NAT. CROAT. | VOL. 17 | No 4 | 273–281 | ZAGREB | December 31, 2008

original scientific paper / izvorni znanstveni rad

TOXICOLOGICAL SURVEY OF FREE RANGING POPULATION OF ROE DEER (*CAPREOLUS CAPREOLUS*) AND RED DEER (*CERVUS ELAPHUS*) BY TEETH EXAMINATION

ANNALISA ZACCARONI[1], DINO SCARAVELLI[1], RENZO DE BATTISTI[2], ALESSANDRO ZANELLA[2] & DONATELLA GELLI[3]

[1]Department of Veterinary Public Health and Animal Pathology, Viale Vespucci 2, Cesenatico (FC) 47042, University of Bologna

[2]Department of Forestry Science, College of Agriculture, Padua University

[3]Department of Veterinary Clinical Sciences, Veterinary School, Padua University

Zaccaroni, A., Scaravelli, D., De Battisti, R., Zanella, A. & Gelli, D.: Toxicological survey of free ranging population of roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) by teeth examination. Nat. Croat., Vol. 17, No. 4, 273–281, 2008, Zagreb.

Heavy metal content was evaluated in the teeth of roe deer and red deer. No differences in heavy metal concentrations between sampling areas or species were observed. A similar deposition process can be considered for the two species, as well as a similar chronic exposure levels.

Keywords: roe deer, red deer, heavy metals, teeth

Zaccaroni, A., Scaravelli, D., De Battisti, R., Zanella, A. & Gelli, D.: Toksikološko istraživanje divlje populacije srne (*Capreolus capreolus*) i jelena (*Cervus elaphus*) ispitivanjem zuba. Nat. Croat., Vol. 17, No. 4, 273–281, 2008, Zagreb.

U zubima srne i jelena mjeren je sadržaj teških metala. Nije primijećena razlika u koncentracijama metala ni između različitih područja prikupljanja uzoraka, niti između vrsta. Za obje vrste može se pretpostaviti sličan proces taloženja metala, kao i slična razina kronične ekspozicije.

Ključne riječi: srna, jelen, teški metali, zubi

INTRODUCTION

Due to their industrial use and high persistence in the environment some heavy metals can be responsible for environmental contamination and can be absorbed through air, water and food, thus being available for bioaccumulation in organisms. Bioaccumulation is greatly evident in the local environment especially in non-migrating species, even if not at the upper trophic level.

Some metals are essential to life in small amounts, but are toxic in higher doses (so called essential elements). Others (toxic metals), such as lead, mercury and cadmium, have no known physiological function in vertebrates. In certain situations, toxic metals display a tendency for marked bioaccumulation, a feature that may be of toxicological significance for the organisms concerned. The most important route of exposure in animals is through ingestion of metal-contaminated food. Cervids have long been used as biomonitors because of their ecological characteristics (CONDER & LANNO, 1999). The kidney is the main target organ for the accumulation of some toxic metals, especially cadmium and mercury, while lead is preferentially accumulated by bone. The evaluation of metals in these organs gives useful information concerning recent exposure to pollutants, because of the high rate of turnover than can be observed. Hard tissues like bone, teeth and antlers can be used to detect accumulation of metals to which they are exposed in a more persistent way than soft tissues, and can thus be used for long term or life-span monitoring of exposure.

Bone, teeth and antlers of the cervids are reliable and sensitive indicators of local pollution (KARSTAD, 1967; MANKOVSKA, 1980; IRWIN *et al.*, 1981; SILEO & BEYER, 1985) and reflect geographical variations in contaminant burden in terrestrial ecosystems (SAWICKA-KAPUSTA, 1979). Most of the available literature refers to the use of bone and antlers for metal monitoring (MCTAGGART *et al.*, 1981; WITKOWSKI *et al.*, 1982; SILEO & BEYER, 1985; SWIERGOSZ *et al.*, 1993; SCHÖNHOFER *et al.*, 1994; MEDVEDEV, 1995; TATARUCH, 1995; KUITERS, 1996; CONDER & LANNO, 1999; KIERDORF & KIERDORF, 2000; BJORÅ *et al.*, 2001; KIERDORF & KIERDORF, 2002a; KIERDORF & KIERDORF, 2002b; LAZARUS *et al.*, 2005). An interesting and little used alternative to these matrices for long term analysis of contaminants could be teeth, which represent an indication of life span accumulation of metals. Present work reports about a first attempt of the use of teeth of cervids for heavy metals monitoring in a North-Eastern areas of Italy.

