Original Scientific Paper

HEAVY METAL LEVELS IN TISSUES OF RED DEER (*CERVUS ELAPHUS*) FROM EASTERN CROATIA*

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The aim of this study was to measure the levels of some toxic and essential metals in the kidney cortex and jawbone of red deer caught in Baranja region, Eastern Croatia. Kidneys and jawbones of 57 red deer (17 males, 40 females), aged six months to ten years, were collected and the concentrations of metals determined using atomic absorption spectrometry (AAS).
The median mass fraction of toxic cadmium, mercury, and lead in the kidney were 0.099 mg kg^{-1}, 0.362 mg kg^{-1}, and 0.578 mg kg^{-1} (wet weight), respectively. In the jawbone, the Pb mass fraction was 0.281 mg kg^{-1}. The median levels of essential elements in the kidney were 35.1 mg kg^{-1} for Zn, 5.20 mg kg^{-1} for Cu, and 108 mg kg^{-1} for Fe. The mass fraction of Zn in the jawbone was 86.8 mg kg^{-1}. Statistical analysis showed age-related differences in the accumulation of Pb in both tissues and of Cd in the kidney. Kidney Zn and Fe also increased with age. Toxic metal levels in the kidney exceeded the levels considered acceptable for human consumption, especially in older animals.

KEY WORDS: *accumulation, age, essential metals, jawbone, kidney, toxic metals*

Many metals are essential to life in small amounts, but are toxic in higher doses. Others, such as lead, mercury and cadmium, probably do not have a physiological function in man. Civilisation, especially in the last century, has caused a sharp increase in the concentration of many metals in the air, soil and water (1). In certain situations, toxic metals display a propensity for marked bioaccumulation, a feature that may be of toxicological significance for the organisms concerned (2). The most important route of exposure for humans and animals is through ingestion of metal-contaminated food.

Organs of wild herbivores, such as red deer (*Cervus elaphus*), can be indicative of the contamination level of their biota. Kidney is known to be the main target organ for the accumulation of some toxic metals, especially cadmium and mercury, as bone is for lead (3). Therefore, their concentrations in the environment should be constantly monitored.

No research has been conducted on natural contamination by metal residues in Croatian population of red deer. This species is traditionally popular big game in Croatia, hunted for antlers and highly appreciated meat. Eastern Croatia (Baranja region) is the most famous habitat of red deer, and their growing number was affected only with the large American liver fluke (*Fascioloides magna*) epidemic in 2000. Legal harvest whose purpose is to estimate the health condition of red deer in an area provides an opportunity to monitor heavy metal levels in various tissues.

We investigated the metal content in the kidneys and jawbones of wild red deer from Baranja region to estimate potential impact of metal levels on the health of the animals. In addition, we estimated metal mass fractions in organs consumed as food to assess potential risk. The results were compared with previously published data from other European and North-American countries.

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MATERIALS AND METHODS

Samples from red deer (N=57) aged six months to approximately ten years were collected during the hunting season from September 2002 to February 2003 in the forestry of Eastern Croatia (Baranja region; wide area around the Nature Park of Kopački rit, Figure 1). Examined because of the infection with the large American liver fluke (*Fascioloides magna*), the deer were subsequently categorised in 10 groups depending on the severity of fascioloidiasis found. Kidney and jawbone samples were packed separately in polyethylene bags, frozen and sent to our laboratory where they were stored at -18°C until analysis.

