Lead concentrations in *Hymenolepis diminuta* adults and *Taenia taeniaeformis* larvae compared to their rat hosts (*Rattus norvegicus*) sampled from the city of Cairo, Egypt

B. SURES[1]*, T. SCHEIBLE[1], A. R. BASHTAR[2]† *and* H. TARASCHEWSKI[1]

[1] *Zoological Institute, Parasitology–Ecology, University of Karlsruhe, Geb. 07.01, D-76128, Karlsruhe, Germany*
[2] *Zoology Department, Faculty of Science, University of Cairo, Cairo, Egypt*

(*Received 12 March 2003; revised 7 May 2003; accepted 7 May 2003*)

SUMMARY

Concentrations of lead, determined by electrothermal atomic absorption spectrometry, were compared between the cestodes *Hymenolepis diminuta* and *Taenia taeniaeformis* and its host rat (*Rattus norvegicus*). Rats were sampled at 2 sites, which differed in respect to lead pollution as quantified from road dust, adjacent to the city of Cairo, Egypt. Comparing lead levels among host tissues and the parasites the significantly highest accumulation was found in *H. diminuta*, followed by rat kidney and larvae of *T. taeniaeformis*. Calculation of bioconcentration factors showed that *H. diminuta* contained 36-, 29-, 6- and 6-fold higher lead levels than intestinal wall, liver, kidney and larvae of *T. taeniaeformis*, at the more polluted site. At the less contaminated site lead bioconcentration factors for *H. diminuta* were found to be 87, 87 and 11 referred to intestine, liver and kidney of the host. Due to a high variability of the lead concentrations in *H. diminuta* it was not possible to indicate differences in metal pollution between both sampling sites. This variability may be influenced by different age structures of cestode infrapopulations. It is likely that younger worms contain lower metal levels than older worms due to a shorter exposure period. Thus, it is necessary to standardize the sampling of worms which should be used for indication purposes. Due to a lack of adequate sentinel species in terrestrial habitats more studies are required to validate and standardize the use of helminths as accumulation bioindicators in order to obtain mean values with low standard deviations. The host–parasite system rat–*H. diminuta* appears to be a useful and promising bioindication system at least for lead in urban ecosystems as rats as well as the tapeworm are globally distributed and easily accessible.

Key words: *Hymenolepis diminuta*, cestode, rat, metal pollution, lead, bioindication, urban environment.

INTRODUCTION

The use of intestinal parasites as indicators of environmental quality attained growing interest in recent years (Sures, Siddall & Taraschewski, 1999; Faulkner & Lochmiller, 2000; Sures, 2001, 2003). A number of studies revealed an exceptionally heavy metal accumulation capacity of acanthocephalans parasitizing fish, and a somewhat lower ability to concentrate metals in archiacanthocephalans from mammals (reviewed by Sures, 2001). Recent studies by Sures and co-workers have shown that acanthocephalans from pigs (Sures, Franken & Taraschewski, 2000*a*) and rats (Scheef, Sures & Taraschewski, 2000; Sures, Jürges & Taraschewski, 2000*b*) had a maximum of 100 times higher element concentrations compared with different tissues of the host. However, adult cestodes from the intestine of birds (Baruš, Tenora & Kráčmar, 2000; Tenora *et al*. 2001) and mammals (Sures, Grube & Taraschewski, 2002) seem to be even more useful indicators for heavy metals than acanthocephalans. In a recent laboratory study the uptake and accumulation of lead was analysed in *Hymenolepis diminuta* from experimentally infected rats (Sures *et al*. 2002). This tapeworm was found to contain 17 times higher lead concentrations than the kidney of the host. Metal levels in all other tissues of the rats ranged below the detection limit. From these laboratory exposure studies we suggested that this parasite may be valuable as a heavy metal bioindicator in terrestrial habitats (Sures *et al*. 2002). In contrast to aquatic environments where a wide variety of organisms is designated as sentinels (e.g. Gunkel, 1994), only a small number of animals appear to match the criteria commonly suggested for bioindicators in terrestrial habitats (for details see Beeby, 2001). Consequently, there is an urgent need for sentinel species reflecting small-scale differences in heavy metal pollution of urban and other habitats (Schubert, 1991).

