Experimental Studies on the Lead Accumulation in the Cestode
Hymenolepis diminuta and its Final Host, Rattus norvegicus

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Accepted 28 May 2002

Abstract. It recently became clear that acanthocephalans parasitizing mammals can bioconcentrate several heavy metals to conspicuously higher concentrations than the tissues of their definitive hosts. As cestodes are more abundant in terrestrial animals than acanthocephalans, and thus potentially more useful in attempts toward passive as well as active biomonitoring, a very common tapeworm and its synanthropic mammalian host were selected for the present study. The tapeworm Hymenolepis diminuta and experimentally infected male Wistar rats of the CD-M-strain were investigated with respect to their lead accumulation. The worms were allowed to grow up for five weeks post infection followed by a five weeks oral lead exposure of the rats. After the exposure period the rats were killed and the metal levels were determined in muscle, liver, intestine, testes and kidney of the rats as well as in the parasites. Lead concentrations were found to be 17 times higher in the cestodes than in kidney, whereas metal levels in all other host tissues were below the detection limit. Thus, this study reveals that lead accumulation also occurs in cestodes parasitizing mammals. Due to a lack of adequate sentinel species in terrestrial habitats the host-parasite-system rat-H. diminuta appears to be a useful and promising bioindication system especially in urban ecosystems.

Keywords: Hymenolepis diminuta; cestodes; lead accumulation; rats; bioindication

Introduction

In environmental impact studies certain organisms provide valuable information about the chemical state of their environment through their ability to concentrate environmental toxins within their tissues. Organisms which have received increasing attention as accumulation indicators of heavy metals are among others (Schubert, 1991; Gunkel, 1994) intestinal parasites of vertebrates (reviewed by Sures and Taraschewski, 1999; Sures et al., 1999a). Mainly acanthocephalans and cestodes of fish, both dwelling in the intestine, were found to bioconcentrate different metals to concentrations surpassing those of established free living sentinel organisms such as the zebra mussel, Dreissena polymorpha (Sures et al., 1997a, 1999b). The phenomenon of metal accumulation in parasites was extensively studied from aquatic host-parasite associations (Sures et al., 1999a) but less information is available on the uptake and accumulation of metals in parasites of terrestrial hosts. This is especially deplorable as there is an urgent need for sentinel species in terrestrial, especially urban, habitats (Schubert, 1991). In a recent series of studies by Sures and co-workers the ability to accumulate metals was investigated with acanthocephalans from pigs (Sures et al., 2000a) and rats (Scheef et al., 2000; Sures et al., 2000b). These
studies revealed element concentrations in the parasites up to 100 times higher compared with different tissues of the hosts. Unfortunately, acanthocephalans in mammals are not as abundant as in fish (see e.g. Sures et al., 1999c; Taraschewski, 2000). A group of parasites frequently found in rodents, even in urban ecosystems, are cestodes, e.g. species of the genus *Hymenolepis*. As it is known that at least tapeworms of fish and birds are able to accumulate high amounts of different heavy metals (Riggs et al., 1987; Sures et al., 1997b, Baruš et al., 2000) the aim of the present study was to investigate the lead accumulation capacity of the cestode *Hymenolepis diminuta* parasitizing rats. After experimental infection of rats with cysticercoids of *H. diminuta* and subsequent oral lead exposure metal accumulation was analysed in host tissues and the parasites by electrothermal atomic absorption spectrometry (ET-AAS).

**Material and methods**

**Maintenance and infection of rats**

Male Wistar CD rats, *Rattus norvegicus*, weighing between 290 and 336 g were obtained from a commercial supplier (Charles River Germany GmbH, Sulzfeld). Rats were free of intestinal helminths as proven by spot checks of the faeces. The animals were kept together in one cage (6.0 m²) at 20 °C, were fed on commercial pellet food (Altromin, Lage Germany) and were allowed to drink *ad libitum*. Rats were additionally fed with biscuits daily to guarantee a sufficient supply of saccharids for the parasite, *H. diminuta*.

To obtain infectious larvae (cysticercoids) of the cestode *H. diminuta*, flour beetles (*Tribolium confusum*), were fed with faeces containing eggs from an infected rat. Twenty days post infection (p.i.) of the beetles, rats were infected with cysticercoids by feeding 3 beetles in sugar solution after the animals were accustomed to drink a glucose solution from an Eppendorf pipette.

**Experimental design**

All rats were randomly divided into four groups and treated according to Table 1. After infection the cestodes were allowed to grow for 5 weeks prior to lead exposure. Lead was added to the sugar solution and administered orally to the rats twice weekly for a period of 5 weeks. The rats were weighed each week to determine the appropriate amount of lead which had to be administered (7 μg/g respectively). Lead solutions were prepared by dissolving lead acetate (Pb(CH₃COO)₂, Merck, Darmstadt, Germany) in distilled water.

**Sampling and analytical procedure**

After exposure rats were killed and dissected. Samples of muscle, liver, kidney, adrenals, testes and intestine as well as parasites were taken with stainless-steel scissors and forceps that had previously been cleaned with 1% ammonium EDTA solution and double-distilled water. The cestodes were removed from the intestine using the same instruments. All samples were frozen at −26 °C until further processing. Prior to metal analysis the tissue samples and helminths were digested using a microwave digestion system (Model MDS 2000, CEM, Mathews, USA). Sample portions between 50 and 200 mg (wet weight) were digested with 1.8 ml (65%) nitric acid (suprapure, Merck, Darmstadt, Germany) as described by Sures et al. (1995).

Lead analysis was performed using a Perkin Elmer Model 4100 ZL atomic absorption spectrometer equipped with a Zeeman effect background system. The metal concentration in each sample was calculated from the corresponding regression line (correlation factor $r \geq 0.99$) using the standard addition method for each tissue and for the cestodes. Lead concentrations in the samples were determined as μg/g wet weight.

**Statistical analysis**

The Mann–Whitney U-Test, Friedman-Test and the Wilcoxon-Test were applied for the statistical

<table>
<thead>
<tr>
<th>Group</th>
<th>n rats</th>
<th>$C_{Pb}^+$ (μg/g)</th>
<th><em>H. diminuta</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>7</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>II Infection</td>
<td>5</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>III Exposure</td>
<td>8</td>
<td>7</td>
<td>–</td>
</tr>
<tr>
<td>IV Exposure and infection</td>
<td>6</td>
<td>7</td>
<td>+</td>
</tr>
</tbody>
</table>

*Table 1. Experimental design*