Figure 1 *Map of Croatia showing the studied area*

About 2 g of the kidney cortex and jawbones were weighed for lead, cadmium, iron, copper and zinc determination and dry-ashed overnight in quartz crucibles in a muffle furnace at 450 °C. The ash residues were subsequently dissolved in concentrated nitric acid, heated and filled up to 10 mL with deionised water. To avoid possible loss of analyte in the case of mercury, samples were digested by wet ashing in a closed system (4). About 1 g of thawed kidney cortex was digested overnight in 2 mL concentrated nitric acid at room temperature in open long-neck glass tubes. The next day digestion was performed at 80 °C in closed tubes for 5 h in a programmed system (DS-40, Tecator, Höganäs, Sweden). After cooling, samples were adjusted to 10 mL with deionised water. Procedural blanks were run with each sample set to control for sample contamination during the dry ashing/digestion procedures. Lead was measured using Varian SpectrAA-300 atomic absorption spectrometer (Mulgrave, Victoria, Australia) equipped with a graphite furnace unit, autosampler and D_2 background correction (5). Argon was used as the purge gas. The determination of Cd, Zn, Fe and Cu was performed by aspirating prepared sample solution into the air-acetylene flame of Varian AA-375 atomic absorption spectrometer (Mulgrave, Victoria, Australia). No matrix modifiers were used for metal analyses. Total mercury was measured according to a modified Farant's method (4) using cold vapour atomic absorption spectrometry in a mercury monitor (LDC, Milton Roy, FL, USA).

At least two replicate determinations were made for each sample. The reliability of the above analytical methods was evaluated through the use of standard reference materials (bovine liver 1577b; NIST, USA and horse kidney H8; IAEA, Austria) in duplicate together with each sample series. All measurements were inside the confidence limits of reference material and obtained results are presented in Table 1.

Table 1 *Accuracy estimated on two reference materials (horse kidney H8, IAEA and bovine liver 1577b, NIST)*

Metal	Method	Reference material	Mass fraction / mg kg^{-1}	
			Determined value[1]	Certified value[1]
Hg	CVAAS	H8	0.974±0.057 (5)	0.910±0.070
Pb	ETAAS	1577b	0.131±0.008 (8)	0.129±0.04
Cd	FAAS	1577b	0.501±0.045 (6)	0.500±0.03
Fe	FAAS	1577b	185±22 (8)	184±15
Zn	FAAS	1577b	128±3 (10)	127±16
Cu	FAAS	1577b	164±6 (10)	160±8

CVAAS = cold vapour atomic absorption spectrometry
ETAAS = electrothermal atomic absorption spectrometry
FAAS = flame atomic absorption spectrometry
[1] *Mean ± standard deviation (number of replicates in parentheses)*

STATISTICAL PROCEDURE

Elementary data were log-transformed to assure normality and homogeneity of variances. Student *t*-test and one-way analysis of variance (ANOVA) were used for the statistical evaluation of the data. Significance was accepted at $p<0.05$. All results are given as mg kg^{-1} on a wet weight basis.

Owing to more than two times large female group (N=40) than male (N=17), sex comparisons were not evaluated statistically, but data from both sexes were pooled.

In relation to age, red deer were classified to 2 age classes using morphological and dental characteristics: animals ≤2 years old (juveniles) and animals >2 years (adults). Only there where significant differences in metal content between age groups were found their levels were separately presented. Otherwise, they are presented jointly for both juvenile and adult deer.

Calculated median values are considered as the best heavy metal distribution representation among wildlife, but mean values were also included to enable the comparisons with other studies where means were used.

RESULTS AND DISCUSSION

In the comparison of this study and other studies, it is important to bear in mind that we took samples of the kidney cortex alone, while most other studies used the whole kidney. There may also be differences in the other studies due to inclusion or failure to include animals' age in the processing of data as well as due to recalculations of data from dry weight.

When statistically compared, the degree of fascioloidiasis had no influence on heavy metal mass fractions in tissue. The only exception was a slight negative correlation of fascioloidiasis with Cu and positive with Pb levels in the kidney ($p<0.05$).

Table 2 shows the mass fractions of Cd, Hg, Pb, Zn, Cu and Fe in samples taken from red deer (N=57) caught in Baranja region. The median values, means with standard deviations (SD) and ranges are given, as well as the percentage of samples exceeding the permitted values of Cd, Hg and Pb in the kidney, if consumed as food [defined by Croatian Regulatory Act (6)].

CADMIUM

Considering Baranja as a nonpolluted area, red deer showed high mean Cd values in the kidney (0.944 mg kg^{-1}), but the median value of 0.099 mg kg^{-1} shows that most samples had low Cd. Still, our mean Cd level is notably lower than reported in other countries (7-14). The mass fractions of Cd found in the kidney cortex of Croatian deer can be approximated to whole kidney mass fractions by dividing our results by a factor of 1.25, as estimated by *Svartengren et al.* (15).