Thus, the aim of the present study was to test the suitability of the cestode *H. diminuta* as an accumulation indicator for lead under field conditions. Accordingly, rats were caught at 2 different sites adjacent to the city of Cairo with its more than

---

* Corresponding author: Zoological Institute, Parasitology–Ecology, University of Karlsruhe, Geb. 07.01, D-76128, Karlsruhe, Germany. Tel: +49 721 6082701. Fax: +49 721 6087655. E-mail: Bernd.Sures@bio-geo.uni-karlsruhe.de

† Present address: Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia.

Parasitology (2003), **127**, 483–487. © 2003 Cambridge University Press
DOI: 10.1017/S0031182003003901 Printed in the United Kingdom

15 000 000 inhabitants. Both places differ in lead levels as quantified from road dust collected at the sites. In addition to infection with adults of *H. diminuta* some rats were also infected with intravisceral larvae of *Taenia taeniaeformis*.

MATERIALS AND METHODS

Study area and sample collection

Rats between 1·5 and 2·5 years old were caught at 2 different sites adjacent to the city of Cairo (Egypt) between March and April 2000 using nets which were placed over the exits of rat burrows. Samples of road dust were collected on several occasions during the 2 month sampling period. Subsequent metal analysis of the dust samples was used to indicate differences in the grade of lead pollution at the sites. As a 'low polluted site' (lower metal concentrations in road dust) 16 rats (weight: 324±63 g) were caught in Abou Rawash, a suburb approximately 10 km in the northwest of Cairo. The rat burrows were situated adjacent to a heavily frequented road which appears to be the main source of pollution. As a 'high polluted site', 24 rats (weight: 270±84 g) were collected in Helwan, situated 30 km in the south of Cairo. This sampling site lies between a heavily frequented road and an industry area with unspecified emissions.

After capture rats were brought to the laboratory, killed immediately, weighed, aged and dissected. Samples of muscle, liver, kidney and intestine as well as the parasites from the liver (larvae of *T. taeniaeformis*) and the intestine (adults of *H. diminuta*) were taken with the aid of stainless steel scissors and forceps which had been previously cleaned with 1% ammonium–EDTA solution and double-distilled water. All samples of host organs and the parasites were frozen at −26 °C until processing for metal analysis.

Analytical procedure

The digestion of the rat samples was carried out with a microwave digestion system (CEM Model MDS-2000, 650±50 W), as described by Sures, Taraschewski & Haug (1995). Up to 200 mg (wet weight) of tissue sample was weighed into a perfluoralkoxy (PFA) vessel (100 ml vol.) and mineralized with 1·8 ml of nitric acid (Suprapur, Merck, Darmstadt, Germany). After digestion was completed, the clear, colourless solution was transferred into a 2 ml volumetric glass flask and brought to volume with double-distilled water. PFA vessels and flasks were cleaned before use by rinsing 3 times with 1 vol.% HNO_3 and 3 times with double-distilled water.

To determine the detection limit analytical blanks were prepared in a similar manner without insertion of a sample. Metal analysis was performed using a Perkin Elmer Model 4100ZL atomic absorption spectrometer equipped with a Zeeman effect background correction system. Lead concentrations in each sample were calculated from the corresponding regression line (correlation factor $r \geqslant 0{\cdot}99$) using the standard addition method. Regression lines were determined for each sample type (blank, parasites and each of the host tissues) using concentrations (ng/ml) and peak areas (Ext s) for the corresponding *xy* values. Lead concentrations in the parasites and host tissues were determined as μg/g wet weight.

After collection the road dust samples were pooled, dried in an oven at a temperature of 50 °C and sieved (grain size <2 mm). Lead concentrations were determined in 3 parallel samples of the dust by TXRF following standard protocols (for details see Kramar, 1997). Additionally, the concentration of biologically available lead was analysed by atomic absorption spectrometry after elution of 3 parallel samples of road dust with NH_4Cl according to DIN 19730.

