czech Republic.

Experimental design

All sheep were randomly divided into groups and treated according to Table 1. Experimental sheep were naturaly infected by *Moniezia expansa* (groups without *Moniezia* infection were dewormed by eprinomectin (Ivomec Eprinex Pour-on sol., Merc).

Lead solutions were prepared by dissolving 2 g lead acetate $Pb(CH_3COO)_2$ in 10 ml of distilled water, and administered orally to the sheep every day for a period of one week (7 days).

Experiment I

The first experiment (Exp.I) comprised three groups (Table 1): the first (A) contained uninfected, exposed sheep; the second (B) contained infected, exposed sheep; and the third (C) consisted of infected, unexposed sheep. We compared individual host tissue (kidney, liver, muscle and blood) burden among individual groups (A, B, C).

Experiment II (group B)

In the second experiment (only group B) we compared burden between host and parasite tissues.

Sampling and analytical procedure

After exposure sheep were killed and dissected. Samples of muscle, liver, kidney and blood as well as parasites were taken with stainless-steel scissors and forceps that had previously been cleaned with double—distilled water. For stabilization of blood samples ethylene diamine tetraacetic acid (EDTA) was applied. The cestodes were removed from the intestine using the same instruments. All samples were frozen at -26°C until freeze drying (Lyovac GT-2, Germany) and further processing.

Pressurized wet ashing: An aliquot (~500 mg of dry matter) of the liver, kidney, muscle and tapeworm sample or 1 ml of EDTA treated blood sample were weighed into a digestion vessel. Concentrated nitric acid (8.0 ml) (Analytika Ltd., Czech Republic), and 30% H_2O_2 (2.0 ml) (Analytika Ltd., Czech Republic) were added. The mixture was heated in an Ethos 1 (MLS GmbH, Germany) microwave assisted wet digestion system for 30 min at 220°C. After cooling the digest was quantitatively transferred into a glass tube and filled up to the volume 20 ml by deionized water. Analytical blanks prepared under the same conditions, but without samples, were used to determine the analytical detection limit (mean ± 3SD of blanks); it was 0.21 ng mL^{-1} Pb. A certified reference material BCR 185R Bovine Liver was applied for the quality assurance of analytical data. In this material, the certified value of lead represents 0.172 ± 0.009 mg kg^{-1} Pb. In our experiment 0.178 mg kg^{-1} of Pb was determined in this material. The total contents of lead in the digests were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, VARIAN VistaPro, Varian, Australia). For determination of low contents of lead (in control samples and in all the muscle digests) flameless atomic absorption spectrometry (GFAAS, VARIAN AA280Z, Varian, Australia equipped by GTA-120 graphite furnace atomizer) was applied.

Statistical analysis

Concentrations of heavy metals were compared among individual tissues and treatments (A, B, C) using one-way analysis of variace (ANOVA, $P \leq 0.05$).

Additionally, the bioconcentration factor as a ratio of the metal concentration in the parasites and the host tissues ($C_{parasite}/C_{host\ tissue}$) was determined according to Sures et al. (1999).

Table 1 Experimental design

Group	*n*	$Pb(CH3COO)_2$	*Moniezia expansa.*
Exposure Pb	3	2 g	–
Exposure Pb and infection	9	2 g	+
Control infection	6	–	+

Results

Experiment I

The first experiment (Exp.I) comprised three treatments (Table 1): the first (A) contained uninfected, exposed sheep; the second (B) contained infected, exposed sheep; and the third (C) consisted of unexposed sheep with controlled infection with *Moniezia expansa*.