MATERIAL AND METHODS

Teeth samples from roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) were collected from mandibles obtained from hunters of two areas of Belluno Province, North-Eastern Italy (Fig. 1). For each mandible, data concerning hunting area, species, and date of killing were available. Due to the small dataset and the relatively balanced situation, age and sex were not included in the analysis.

The last molar (M3) was extracted from each mandible and was carefully ground in a marble mortar. The obtained powder (0.7 g) was microwave digested and analysed using an ICP-OES technique for As, Cd, Co, Cr, Hg, Ni, Pb and Se.

Statistical analysis was performed using STATISTICA 6.0© Program (StatSoft Italia s.r.l.) performing Student's t test for comparison of species and sampling areas.

Fig. 1. Sampling areas for roe deer and red deer.

Whenever a toxicological result was below the limit of detection it was substituted by a random number between the detection limit and one-half the detection limit (TRAVIS & LAND 1990). The rejection limit was established at P<0.05 unless otherwise noted.

RESULTS AND DISCUSSION

Obtained data, reported as µg/g ± s.e. dry weight and minimum and maximum values observed, are shown in Tab. 1 as concentration in deers and in areas, and in Tab. 2 as differences between species in the two sites.

Chromium was the metal found in the highest amounts, followed by arsenic, cadmium, mercury and lead. Nickel and cobalt were present in very small amounts, close to the limits of detection, while selenium was always below detection limits; data concerning selenium and nickel are not reported in tables and figures and will not be discussed in this paper.

Statistical analysis of data by sampling area revealed no significant difference in heavy metal content between the two sampling areas in roe deer, while some differences were found in red deer for As (p<0,05) and Cr (P<0,01), with Sedico showing the highest concentrations of both elements (Tab. 2). Considering this lack of significant differences, data were aggregated in order to check for differences between species and for those between areas. No significant difference was found for any of the parameters considered.

Tab. 1. Heavy metals in teeth of roe deer and red deer as function of species and of sampling areas.

Metal (µg/g)	Roe deer		Red deer	
	San Vito di Cadore	Sedico	San Vito di Cadore	Sedico
As	0.631 ± 0.136 0.164-1.933	0.667 ± 0.084 0.037-2.047	0.478 ± 0.086[a] 0.219-0.758	0.804 ± 0.0758[a] 0.312-1.345
Cr	4.295 ± 0.408 0.996-6.856	4.595 ± 0.255 0.954-6.678	3.657 ± 0.577[b] 0. 996-4.895	5.489 ± 0.218[b] 4.214-6.338
Pb	0.046 ± 0.020 BLD-0.278	0.138 ± 0.067 BLD-1.75	0.023 ± 0.014 BLD-0.067	0.092 ± 0.060 BLD-0.706
Co	0.010 ± 0.003 BLD-0.052	0.012 ± 0.005 BLD-0.170	0.014 ± 0.008 BLD-0.052	0.018 ± 0.013 BLD-0.171
Cd	0.072 ± 0.015 0.026-0.234	0.088 ± 0.017 0.010-0.598	0.076 ± 0.032 0.026-0.234	0.061 ± 0.019 0.023-0.236
Hg	0.057 ± 0.005 0.022-0.089	0.066 ± 0.004 0.023-0.175	0.062 ± 0.009 0.036-0.089	0.054 ± 0.021 0.024-0.092

a: P< 0,05
b: P< 0.01

Tab. 2. Heavy metals in teeth of roe deer and red deer as function of species in sampling areas.