Statistical analysis

For statistical analysis the Kruskal–Wallis test and Mann–Whitney *U*-test were applied to check for significant differences between metal concentrations in rat tissues depending on the status of infection (uninfected, infected with adult cestodes, infected simultaneously with adult and larval cestodes). Friedman-Test and Wilcoxon-Test were applied to investigate differences of metal concentrations among the tissues and parasites of rats collected at each site. For all tests a significance level of $P \leqslant 0{\cdot}05$ was applied. Additionally, the ratio of the metal concentration in the parasites to that in different host tissues ($C_{[\text{parasite}]}/C_{[\text{host tissue}]}$) was determined according to the method described by Sures *et al.* (1999).

RESULTS

Metal concentrations in road dust

TXRF analysis revealed that total lead levels differed clearly among places, with 73±5 μg/g in dust sampled at Abou Rawash as compared with 283±29 μg/g in dust from Helwan. Accordingly, also the concentration of biologically available (NH_4Cl soluble) lead was lower in Abou Rawash (1·5±0·1 ng/ml) than in Helwan (58±5 ng/ml). Thus, the latter site appears to be more polluted than Abou Rawash which served as the low polluted site.

Infection of rats with cestodes

The prevalence of *H. diminuta* was 79% in rats caught at Helwan with a mean intensity (±S.D.) of

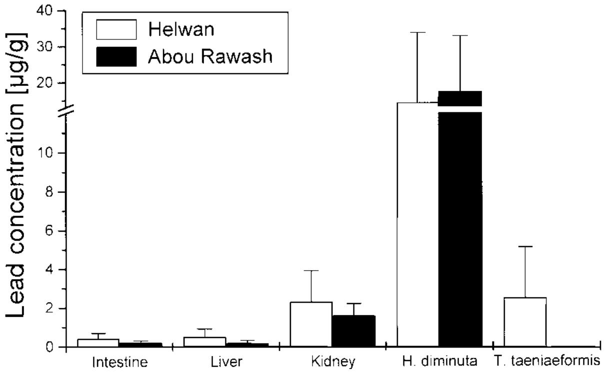

Fig. 1. Lead concentrations (mean ± S.D.) in different organs of rats and its cestode parasites *Hymenolepis diminuta* and *Taenia taeniaeformis* sampled in 2 different areas adjacent to the city of Cairo; muscle lead levels ranged below the detection limit.

3·5 ± 4·2, whereas only 50% of the rats sampled in Abou Rawash were infected with this cestode (mean intensity ± S.D.: 3·0 ± 1·1). Larval *T. taeniaeformis* were only found in rats from Helwan with a prevalence of 33% and a mean intensity (± S.D.) of 2·7 ± 1·2.

Metal distribution among host tissues and parasites

In all samples of rat muscle lead concentrations ranged below the detection limit of 3 ng/ml. Accordingly, lead was only detected in the parasites and in liver, kidney and intestine of the rats (see Fig. 1). Comparing lead levels among host tissues and the parasites the significantly highest accumulation was found in *H. diminuta*, followed by rat kidney and larvae of *T. taeniaeformis* which differ insignificantly from each other. Intestine was always found to contain the significantly lowest lead concentration of all organs and parasites investigated, followed by liver which had the second lowest level.

Statistical analyses concerning the effects of infection on lead concentrations in host tissues revealed that metal concentrations in rat organs were not significantly different between uninfected rats and those rats being infected with either only adult or adult and larval cestodes. Thus, infection of rats with either of the parasites does not affect metal burdens in host tissues.