We compared individual host tissues (kidney, liver, muscle) and blood among individual groups (A, B, C). The

Table 2 Concentrations of lead in the blood (μg/ml) and in the tissues (muscle, liver, kidney) of *Ovis aries* and *Moniezia expansa* (mg/kg of dry weight; mean ± SD) after 1 week Pb exposition

Treatment	*Ovis aries*				*Moniezia expansa*
	Blood	Muscle	Liver	Kidney	
A	2.90 ± 0.145[a]	0.179 ± 0.010	22.3[c]	39.1 ± 1.6[e]	–
B	**1.31 ± 0.64[a,b]**	**0.186 ± 0.007**	**16.3 ± 0.7[c,d]**	**19.9 ± 2.5[e,f]**	**85.2 ± 39.9[g]**
C	0.080 ± 0.000[b]	0.169 ± 0.010	0.518 ± 0.066[d]	0.652 ± 0.145[f]	0.145 ± 0.039[g]

[a, b, c, d, e, f, g] Indicate statistical differences ($P \leq 0.05$)

mean Pb concentrations in the blood, muscle, liver and kidney of the uninfected animals with Pb exposure (treatment A) were 2.904 μg/ml, 0.179, 22.296 and 39,109 mg/kg, respectively whilst the infected sheep with Pb exposure (treatment B) had a mean lead level of 1.312 μg/ml, 0.186, 16,257 and 19,877 mg/kg, respectively. The mean Pb concentrations of the infected animals without Pb exposure (treatment C) were 0.080 μg/ml, 0,169; 0,518 and 0,652 mg/kg, respectively (Table 2). These differences (treatments A versus B versus C) were found to be significant (ANOVA $P \leq 0.05$).

Considering the parasites, the mean Pb concentration in the tapeworms of the exposed sheep was 85.233 mg/kg dry weight (group B) whilst the tapeworms from sheep without Pb exposure (group C) had a mean lead level of 0,145 mg/kg dry weight only (Table 2). There were significant differences ($P \leq 0.05$) in heavy metal burdens between tapeworms from treatments B and C. There were also significant differences ($P \leq 0.05$) in blood heavy metal burdens among individuals treatments A, B and C (Table 2).

Experiment II

Lead concentrations in *Moniezia expansa* and in the tissues and blood of the sheep (group B) are shown in Table 2.

The average lead concentration of *Moniezia expansa* was significantly higher (ANOVA, $P \leq 0.05$) than in the sheep tissues (Table 2). Bioconcentration factors for lead in *Moniezia expansa* ($Pb_{parasite}/Pb_{host}$) were 458, 5, 4, compared to the host muscle, liver, and kidney, respectively (Exp. II). The highest lead concentrations were found in the *Moniezia expansa*, with a mean value of 85.2 mg/kg dry weight. The lead burden was significantly higher ($P \leq 0.05$) in *Moniezia expansa* than in host tissues (Table 2-treatment B).

Discussion

Informations regarding the impact of parasites on the metal uptake by their hosts are contradictory. In the Sures and Siddall (1999) study it was conclusively shown that infection of chub (*Leuciscus cephalus*) with *Pomphorhynchus laevis* resulted in significantly lower lead concentrations in the intestine of infected fish compared to uninfected controls. On the other hand, in an experimental study on the lead uptake by *Moniliformis moniliformis* no reduction of the lead concentration in the kidneys of the rat was observed (Sures et al. 2000b).

As was described by Sures and Siddall (1999), lead ions from hosts GIT (gastrointestinal tract) are able to pass across the epithelial membrane by paracellulardiffusion and enter the bloodstream. There they bind to the membrane of erythrocytes and are transported by the circulatory system to various organs in the body. Lead ions are passed from the GIT and then through the portal *circulation* to the *liver.* In the liver the majority of lead is removed from the blood and excreted into the intestine via the bile. The bile contains steroids with which the heavy metal ions form organometallic complexes that then pass down the bile duct into the small intestine (Sures and Siddall 1999). This bile-bound lead can then be taken up by tapeworms, or may be reabsorbed by the intestinal wall, or can be excreted in the feces of the host. The bile may play an important role in the uptake and accumulation of lead in parasites (Sures et al. 1998).

Tapeworms can absorb bile-bound lead through its tegument. The cestode tegumentary surface is functionally equivalent to the brush border of the vertebrate intestinal mucosa (Starling 1975). Cestode tegument serves as a highly efficient digestive-absorptive layer that competes with the vertebrate mucosa for nutrients including heavy metals (Dalton et al. 2004).