Metal (µg/g)	Species		Sampling area	
	Roe deer	Red deer	San Vito di Cadore	Sedico
As	0.637 ± 0.103 0.037-2.048	0.695 ± 0.067 0.219-1.345	0.631 ± 0.136 0.164-1.932	0.668 ± 0.084 0.037-2.047
Cr	4.313 ± 0.282 0.955-6.856	4.878 ± 0.311 0.996-6.338	4.295 ± 0.408 0.996-6.856	4.595 ± 0.255 0.954-6.678
Pb	0.135 ± 0.070 BLD-1.748	0.069 ± 0.040 BLD-0.706	0.046 ± 0.020 BLD-0.278	0.138 ± 0.067 BLD-1.748
Co	0.009 ± 0.003 BLD-0.086	0.016 ± 0.009 BLD-0.170	0.010 ± 0.003 BLD-0.051	0.012 ± 0.005 BLD-0.170
Cd	0.084 ± 0.018 0.010-0.598	0.066 ± 0.016 0.023-0.235	0.071 ± 0.015 0.026-0.234	0.080 ± 0.017 0.010-0.598
Hg	0.066 ± 0.005 0.022-0.175	0.057 ± 0.005 0.024-0.092	0.057 ± 0.005 0.022-0.089	0.066 ± 0.004 0.023-0.175

Few works report heavy metal concentrations in cervids (MANKOVSKA, 1980; WITKOWSKI *et al.*, 1982; SILEO & BEYER, 1985; MEDVEDEV, 1995), and those published tend to focus on Cd and Pb. Some additional work has been done on human, swine, rat and bovine teeth, mainly focusing on mineral composition of this tissue or on the evaluation of past exposure to cadmium and lead in the diet (COUSINS *et al.*, 1973; JOHNSON & SHEARER, 1979; NEEDLEMAN *et al.*, 1979; SHEARER *et al.*, 1980; MALARA *et al.*, 2006; SABER-TEHRANI *et al.*, 2007).

Data here presented concerning Cd are well below those reported by MEDVEDEV (1995) in reindeer and those of MANKOVSKA (1980) in roe deer. Both two studies refer to polluted areas and it can thus be speculated that in those areas a notable pollution by cadmium was present.

By contrast, our data are in agreement with those of SILEO & BEYER (1985) in white tailed deer and can be considered as indicative of little or no exposure to Cd. Indeed, the authors consider observed levels in teeth (0.18–0.21 μg/g) as indicative of little exposure to cadmium and they also speculate that probably most of the body burden of Cd could be in soft tissues, particularly liver and kidney. Starting from the data of COUSINS *et al.* (1973) and SHEARER *et al.* (1980) in teeth of rats and swine, a daily intake lower than 30 ppm in the diet for cadmium can be considered.

Such a consideration is confirmed by the data of GDULA-ARGASINSKA *et al.* (2004) in bank voles from different areas of Poland. These authors found highest levels of cadmium in the teeth of bank voles from highly polluted areas, while burdens in the teeth of animals from uncontaminated areas and from laboratory housed bank voles were comparable to those found in the present study (0.03–0.22 μg/g vs. 0.061–0.088 μg/g in GDULA-ARGASINSKA and our studies respectively)

Again, OUTRIDGE *et al.* (1997) and OUTRIDGE (2005) found comparable mean values (0.01 μg/g) in beluga whale and walrus teeth and consider mean concentrations as indicative of exposure to »natural« or background levels. Even if species and environment considered are completely different, such a speculation can be done also for our result.

Some other information is available on lead concentrations in cervids and in other wildlife species (MANKOVSKA, 1980; WITKOWSKI *et al.*, 1982; SILEO & BEYER, 1985; MEDVEDEV, 1995; OUTRIDGE *et al.*, 1997; GDULA-ARGASINSKA *et al.* 2004).

The analysis of data reported in Tab. 1 underlines how mean values of lead as function of both species and sampling area seem to be highly different (0.135 vs. 0.069 μg/g between roe deer and red deer; 0.046 vs. 0.138 μg/g between San Vito di Cadore and Sedico, respectively). Anyway, the huge variability observed in lead values (the range being BLD-1.748 μg/g) made the differences observed not statistically significant ($P>0.05$). A similar high variation in data was observed by KIERDORF *et al.* (2007) in roe deer from Germany, thus minimizing the hypothesis of analytical mistakes in our study.

