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Trace elements in reindeer from Rybatsjij Ostrov, north western Russia

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Abstract: Reindeer (*Rangifer tarandus tarandus*) grazing the Rybatsjij Ostrov peninsula, north western Russia, north-east of the industrial towns of Nikel and Zapoljarnij, were analysed for hepatic concentrations of trace elements [arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), selenium (Se), and zinc (Zn)] by atomic absorption spectroscopy. The median (range) concentrations (µg/g wet weight) determined in liver samples from 40 reindeer with even sex ratio and representation from different age classes were As 0.035 (0.017-0.048), Cd 0.34 (0.15-1.2), Cr 0.008 (<0.002-0.022), Co 0.09 (0.06-0.12), Cu 98 (29-220), Pb 0.56 (0.23-1.0), Hg 0.16 (0.08-0.31), Ni 0.027 (<0.020-0.13), Se 0.88 (0.56-1.3) and Zn 37 (24-105). The concentrations of Cd increased and Ni decreased with age. The measured liver concentrations were below levels of toxicological significance to the animals. It can be inferred that there is no risk with the measured trace elements to human health associated with the consumption of meat from these reindeer.

Key words: *Rangifer tarandus*, liver, arsenic, cadmium, chromium, cobalt, copper, lead, mercury, nickel, selenium, zinc.

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Introduction

The nickel (Ni) smelters in Nikel and Zapoljarnij in north-west Russia (Fig. 1) emit large amounts of dust, sulphur dioxide and trace elements, and the environment in the area is heavily affected by pollution (Tømmervik *et al.*, 1995). Considerably higher trace element concentrations, particularly of Ni, arsenic (As), copper (Cu), and cobalt (Co), are found in air and precipitation near the Russian-Norwegian border than are present in southern Norway (Hagen *et al.*, 1996). Elevated concentrations of Ni and As, in particular, have been reported in livers of reindeer (*Rangifer tarandus tarandus*) at Jarfjord and Pasvik in eastern Finnmark County, Norway (north and west, respectively, of Nikel and Zapoljarnij) relative to concentrations in reindeer in the western part of Finnmark County (Sivertsen *et al.*, 1995; Løvberg & Sivertsen, 1997). Western Finnmark is not contaminated by emissions from the smelters in Nikel and Zapoljarnij (Steinnes *et al.*, 1994).

Semi-domesticated reindeer are very common in parts of north western Russia, as in north eastern Norway. Trace element concentrations in bone, tooth, antler, muscle and hair samples from reindeer in regions of Karelia and Archangelsk in

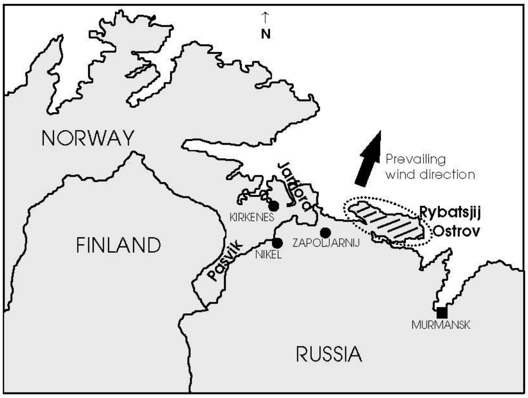

Fig. 1. Map of the Norwegian-Russian border area showing the industrial towns Nikel and Zapoljarnij, and Rybatsjij Ostrov where the sampled reindeer were grazing.

Russia have been published (Medvedev, 1995; 1999). However, these reindeer grazed too far from Nikel and Zapoljarnij to be detectably contaminated by emissions from the smelters. Also livers were not collected, and Ni was only determined in hair samples, and As was not determined. Therefore, the results from the previous Russian studies are not directly comparable with the present study.

Due to the finding of higher trace element concentrations in reindeer in Norwegian border areas north and west of Nikel and Zapoljarnij, a study of reindeer from Russia was initiated. This study presents trace element concentrations in liver samples of semi-domesticated reindeer from the Rybatsjij Ostrov peninsula, Russia, north-east of the smelters. Liver levels are considered to be optimal indicators for the status of trace elements in the body and directly comparable with corresponding results from most studies. The study was designed to determine whether age and sex affected the trace element concentrations.