Nordberg et al. (16) showed that Cd in reindeer used for human food is bound to metallothionein-like proteins which enter the blood plasma and are efficiently transported to the kidney. The accumulation of Cd in the kidney without apparent toxic effect is possible because of the formation of the above mentioned metal protein complex (3). Accordingly, kidney Cd burden in red deer investigated in this study, although high, is non-toxic, as it does not produce measurable adverse health effects in animals (from 100 mg kg^{-1} to 300 mg kg^{-1}), according to *Cooke and Johnson* (17).

Statistics showed no significant differences in the kidney Cd levels between the two age groups, although they were slightly higher in adult deer. The main cause for this elevation is the extremely long retention period, reaching 10 to 30 years in the kidney of mammals (17). The accumulation of Cd and Pb in red deer kidney with age was reported in the Veluwe area, the Netherlands, but no significant differences between age groups was observed for the bones (13). Similar age-related increases in the kidney Cd burden have also been documented in South Ontario (14).

There are no published data concerning heavy metal levels in red deer tissues from Croatia. However, *Pompe-Gotal et al.* (18) and *Olujić* (19) determined Cd ranges and median renal values in roe deer (*Capreolus capreolus*) from central Croatia. These ranges were from 1.39 mg kg^{-1} to 27.7 mg kg^{-1} (median 6.15 mg kg^{-1}) and from 0.223 mg kg^{-1} to 27.7 mg kg^{-1} (median 2.28 mg kg^{-1}), respectively. In comparison with our data (Table 2), Cd levels were much higher, as expected, because roe deer is known to accumulate Cd at a higher rate than red deer (20). Some authors (21) found higher Cd residues in red deer. *Grdiša* (22) differentiated kidney Cd levels in calves (median 0.195 mg kg^{-1}) and adult (median 0.796 mg kg^{-1}) fallow deer (*Dama dama*) from the

Table 2 *Mass fractions of Cd, Hg, Pb, Zn, Cu, and Fe (mg kg^{-1} wet weight) in tissues of red deer (N=57) collected in Baranja region in the hunting season 2002/2003*

Element	Mass fraction / mg kg^{-1}			Excess* / %
	Median	Range	Mean ± Standard deviation	
Cd				
kidney	0.099	0.017-8.57	0.944±1.80	30
Hg				
kidney	0.362	0.092-0.883	0.375±0.174	98
Pb				
kidney	0.578	0.026-12.1	2.28±3.36	51
bone	0.281	0.056-3.00	0.490±0.602	/
Zn				
kidney	35.1	21.6-242	50.7±47.1	/
bone	86.8	51.4-124	86.4±14.8	
Cu				
kidney	5.20	2.59-8.60	5.16±1.00	/
Fe				
kidney	108	25.9-353	121±66	/

**The percentage of samples exceeding the permitted values for kidney for food defined by Croatian Regulatory Act (6) given as: Cd and Pb: 0.5 mg kg^{-1}; Hg: 0.1 mg kg^{-1}; Zn, Cu and Fe: not specified*

Croatian island of Brijuni, both higher than the levels observed in our study (Table 2).

Domestic animals are less contaminated with heavy metals than wild animals, principally due to their food intake. Cadmium in cattle kidneys ranged from 0.300 mg kg^{-1} to 0.650 mg kg^{-1} (23) and *Sapunar-Postružnik et al.* (24) reported median Cd bovine and porcine content to be 0.175 mg kg^{-1} (up to 2.40 mg kg^{-1}) and 0.270 mg kg^{-1} (up to 10.4 mg kg^{-1}), respectively.

MERCURY

Kidneys contain the greatest mass fractions of Hg following exposure to inorganic salts of mercury and mercury vapour (3). Available data on kidney Hg levels in red deer are scarce for the purpose of comparison. Relatively high levels (Table 2) exceeded the comparable levels of Hg in deer from North Poland (mean 0.018 mg kg^{-1}) and West Germany (mean 0.027 mg kg^{-1}) reported by *Falandysz* (7) and *Rimkus and Wolf* (25), respectively. Unlike Cd, Hg did not show a trend of accumulation in the kidney of red deer from Baranja region. Roe deer from central Croatia showed four times lower levels of Hg in the kidney (18, 19).