All reported values are much higher than those found in the present study and make it possible to state that roe deer and red deer lead content are indicative of background exposure to the metal. Indeed, SILEO & BEYER (1985) consider concentrations up to 5.5 μg/g as indicative of a normal range of exposure, and OUTRIDGE *et al.* (1997) stated that 0.3 μg/g of lead in beluga whales should be considered as indicative of background exposure. More recently, KIERDORF *et al.* (2007) found levels of Pb as low as 0.2 μg/g in roe deer from German lead-polluted areas. These data thus confirm our conclusion concerning the low contamination exposure of Italian animals.

Again, GDULA-ARGASINSKA *et al.* (2004) report about mean lead concentration of 1.31 ± 1.92 µg/g and 0.56 ± 0.06 µg/g in back vole teeth from unpolluted areas and from laboratory animals respectively.

The significant differences observed for chromium and arsenic were unexpected. No information is available concerning the presence of these two metals in teeth. Anyway, some hypothesis can be done regarding possible causes producing such differences.

First of all, differences in feeding habits can produce a different body burden in roe deer and red deer. Anyway, the fact that only in red deer differences among areas can be observed implies not only a differential exposure to both arsenic and chromium via food, but also some differences in metal metabolism or deposition in hard tissues. Even if they share the same environment, roe deer and red deer select different food items, which can be responsible for a differential exposure to contaminants.

The fact that As and Cr in roe deer are highly comparable between the two areas seems to make the combination of these two hypotheses the most probable explanation of the results obtained. The already mentioned lack of information regarding As and Cr presence in teeth make any verification of this hypothesis speculative.

Differences in environmental contamination between the two areas should be considered as well, starting from the data in red deer. Considering the geographical position of the Sedico hunting fields with respect to those of S. Vito di Cadore, the highest concentrations found (0.804 ± 0.0758 µg/g vs. 0.478 ± 0.086 µg/g for As and 5.489 ± 0.218 µg/g vs. 3.657 ± 0.577 µg/g for Cr in Sedico and San Vito di Cadore respectively) can be probably related to a closer proximity to lowlands where agricultural and industrial activities are present.

Further and more in-depth studies concerning As and Cr burden in teeth of roe deer and red deer are necessary in order to better understand the exact mechanism of and the causes underlying such differences, possibly coupling metal determination in teeth to quantification in soft tissue. Finally, when mercury is of concern, it should be noted that in all samples the metal was over the limit of detection of the method, and this was not expected. Obtained data are to be considered as indicative of exposure to background levels, in agreement to data reported by OUTRIDGE (2005) in beluga whales and by EIDE *et al.* (1995) in control rats of an exposure trial to 20 µg/g $HgCl_2$ or 20 µg/g CH_3HgCl every two days with drinking water. In EIDE's experiment exposed rats presented levels as high as 0.888 µg/g Hg after 4 weeks exposure, while control rats had a mean concentration 0.010–0.021 µg/g.

The present study focused on the monitoring of various metals in deer teeth, defining a low exposure of the studied species to heavy metals, making it possible to consider the selected areas unpolluted.

Species and areas differences observed for some metals make it possible to consider teeth a reliable monitoring tool for both long-term and short-term exposure to heavy metals.

To obtain a more effective result and better define the role of teeth as a monitoring tool it would be important, taking into account soft tissues as well, to compare and correlate tissue burden and teeth concentration of heavy metals. The definition of such correlations will allow the creation of predictive models for monitoring activities.

REFERENCES

BJORÅ, R., FALCH, J. A., STAALAND, H., NORDSLETTEN, L. & GJENGEDAL, E., 2001: Osteoporosis in the Norwegian moose. Bone **29**, 70–73.

CONDER, J. M. & LANNO, R. P., 1999: Heavy metal concentration in mandibles of white-tailed deer living in the Pincher mining district. Bulletin Environmental Contamination 63, 80–86.