Materials and methods

The mandibles and samples of livers from 6 male and 6 female 10 months old calves, and 14 male and 14 female adult reindeer (22 months or older) were randomly collected during slaughter in Varangerbotn, East-Finnmark, in March 1997 immediately after the animals were transported live from Rybatsjij Ostrov (Opk Vashod reindeer research herd), Russia (Fig. 1). The liver samples (ca 50 g) were stored in plastic vials and kept frozen at –20 °C (C until analysed for As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Se, and Zn. In order to prevent contamination from alloys in the laboratory, disposable plastic utensils were used. For the analysis of Cd, Co, Cr, Cu, Ni, Pb and Zn, sample (1 g) digestion was carried out in closed teflon beakers by microwave heating with a mixture of super purity nitric acid (65%, 4 ml) (Romil, Cambridge, England) and hydrogen peroxide (30%, 1 ml) (Merck, Darmstadt, Germany) (Jorhem & Engman, 1996). Samples (1 g) for analysis of As, Hg and Se were digested in a mixture of pro analysis nitric and perchloric acids (3+1 v/v, 16 ml) (Merck, Darmstadt, Germany) (Norheim & Haugen, 1986). Analyses of all elements were performed by atomic absorption spectroscopy (SpectrAA400, Varian Inc., Mulgrave, Australia) after dilution to 25 ml with deionised water (resistivity >10MΩ x cm). A graphite furnace was used for analyses of Cd, Co, Cr, Ni, and Pb, flame atomisation was used for analyses of Cu and Zn, and a hydride generator system was used for analyses of As, Hg and Se. Detection limits per g liver were 0.001 µg Cd, 0.002 µg Cr, 0.01 µg Co, 0.01 µg Pb, and 0.02 µg Ni (graphite furnace elements), 0.5 µg Cu and Zn (flame atomisation elements), and 0.003 µg As, 0.06 µg Hg, and 0.01 µg Se (hydride generator elements). All results are reported on a wet weight (ww) basis. The quality control samples Bovine liver BCR 185 and SRM 1577b, and Dogfish liver DOLT-2 were utilized as reference materials and the values were in agreement to certified values. All analyses were performed by accredited methods (Norwegian Accreditation (P110)).

Age determination was carried out by microscopic inspection of the annualation of the cementum of the first incisor (Reimers & Nordby, 1968).

Statistical calculations were performed using JMP statistical software (SAS Institute Inc., Cary, North Carolina, USA). The level of significance was set at $P \leq 0.05$. Analytical results below detection limits were given a numeric value of half the detection limit for statistical calculations. Shapiro-Wilk *W* testing of the data revealed lack of normality for most results (distributions of Cd, Co, Cr, Hg, Ni, Se and Zn concentrations, and age), hence, non-parametric methods were used in all statistical testing. The Wilcoxon rank sum method was employed for comparisons of element concentrations in groups with regard to age and sex. The Bonferroni method for adjustment of the significance level was employed for comparison of element concentrations in the three groups: calves, adult females and adult males; each element was tested three times, and gave P (0.05:3) =0.017. Spearman´s rank correlation coefficient (r_s) was used to evaluate correlations between element concentrations and age.

Table 1. Median (ranges) hepatic element concentrations (µg/g wet weight) and ages (months) for reindeer from Rybatsjij Ostrov, Russia.

	All reindeer	Calves	Adult females	Adult males
n	40	12	14	14
Age	22 (10-106)	10	34 (22-106)	40 (22-70)
As	0.035 (0.017-0.048)	0.033 (0.019-0.046)	0.039 (0.023-0.048)	0.030 (0.017-0.046)
Cd	0.34 (0.15-1.2)	0.23 (0.15-0.49)	0.43^{Y} (0.20-1.2)	0.49^{Y} (0.22-0.86)
Co	0.09 (0.06-0.12)	0.10^{AF} (0.08-0.12)	0.09 (0.06-0.10)	0.09 (0.07-0.12)
Cr	0.008 (<0.002-0.022)	0.008^{AF} (0.007-0.022)	0.007 (<0.002-0.18)	0.009 (<0.002-0.013)
Cu	98 (29-220)	100^{AF} (76-175)	72 (29-220)	135^{AF} (54-185)
Hg	0.16 (0.08-0.31)	0.18 (0.09-0.30)	0.16 (0.12-0.24)	0.20 (0.08-0.31)
Ni	0.027 (<0.020-0.13)	$0.043^{AF/AM}$ (0.020-0.072)	0.024 (<0.020-0.13)	0.021 (<0.020-0.050)
Pb	0.56 (0.23-1.0)	0.53 (0.31-0.84)	0.48 (0.31-0.96)	0.61 (0.23-1.0)
Se	0.88 (0.56-1.3)	1.1 (0.72-1.3)	0.78 (0.56-1.3)	0.92 (0.73-1.3)
Zn	37 (24-105)	38 (30-96)	44 (25-65)	32 (24-105)
% dry mat.	29.7 (26.1-38.6)	29.9 (28.9-33.7)	29.8 (26.1-38.6)	29.4 26.6-31.7)