Bioconcentration factors were determined as described by Sures *et al.* (1999), which revealed 36-, 29-, 6- and 6-fold higher lead levels in *H. diminuta* as compared with intestine, liver, kidney and larvae of *T. taeniaeformis*, respectively, for Helwan. The lead bioaccumulation capacity of the extraintestinal larval cestodes was much lower with bioconcentration factors of 8, 6 and 1 as compared with host's intestine, liver and kidney. Thus, the ability to take up and bioconcentrate lead in parasites is clearly related with the parasite's microhabitat within the host and/or the developmental stage. In Abou Rawash bioconcentration factors for *H. diminuta* were found to be 87, 87 and 11, respectively, in intestine, liver and kidney of the host.

A comparison of the metal levels in rat tissues and parasites between both sampling areas revealed significant differences only for the lead content of the intestine although the lead levels in road dust as well as the bioavailable portion was considerably higher in Helwan than in Abou Rawash. Furthermore, *H. diminuta* had even slightly higher lead concentrations at the site which was classified as less polluted according to the road dust samples.

DISCUSSION

In terrestrial mammals the kidney is known as one of the main accumulation organs for metals (Merian, 1991). Accordingly, in the present study the kidney of the rats exhibited the highest lead concentrations compared with the other host tissues investigated. But, considering also the parasites, the highest concentration of lead was recorded for *H. diminuta*. However, *H. diminuta* was not suitable to indicate local differences in metal levels between the 2 sampling areas as reflected by the respective road dust samples. The rat tissues showed at least the same tendency in lead levels as road dust. Using intestinal samples differences of the lead levels were found to be significantly different at the two locations. However, as the lead concentrations in the intestine were very low, this tissue appears to be unsuitable for bioindicating purposes (Beeby, 2001).

No effect of an infection with *H. diminuta* on metal levels in host tissues was detected, which is in contrast to recent experimental studies where it appears that the presence of *H. diminuta* reduces the metal accumulation in host kidney (Sures *et al.* 2002). Information about the impact of parasites on the metal uptake by their respective hosts is heterogenic. In a study by Sures & Siddall (1999) it was shown that infection of chub (*Leuciscus cephalus*) with *Pomphorhynchus laevis* resulted in significantly lower lead concentrations in the intestine of infected fish compared with uninfected controls. On the other hand, in an experimental study on the lead uptake by *Moniliformis moniliformis* in rats no reduction of the lead concentration in the kidney of the host was observed (Sures *et al.* 2000*b*). Thus, mechanistical studies are necessary to decide whether intestinal parasites of mammals are able to affect metal levels in the tissues of their hosts as described from fish infected by acanthocephalans.

Our results provide additional evidence for the conclusion that mainly adult parasites inside their final host are able to take up and accumulate metals (Sures & Taraschewski, 1995; Siddall & Sures, 1998; Sures & Siddall, 1999). Most likely this phenomenon is related to the microhabitat of the parasite within its host rather than to the developmental stage of the parasite. Inside the intestine, adult parasites have access to bile acids, which are able to form organometallic complexes with metal ions running through the hepatic–intestinal cycle (Sures & Siddall, 1999). As parasites are known to be extremely efficient in taking up bile salts from the intestinal lumen of the host, this could explain the significantly higher lead concentrations detected in adults of *H. diminuta* as compared with larvae of *T. taeniaeformis*. The latter are located in cysts within liver tissue and thus have no access to bile acids which are produced in the liver.

From a recent experimental study it emerged, that the cestode *H. diminuta* could be a promising bioindicator for lead due to its enormous metal accumulation capacity (Sures *et al*. 2002). The accumulation capacity was confirmed in the present field study. Concerning the metal uptake of helminths in the gut of naturally infected mammals it appears that cestodes are about as effective as acanthocephalans. The acanthocephalans *Moniliformis moniliformis* and *Macracanthorhynchus hirudinaceus* showed elevated Pb levels in comparison with their hosts rat and pig, respectively (Sures *et al*. 2000*a*, *b*). As it is not easy to decide which sort of environmental contamination could be used as a measure of pollution, it is not appropriate to calculate the ratio of lead in *H. diminuta* to that in road dust. In mammals metal accumulation results from a complex uptake via food, water and air (Merian, 1991) and the road dust levels can only be used as an indication of the contamination around the sampling sites. Therefore, it is useful to calculate bioconcentration factors between metal levels in the parasites and host tissue concentrations. A bioconcentration factor ($C_{[\text{parasites}]}/C_{[\text{host kidney}]}$) of 6 for lead in the present study was in the upper range of factors determined in earlier studies for acanthocephalans which were found to be 5 for *M. moniliformis* (Sures *et al*. 2000*b*) and 56 for *M. hirudinaceus* (Sures *et al*. 2000*a*).