COUSINS, R. J., BARBER, A. K. & TROUT J. R., 1973: Cadmium toxicity in growing swine. Journal of Nutrition **103**, 964–972.

EIDE, R., SCHIØNNING, J. D., ERNST, E., HANSEN, I. M. & WESENBERG, G. R., 1995: Mercury content in rat teeth after administration of organic and inorganic mercury. Acta Odontologica Scandinavica **53**, 12–16.

GDULA-ARGASINSKA, J., APPLETON, J., SAWICKA-KAPUSTA, K. & SPENCE, B., 2004: Further investigation of the heavy metal content of the teeth of the bank vole as an exposure indicator of environmental pollution in Poland. Environmental Pollution **131**, 71–79.

JOHNSON, J. R. & SHEARER T. R., 1979: Selenium uptake into teeth determined by fluorimetry. Journal Dental Research **58**, 1836–1839.

KARSTAD, L., 1967: Fluorosis in deer (*Odocoileus virginianus*). Bulletin Wildlife Disease Association **3**, 42–46.

KIERDORF, H. & KIERDORF, U., 2000: Roe deer antlers as monitoring units for assessing temporal changes in environmental pollution by fluoride and lead in a German forest area over a 67-year period. Archives Environmental Contamination and Toxicology **39**, 1–6.

KIERDORF, H. & KIERDORF, U., 2002a: Reconstruction of a decline of ambient lead levels in the Ruhr area (Germany) by studying lead concentrations in antlers of roe deer (*Capreolus capreolus*). Science of the Total Environment 296, 153.158.

KIERDORF, U. & KIERDORF, H., 2002b: Temporal variation of fluoride concentration in antlers of roe deer (*Capreolus capreolus*) living in an area exposed to emissions from iron and steel industry, 1948–2000. Chemosphere **52**, 1677–1681.

KIERDORF, H., ÅBERG, G. & KIERDORF, U., 2007: Lead concentration and lead and strontium stable isotope ratios in teeth of European roe deer (*Capreolus capreolus*). Proceedings of the 2nd International Symposium »Game and Ecology«, Plitvice Lakes National Park, Republic of Croatia, 17–20 October 2007.

KUITERS, A. T., 1996: Accumulation of cadmium and lead in red deer and wild boar at the Veluwe, The Netherlands. Veterinary Quarterly **18** (S3), S134–S135.

LAZARUS, M., VICKOVIĆ, I., SOSTARIĆ, B. & BLANUSAI, M., 2005: Heavy metal levels in tissues of red deer (*Cervus elaphus*) from Eastern Croatia. Arh Hig Rada Toksikol **56**, 233.240

MALARA, P., KWAPULINSKI, J. & MALARA, B., 2006: Do the levels of selected metals differ significantly between the roots of carious and non-carious teeth? Science of the Total Environment 369, 59–68.

MANKOVSKA, B., 1980: The concentration of four toxic elements in the teeth of roe deer from the area of an aluminium plant. Biologia (Bratislava) **35**, 819–822.

MCTAGGART, H. S., LOWE, V. P., BARDEN, P. J. & GELLATLY J. B., 1981: Copper status of red deer on the island of Rhum. Veterinary Records **109**, 155–157.

MEDVEDEV, N., 1995: Concentration of cadmium, lead and sulphur in tissues of wild, forest reindeer from North-West Russia. Environmental Pollution **1**, 1–5.

NEEDLEMAN, H. L., GUNNOE, C., LEVITRON, A., REED, R., PERESIE, H., MAHER, C. & BARRETT P., 1979: Deficits in psychologic and classroom performance of children with elevated dentine lead levels. N. England Journal Medicine 300, 689–695.

OUTRIDGE, P. M., EVANS, R. D., WAGEMANN, R. & STEWART, R. E. A., 1997: Historical trends of heavy metals and stable lead isotopes in beluga (*Delphinapterus leucas*) and walrus (*Odobenus rosmarus rosmarus*) in the Canadian Arctic. The Science of the Total Environment 203, 209–219.