Mercury residues which deer take in through plants could originate from the Danube sediments, which flood this area twice a year. When the river retreats to its bed, it leaves behind great amounts of mud. Plant Hg shows even greater toxic effects than Hg from salt waters (26).

LEAD

Baranja does not have big local pollution sources and industry. However, high kidney lead mass fraction (mean 2.28 mg kg^{-1}) found in our study could originate from traffic, long-range aerial transport, and deposition on the surfaces of leaves (7). Some high kidney levels of Pb may be due to the secondary contamination caused by dust from shots or by intensive pollution during the war in the nineties. Lead levels found in the jawbone (median 0.281 mg kg^{-1}) were lower than those in the kidney (median 0.578 mg kg^{-1}). The opposite was found in red deer from South Ontario (14). The skeleton is the largest and kinetically the slowest pool of Pb with a half-life of more than 20 years, whereas the soft tissue pool (3) is much more labile, with the half-life measured in weeks. Therefore, Pb level in the red deer kidney indicates present exposure of animals.

Table 3 shows the median values of the kidney and bone Pb, followed by ranges in two age groups (juveniles and adults). In contrast to Cd and Hg levels, Pb mass fractions were significantly higher in adult kidneys and bones than in juvenile tissues ($p<0.01$ and $p<0.05$, respectively). In juvenile deer, 70 % of kidney samples had Pb levels under 0.500 mg kg^{-1} [permitted level of Pb in kidney for food (6)] and were appropriate for consumption, unlike most of the adult samples, which exceeded the acceptable level of Pb.

Table 3 *Median and range values of Pb and Zn (mg kg^{-1} wet weight) in the kidney cortex and jawbone calculated separately for juvenile (N=22) and adult (N=35) animals*

Metal	Age group[1]	Mass fraction / mg kg^{-1}	
		Kidney cortex	Jawbone
Pb	Juveniles		
	Median	0.281**	0.236*
	Range	0.026-5.16	0.056-0.782
	Adults		
	Median	1.93**	0.341*
	Range	0.046-12.0	0.072-3.00
Zn	Juveniles		
	Median	32.8*	84.3
	Range	21.5-55.5	66.7-104
	Adults		
	Median	37.1*	90.2
	Range	22.2-242	51.4-124

[1]*Juveniles: animals ≤2 years old; adults: animals >2 years old*
**Age group differences significant at p<0.05*
***Age group differences significant at p<0.01*

When comparing our values obtained in the kidney of red deer (Table 2) with the only available data for big game animals from Croatia (18, 19), roe deer showed comparable or lower kidney levels of Pb. Lead content found in bovine and porcine kidney (24) was lower than in red deer (Table 2) and their means were 0.137 mg kg^{-1} and 0.183 mg kg^{-1}, (up to 1.10 mg kg^{-1} and 2.07 mg kg^{-1}), respectively.

Toxic Cd, Hg and Pb levels in edible red deer kidney may be relatively unimportant in terms of dietary intake of the Croatian population in general, but it may have a significant impact when consumed on a regular basis (hunters), especially considering the background factors which influence Cd uptake and bioavailability (sex, age, living environment, smoking habits, etc.). The FAO/WHO Expert Committee on Food Additives established a Provisional Tolerable Weekly Intake (PTWI) of 7 µg kg^{-1} b. w. for Cd (28) and 25 µg kg^{-1} b. w. for Pb (29). *Vahteristo et. al.* (27) estimated that a median daily intake of Cd from consumption of moose kidneys in Finland was 4.80 µg per a 60 kg user (8 % PTWI) while the average daily intake of Cd in Finland is about 10 µg. With an even higher estimated mean weekly dietary intake of Cd and Pb (24) per person in Croatia (121 µg, corresponding to 24.8 % PTWI and 701 µg, corresponding to 40.1 % PTWI, respectively) and more than a hundred years old tradition of hunting, Croatian hunters or any other sensitive individuals consuming large amounts of adult deer kidney can significantly elevate the toxic metal body burden. Other authors (30) calculated Cd and Pb intake in Croatia to be less than 23 % and 25 % PTWI, respectively. Consequently, the possibility of further increase of Cd and Pb intake to hazardous levels by eating internal organs is lower, but still, only moderate consumption of deer kidneys is recommended.