^{Y} significantly higher concentration than in calves.
^{AF} significantly higher concentration than in adult females.
$^{AF/AM}$ significantly higher concentration than in adult females and adult males.

Results

The median concentration and the range for each element measured in liver samples from reindeer from Rybatsjij Ostrov, Russia, are presented in Table 1. The element concentrations in male and female calves were not different (P>0.05), hence, calf concentrations are presented as one group. The median concentration of Cd in calves was about half of that in adult reindeer, and Cd concentration was positively correlated with age (r_s=0.68, P<0.001) (Fig. 2). The median concentration of Ni in calves was about twice that in the adults, and Ni was negatively correlated with age (r_s=-0.60, P<0.001) (Fig. 3). The concentrations of Co, Cr and Cu in calves were significantly higher than in adult females (P= 0.009, 0.009, 0.016, respectively) but not different from those in adult males. The concentration of Cu was also significantly higher in adult males than in adult females (P=0.003). There were no significant differences between age and sex groups for the concentrations of As, Hg, Pb, Se and Zn.

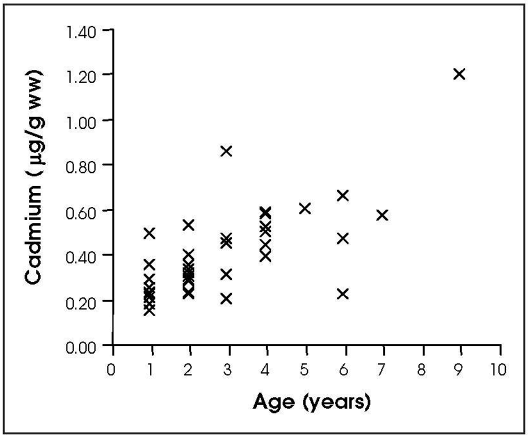

Fig. 2. Hepatic cadmium concentration versus age for 40 reindeer from Rybatsjij Ostrov, Russia. The Spearman´s rank correlation coefficient, r_S, was 0.68 (P < 0.001).

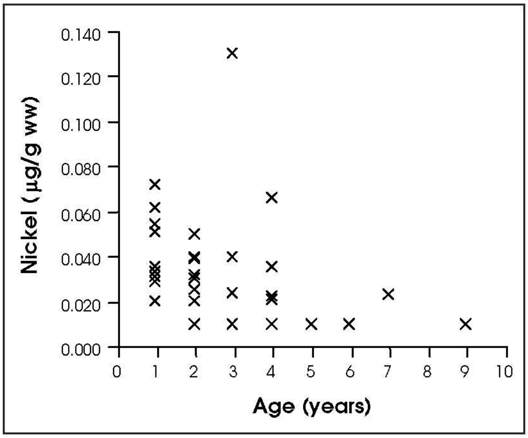

Fig. 3. Hepatic nickel concentration versus age for 40 reindeer from Rybatsjij Ostrov, Russia. The Spearman´s rank correlation coefficient, r_S, was -0.60 (P < 0.001).

Discussion

The element levels

The levels of emissions from the smelters in Nikel and Zapoljarnij in north western Russia have not improved in recent years (Hagen *et al.*, 1996). Thus, it was anticipated that the trace element concentrations in Rybatsjij Ostrov reindeer sampled in 1997 would be comparable with those of reindeer sampled in 1990-1991 and in 1995 in Finnmark County, Norway (Sivertsen *et al.*, 1995; Løvberg & Sivertsen, 1997).