However, the variability of the lead concentrations showing relative standard deviations between 112 to 135% was very high in *H. diminuta*. This phenomenon has been described previously from other host–parasite associations (Sures, 2001). For example, for the lead accumulation in the caryophyllid cestode *Monobothrium wageneri* parasitizing tench a relative S.D. of 50% was determined (Sures, Taraschewski & Rokicki, 1997). But if parasites should be used as sentinels they have to indicate at least the same pollution pattern as the ambient matrices. The results on lead in *H. diminuta* in the present study which do not reflect the finding from road dust may be related to the fact that the sampled helminth tissues (proglottids) were of different age and that gravid cestodes constantly destrobilize the posterior parts of their bodies. From the few studies published on metals in cestodes it appears that elements like lead, cadmium and selenium are not distributed evenly in the worms, but concentrate especially in gravid proglottids of the strobila (Riggs, Lemly & Esch, 1987; Sures *et al*. 1997). Therefore, it is likely that differences in the grade of pollution between sites might be obscured if the analysed proglottids are not of a comparable age. Accordingly, it is necessary to standardize the sampling procedures for cestodes used for indication purposes. It should be inevitable to use only worms of approximately the same length which might stand as a measure of age. But as cestodes are not very compact organisms it is not easy to collect worms which are not broken into several parts. Therefore, we suggest that only those parts of the strobila should be taken which are similar in size and have gravid proglottids.

In conclusion it appears that cestodes might be promising indicators at least for lead. They meet a couple of criteria commonly suggested for accumulation indicators (see Sures, 2003). Cestodes are much more widespread and common parasites of mammals than acanthocephalans (Smyth, 1994), they are easily identified and provide enough tissue for metal analysis. Rats, the final hosts of *H. diminuta* are widely distributed and abundant in all kinds of terrestrial habitats, especially urban ecosystems. Thus, rats and their cestodes can be applied as a very useful tool in environmental monitoring. However, there are also a couple of characteristics suggested for accumulation indicators which need to be studied in the future to decide whether cestodes meet these criteria (see Discussion section in Sures, 2003). Especially more field and experimental studies are required to evaluate the relationship between parasite bioaccumulation and environmental metal exposure. If a linear relationship exists between cestode and environmental concentrations over a wide range of ambient pollution levels *H. diminuta* would be a suitable bioindicator in terrestrial habitats.

The authors express thanks to Fa. UMEG (Gesellschaft für Umweltmessungen und Umwelterhebungen, Karlsruhe) for providing the atomic absorption spectrometer PE 4100ZL.

REFERENCES

BARUŠ, V., TENORA, F. & KRÁČMAR, S. (2000). Heavy metal (Pb, Cd) concentrations in adult tapeworms (Cestoda) parasitizing birds (Aves). *Helminthologia* **37**, 131–136.

BEEBY, A. (2001). What do sentinels stand for? *Environmental Pollution* **112**, 285–298.

DIN 19730, Ausgabe: 1997–06. Bodenbeschaffenheit – Extraktion von Spurenelementen mit Ammoniumnitratlösung.

FAULKNER, B. C. & LOCHMILLER, R. L. (2000). Ecotoxicity revealed in parasite communities of *Sigmodon hispidus* in terrestrial environments contaminated with petrochemicals. *Environmental Pollution* **110**, 135–145.

GUNKEL, G. (1994). *Bioindikation in aquatischen Ökosystemen*. Gustav Fischer Verlag, Jena, Stuttgart.