OUTRIDGE, P. M., HOBSON, K. A. & SAVELLE, J. M., 2005: Changes in mercury and cadmium concentrations and the feeding behaviour of beluga (*Delphinapterus leucas*) near Somerset Island, Canada, during the 20th century. The Science of the Total Environment 350, 106–118.

OUTRIDGE, P. M., WAGEMANN, R. & MCNEELY, R., 2000: Teeth as biomonitors of soft tissues mercury concentrations in beluga, *Delphinapterus leucas.* Environmental Toxicology and Chemistry **19**, 1517–1522.

SABER-TEHRANI, M., GIVIANRAD, M. H. & KAHKASHAN, P., 2007: Assessment of some elements in human permanent healthy teeth, their dependence on number of metallic amalgam fillings and interelements relationships. Biological Trace Element Research **116**, 155–169.

SCHÖNHOFER, F., TATARUCH, F. & FRIEDRICH, M., 1994: Strontium-90 in antlers of red deer: an indicator of environmental contamination by strontium-90. Science of the Total Environment 157, 323–332.

SHEARER, T. R., BRITTON, J. L. & DESART, D. J., 1980: Influence of post-developmental cadmium on caries and cariostosis by fluoride. Environmental Health Perspectives **34**, 219–221.

SILEO, L. & BEYER, W. N., 1985: Heavy metals in white-tailed deer living near a zinc smelter in Pennsylvania. Journal Wildlife Diseases **21**, 289–296.

SWIERGOSZ, R., PERZANOWSKI, K., MAKOSZ, U. & BIŁEK, I., 1993: The incidence of heavy metals and other toxic elements in big game tissues. Science of the Total Environment S1, 225–231.

TATARUCH, F., 1995: Red deer antlers as biomonitors for lead contamination. Bulletin Environmental Contamination and Toxicology **55**, 332–337.

TRAVIS, C. C. & LAND, M. L., 1990: Estimating the mean of data sets with nondetectable values. Environmental Science and Technology **24**, 961.

WITKOWSKI, S. A., AULT, S. R. & FIELD, R. W., 1982: Lead concentrations in white-tailed deer mandibles and teeth. Bulletin Environmental Contamination and Toxicology **28**, 561–565.

Summary

Toxicological survey of free ranging population of roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) by teeth examination

A. Zaccaroni, D. Scaravelli, R. De Battisti, A. Zanella & D. Gelli

Cervids have long been used as biomonitoring tools, as they are non-migratory and have small annual home ranges. Bone, teeth and antlers of the cervids are reliable and sensitive indicators of local pollution and reflect geographical variations in the contaminant burden in a terrestrial ecosystem. The present work reports the use of the teeth of cervids for heavy metal monitoring in a North-East area of Italy. Teeth samples from roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) were collected from mandibles obtained from hunters of two areas of Belluno Province, North-Eastern Italy. The last molar (M3) was extracted from each mandible and was analysed using an ICP-OES technique for As, Cd, Co, Cr, Hg, Ni, Pb and Se determination. Chromium was the metal found in the highest amounts, followed by arsenic, cadmium, mercury and lead. Statistical analysis of data by sampling area revealed no significant difference in heavy metal content between the two sampling areas in roe deer, while some differences were found in red deer for As and Cr, Sedico showing the highest concentrations of both two elements. Data concerning Cd are well below those reported in reindeer and roe deer from polluted areas and it can thus be speculated that in those areas a notable pollution by cadmium was present. Data obtained agree with those published on white tailed deer and can be considered indicative of little or no exposure to Cd. Lead values reported in the literature are much more higher than those found in the present study and make it possible to state that roe deer and red deer lead contents are indicative of background exposure to the metal. The data obtained define a low exposure of studied cervids to heavy metals, making it possible to consider the selected areas pollution-free. Species and area differences observed for some metals make it possible to consider teeth a reliable monitoring tool for both long-term and short-term exposure to heavy metals. To obtain a more effective result and to better define the role of teeth as monitoring tool it would be important taking into account soft tissues, to compare and correlate the tissue burden and the teeth concentration of heavy metals. The definition of such correlations will allow the creation of predictive models for monitoring activities.