No trace elements were found at higher concentrations in the livers of reindeer from Rybatsjij Ostrov, north-east from the smelters, than previously found in livers from reindeer from Jarfjord and Pasvik, eastern Finnmark, Norway, north and west of the smelters, respectively (Sivertsen *et al.*, 1995; Løvberg & Sivertsen, 1997). As and Ni were the elements of most elevated concentrations in reindeer from Jarfjord and Pasvik compared to a background level in reindeer in western Finnmark (Sivertsen *et al.*, 1995). The median As and Ni levels in Rybatsjij Ostrov reindeer were >3 times higher than in reindeer from western Finnmark, similar to the levels from Pasvik reindeer, and approximately 0.3 times those from Jarfjord reindeer. The levels of Co, Cu, Se and Zn were also increased in Jarfjord and Pasvik reindeer, and their median levels in the Rybatsjij Ostrov reindeer were 1.3-1.8 times those previously found in reindeer from western Finnmark, similar to the Pasvik reindeer levels, and 0.3-0.8 times the corresponding levels in Jarfjord reindeer (Sivertsen *et al.*, 1995; Løvberg & Sivertsen, 1997).

A geographical comparison of element levels in the reindeer herds as described above, is also dependent on comparable seasons for collecting the samples and comparable age distributions of the animals. The age distribution of the animals investigated in the present study was comparable to that in the Finnmark studies (Sivertsen *et al.*, 1995; Løvberg & Sivertsen, 1997). However, the Finnmark reindeer were sampled in November to January and the Russian reindeer in March. Lichens accumulate large amounts of elements from air and are important in the winter diet of the reindeer. The amount of lichen in the diet will influence the amount of trace element residues in the reindeer and is regarded as the key factor determining seasonal variation of element levels (Frøslie *et al.*, 1984; Aastrup *et al.*, 2000). Differences in the intake of lichens between reindeer herds could mask differences in hepatic element levels due to pollution. However, the levels of Cd, Hg and Pb were similar in reindeer from Rybatsjij Ostrov and in reindeer from eastern and western Finnmark and these elements are not associated with the smelters in Nikel and Zapoljarnij (Hagen *et al.*, 1996). Therefore this observation may be interpreted as showing that there were no obvious differences in the lichen dietary between the compared Norwegian and Russian reindeer herds.

The prevailing wind direction in the area is south/south-west, which is the direction from Nikel to Jarfjord, and from Zapoljarnij to Rybatsjij Ostrov (Hagen *et al.*, 1996). The distance from these Russian industrial towns to Rybatsjij Ostrov is, however, longer than the average distance from the two towns to Jarfjord and to Pasvik. This may explain why reindeer in the Russian Rybatsjij Ostrov area seem less contaminated by local pollution than reindeer living in the Norwegian Jarfjord area.

Comparable results for As or Ni in reindeer or caribou from other areas are scarce. In reindeer sampled at different areas in Norway in autumn (Frøslie *et al.*, 1984), the mean hepatic As level in southern, central and northern (western Finnmark) Norway were similar, 0.1-0.4 times, and 0.3 times, respectively, to the As level in Rybatsjij Ostrov reindeer. These Norwegian results reflect the atmospheric deposition of As. Reindeer in western Finnmark were also sampled at winter pasture (Frøslie *et al.*, 1984) and had a hepatic As level 0.5 times that in Rybatsjij Ostrov reindeer. The study of Frøslie *et al.* (1984) did not measure Ni. However, Ni concentrations have been published in hair samples from Karelian reindeer, Russia (mean level 0.16 µg/g dry weight) (Medvedev, 1999), in muscle and kidney samples from reindeer in Finnish Lapland (mean levels 0.01-0.05 µg/g wet weight) (Rintala *et al.*, 1995), in liver and kidney samples from caribou herds in the Northwest Territories, Canada (mean levels 0.13-0.49 and 0.24-1.33 µg/g dry weight, respectively) (Elkin & Bethke, 1995). Lower Ni levels were presented in a recent publication on heavy metal levels in kidneys of other caribou herds in Northwest Territories (average <0.14 µg/g dry weight) (Larter & Nagy, 2000). The Ni levels in hair, kidney and muscle are not easily comparable with the liver Ni level in the present study. However, the range of mean hepatic levels determined in Canadian caribou herds, sampled in the autumn and based on even sex ratio and representation from different age classes, were all above the median hepatic Ni level in the Russian reindeer (0.091 µg/g dry weight).