ZINC, COPPER AND IRON

Similar to Pb, age-related differences were established for Zn content ($p<0.05$), as shown in Table 3. Higher kidney and jawbone Zn levels were found in adult deer, but these differences were not statistically significant in the jawbone. *Parker and Hamr* (14) found that adult red deer had lower Zn levels than calves of the same species in South Ontario. They reported higher levels of Zn in the kidney and bone than we found in the Croatian red deer.

Mean kidney Zn and Cu mass fractions (Table 2) were comparable to deer from the North (7) and South Poland (8) while Fe mean values in Baranja deer were two to three times higher. Likewise, Cu and Fe levels in Baranja red deer were twice as high as in the animals from South Ontario (14).

CONCLUSION

This preliminary study of toxic and essential metal levels in red deer kidney and bone in Eastern Croatia generally shows higher values of lead and mercury than reported in other countries. Other elements were mostly in the same range with the levels reported. Toxic metals accumulated in tissues of interest indicate the need of further and more detailed monitoring of heavy metals. Based on our data, we recommend moderate consumption of deer kidney, especially those from older animals.

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Sažetak

SADRŽAJ TEŠKIH METALA U TKIVIMA OBIČNOG JELENA (*CERVUS ELAPHUS*) IZ ISTOČNE HRVATSKE

Divlje životinje u svom su okolišu stalno izložene različitim izvorima onečišćenja. Svrha ovog istraživanja bila je izmjeriti masene udjele kadmija, olova, žive, cinka, bakra i željeza u bubregu te olova i cinka u kosti običnog jelena (*Cervus elaphus*) ulovljenog na području Baranje u istočnoj Hrvatskoj, u blizini Parka prirode "Kopački rit". Uzorkovane su jedinke odstrijeljene sanitarnim odstrelom zbog pojave velikog američkog jetrenog metilja (*Fascioloides magna*). Skupljeni su bubrezi i mandibule 57 jedinki (17 mužjaka, 40 ženki), dobi od 6 mjeseci do 10 godina. Sadržaj metala izmjeren je metodom atomske apsorpcijske spektrometrije (AAS). Za određivanje Cd, Zn, Cu i Fe rabila se plamena AAS, za Pb elektrotermička AAS tehnika, dok je tehnikom hladnih para određivana Hg.
Medijani izmjerenih masenih udjela toksičnih metala Cd, Hg i Pb u bubregu su 0.099 mg kg^{-1}, 0,362 mg kg^{-1} i 0,578 mg kg^{-1}, dok je medijan Pb u kosti 0,281 mg kg^{-1} (mokre težine). Medijani masenih udjela esencijalnih metala u bubregu su 35,1 mg kg^{-1} za Zn, 5,20 mg kg^{-1} za Cu i 108 mg kg^{-1} za Fe. U kosti je izmjeren maseni udio Zn od 86,8 mg kg^{-1}. Statistička analiza pokazala je razlike u akumulaciji Pb s obzirom na dob u oba tkiva te Cd u bubregu, kao što se i očekivalo, ali razlika u slučaju Cd nije bila značajna. Sadržaj Zn i Fe u bubregu također je pokazao trend rasta s dobi. Dobiveni rezultati uspoređeni su sa sličnim podacima autora iz drugih zemalja. Nije nađena povezanost stupnja fascioloidoze i količine teških metala, s iznimkom Cu i Pb u bubregu. Razine toksičnih metala u bubregu, posebno kod starijih životinja, više su od dopuštenih razina u namirnicama.

KLJUČNE RIJEČI: *bubreg, dob, esencijalni metali, mandibula, nakupljanje, toksični metali*

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