KRAMAR, U. (1997). Advances in energy-dispersive X-ray fluorescence. *Journal of Geochemical Exploration* **58**, 73–80.

MERIAN, E. (1991). *Metals and their Compounds in Environment and Life. Occurence, Analysis and Biological Relevance*. Verlag Chemie Weinheim, New York.

RIGGS, M. R., LEMLY, A. D. & ESCH, G. W. (1987). The growth, biomass and fecundity of *Bothriocephalus acheilognathi* in a North Carolina cooling reservoir. *Journal of Parasitology* **73**, 893–900.

SCHEEF, G., SURES, B. & TARASCHEWSKI, H. (2000). Cadmium accumulation in *Moniliformis moniliformis* (Acanthocephala) from experimentally infected rats. *Parasitology Research* **86**, 688–691.

SCHUBERT, R. (1991). *Bioindikation in terrestrischen Ökosystemen*. Fischer Verlag, Jena, Stuttgart.

SIDDALL, R. & SURES, B. (1998). Uptake of lead by *Pomphorhynchus laevis* cystacanths in *Gammarus pulex* and immature worms in chub (*Leuciscus cephalus*). *Parasitology Research* **84**, 573–577.

SMYTH, J. D. (1994). *Introduction to Animal Parasitology*. Cambridge University Press, Cambridge.

SURES, B. (2001). The use of fish parasites as bioindicators of heavy metals in aquatic ecosystems: a review. *Aquatic Ecology* **35**, 245–255.

SURES, B. (2003). Accumulation of heavy metals by intestinal helminths in fish: an overview and perspective. *Parasitology* (in the Press).

SURES, B. & SIDDALL, R. (1999). *Pomphorhynchus laevis*: the intestinal acanthocephalan as a lead sink for its fish host, chub (*Leuciscus cephalus*). *Experimental Parasitology* **93**, 66–72.

SURES, B. & TARASCHEWSKI, H. (1995). Cadmium concentrations of two adult acanthocephalans (*Pomphorhynchus laevis*, *Acanthocephalus lucii*) compared to their fish hosts and cadmium and lead levels in larvae of *A. lucii* compared to their crustacean host. *Parasitology Research* **81**, 494–497.

SURES, B., FRANKEN, M. & TARASCHEWSKI, H. (2000*a*). Element concentrations in the archiacanthocephalan *Macracanthorhynchus hirudinaceus* compared with those in the porcine host from a slaughterhouse in La Paz, Bolivia. *International Journal for Parasitology* **30**, 1071–1076.

SURES, B., GRUBE, K. & TARASCHEWSKI, H. (2002). Experimental studies on the lead accumulation in the cestode *Hymenolepis diminuta* and its final host, *Rattus norvegicus*. *Ecotoxicology* **11**, 365–368.

SURES, B., JÜRGES, G. & TARASCHEWSKI, H. (2000*b*). Accumulation and distribution of lead in the acanthocephalan *Moniliformis moniliformis* from experimental infected rats. *Parasitology* **121**, 427–433.

SURES, B., SIDDALL, R. & TARASCHEWSKI, H. (1999). Parasites as accumulation indicators of heavy metal pollution. *Parasitology Today* **15**, 16–21.

SURES, B., TARASCHEWSKI, H. & HAUG, C. (1995). Determination of trace metals (Cd, Pb) in fish by electrothermal atomic absorption spectrometry after microwave digestion. *Analytica Chimica Acta* **311**, 395–399.

SURES, B., TARASCHEWSKI, H. & ROKICKI, J. (1997). Lead and cadmium content of two cestodes *Monobothrium wageneri* and *Bothriocephalus scorpii*, and their fish hosts. *Parasitology Research* **83**, 618–623.

TENORA, F., KRÁČMAR, S., PROKEŠ, M., BARUŠ, V. & SITKO, J. (2001). Heavy metal concentrations in tapeworms *Diploposthe laevis* and *Microsomacanthus compressa* parasitizing aquatic birds. *Helminthologia* **38**, 63–66.