Comparisons of other elements elevated in Rybatsjij Ostrov reindeer relative to those from western Finnmark (Sivertsen *et al.*, 1995), Cu, Se, Zn and Co, with published hepatic reindeer or caribou levels, show higher Cu and Se levels in the present study than in most other investigated reindeer or caribou populations in Norway (Frøslie *et al.*, 1984), Finnish Lapland (Rintala *et al.*, 1995), Svalbard (Borch-Iohnsen *et al.*, 1996), Greenland (Aastrup *et al.*, 2000), and Northwest Territories, Canada (Elkin & Bethke, 1995; Dietz *et al.*, 1998). Hepatic levels of Cu and Se are known to vary naturally over a wide range in wild cervids (Frøslie *et al.*, 1987). The Zn level in the Russian reindeer was within the range found in reindeer from different areas in Norway (Frøslie *et al.*, 1984) and in Svalbard (Borch-Iohnsen *et al.*, 1996), and somewhat above the level found in reindeer from Greenland (Aastrup *et al.*, 2000) and Northwest Territories (Elkin & Bethke, 1995). No comparable results from reindeer or caribou were available for Co, but the level in the present study was within normal ranges for sheep (Radostits *et al.*, 1994).

For Cd, Hg and Pb, the hepatic levels in the Rybatsjij Ostrov reindeer were within the range found in reindeer from different areas in Norway (Frøslie *et al.*, 1984) and Greenland (Aastrup *et al.*, 2000). The levels of Hg and Pb were also within the range found in Canadian caribou, while the Cd level was below those in Canada (Elkin & Bethke, 1995). Furthermore, the present results on Cd and Pb were below those in Svalbard reindeer (Borch-Iohnsen *et al.*, 1996). Comparable Cr levels in reindeer or caribou are not available but the present hepatic reindeer level was below the normal range for bovine liver (Kerr & Edwards, 1981).

Effect of age and sex

In studies of trace elements in reindeer from Finnmark County (Sivertsen *et al.*, 1995; Løvberg & Sivertsen, 1997) only the concentration of Cd correlated with the age of the animals. In the present study, both Cd and Ni concentrations were found to correlate with the age of the animals, as Cd increased, whereas Ni decreased with age. The increase in hepatic Cd concentration with age reflects the specific accumulation of that element in kidney and liver (Kostial, 1986). The finding that Ni levels decreased with age could be due to suckling of the young, as milk of various mammals has been found to contain considerable concentrations of Ni (Nielsen, 1987). For example, children below one year of age are found to contain higher concentrations of Ni than older humans (Schneider *et al.*, 1980).

Excretion of trace elements in the milk, with transfer to the sucklings, may also be the most plausible explanation for the finding of some lower levels of Co, Cr and Cu in adult females than in calves. It is well known that the essential elements Co, Cr, Cu and Se are easily excreted in milk and also cross the placenta (Levander, 1986; Anderson, 1987; Davis & Mertz, 1987; Smith, 1987). Thus, in the present study also the Se level in adult females tended (P=0.04) to be lower than in the calves. Only small amounts of As, Cd, Pb and inorganic Hg are secreted in milk (Anke, 1986; Kostial, 1986; Quarterman, 1986; Clarkson, 1987). The results presented in Table 1 are also in accord with these findings, since As, Cd, Pb, and Hg were not found in higher levels in calves than in adults. Zinc, however, is easily transported via milk. The finding of similar zinc levels in young and adult reindeer may be due to homeostatic regulation of the zinc body burden (Hambidge *et al.*, 1986).

Toxicological consideration and conclusions

The hepatic element levels are considered to be optimal indicators of the status of the elements in the body, and are higher than corresponding concentrations in muscle tissue (Mertz, 1986; 1987). Both veterinary toxicological and food safety assessment may therefore be based on the liver levels. As and Ni were the elements with relatively high concentrations in the Rybatsjij Ostrov reindeer. However, these reindeer had As level similar to that in reindeer from south Norway and lower Ni level than in Canadian caribou. The concentrations of all trace elements investigated were below levels considered to be of toxicological significance to the animals (Mertz, 1986; 1987; Humphreys, 1988). It can be inferred that there is no risk associated with the measured elements for human health from the consumption of meat from reindeer grazing the Rybatsjij Ostrov area.

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