The main object of the present study was to monitor the relationship of farm animals (sheep), to their lead burden and their cestode parasites. The lead bioaccumulation by cestode parasites of farm animals from the host tissues is a new way of natural detoxication for organisms. In our experiment, we found that 3,336% of lead, from the total amount administered to the sheep (14 g of lead acetate) was taken up by the sheep tapeworms (*Moniezia expansa*).

Due to the fact that muscle tissue makes up the bulk of a carnivorous diet, and biologically incorporated heavy metals (lead above all) transfers upwards in the food chain only in scarce amounts, the herbivorous species may be more susceptible to lead and other heavy metals poisoning than predatory species (Kålås et al. 2000).

When comparing lead levels of exposed sheep with *Moniezia* infection (treatment B), it appears that *Moniezia expansa* reduces the metal accumulation in host blood, liver and kidneys (Table 2).

Another purpose of this study was to test the potential suitability of the cestode/sheep model *Moniezia expansa/Ovis aries* as another promising bioindicator system for lead pollution under farm and natural conditions. Sheep are also used as a low-cost means of maintaining the landscape (through grass-feeding). These species were chosen considering that there are very few models for farming condition, such as Sures et al. (1998) for the digenean *Fasciola hepatica*/cattle (*Bos primigenius* f. *taurus*), Sures et al. (2000a) for the archiacanthocephalan *Macracanthorhynchus hirudinaceus*/pig (*Sus scrofa* f. *domestica*).

Sures et al. (2002a, 2003) proposed a cestode/rodent model successfully evaluated for urban areas (*Hymenolepis diminuta/Rattus rattus*). Torres et al. (2004, 2006) and Jankovská et al. (2009) proposed models for non urban areas (*Gallegoides arfaai/Apodemus sylvaticus*, *Skrjabinotaenia lobata/Apodemus sylvaticus* and *Paranoplocephala* spp./*Microtus agrestris*). Also the cestode/carnivore model (*Mesocestoides* spp./*Vulpes vulpes*) is the suitable bioindicator system (Jankovská et al. 2010) while Eira et al. (2005) verified the inadequacy of cestode/lagomorph model (*Mosgovoyia ctenoides/Oryctolagus cuniculus*) as a bioindicator system.

Sures et al. (2002a) revealed that the bioconcentration factor of the host-parasite-system rat-*Hymenolepis diminuta* had approximately 17 times higher lead burdens in the tapeworms compared with the rat kidney. In terrestrial mammals the kidney is known as one of the main accumulation organs for metals (Merian 1991; Sures et al. 2002a, b). Accordingly, in our study, the kidney of the sheep both with or without *Moniezia expansa* infection exhibited the highest lead content among all the investigated tissues (Table 2). The kidney lead content was 19.9 mg kg^{-1} dry weight compared with the liver and muscle which contained lower (16.3 and 0.186 mg kg^{-1} dry weight lead levels, respectively (treatment B). Lead in the sheep muscles showed approximately 108 times lower metal concentration than in the sheep kidney. Although when taking into account the parasites, the highest concentration of lead within the host-parasite system was recorded for *Moniezia expansa* (85.2 mg kg^{-1} dry weight) from exposed sheep (Table 2).

A significantly higher accumulation of lead was recorded for the cestode parasite *Moniezia expansa* compared to the organs of its host. It resembled earlier results in the host—acantocephalan parasite system studied by Sures (2001, 2004, 2006), Sures and Siddall (1999), Sures et al. (2000a, b, 2002a, b). Bioconcentration factors for lead in *Moniezia expansa* ($Pb_{parasite}/Pb_{host}$) were 432, 5, 4 compared to the host muscle, liver, kidney, respectively (treatment B, Exp. II).

The results presented here demonstrate that sheep tapeworms (*Moniezia expansa*) accumulate much higher lead levels than in the host tissues. Thus, *M. expansa* might be used as an accumulation indicators for heavy metals in terrestrial biotopes especially as it is a very abundant parasite of sheep and cattle. Further investigations are necessary to decide whether intestinal parasites of mammals are able to affect metal levels in the host tissues or not.

Acknowledgements This study was supported by a project no. MSM 6046070901 provided by the Ministry of Education, Youths and Sports and by a project no. MZE 0002701404. Performed experiments comply with current laws of the Czech Republic.

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