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Cadmium concentration in cattle tissues in the Czech Republic

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Abstract: The aim of this paper was to evaluate the cadmium concentrations in the muscles, liver, and kidney of three age categories (≤ 8 months; ≤ 2 years; ≥ 2 years) of cattle during the period of years 2014–2019 and to determine the age limit at which the concentration of cadmium in an organ with the highest accumulation increases health risk for consumers. In cattle above two years of age, there was a higher average cadmium concentration in the liver (0.10 mg/kg) and kidney (0.62 mg/kg), when compared with cadmium concentration in the liver (0.06 mg/kg) and kidney (0.24 mg/kg) of cattle under two years of age. A paired correlation coefficient r = 0.825 8 (P < 0.006) and Spearman's coefficient ρ = 0.92 (P < 0.000 1) were calculated for the dependence of the cadmium concentration on the age. The correlation analysis statistically demonstrated a significant positive correlation between the concentration of cadmium in the kidney and the age of the cattle. A non-significant difference between the maximum limit and the average concentration of the cadmium in the kidney of the cattle from the age of 6 years (P = 0.029) was demonstrated using the t-test. The cadmium concentration in the muscles was low and was not significantly affected by the cattle's age.

Keywords: accumulation; cows; kidney; toxic metal

Ruminants are exposed to contamination with cadmium (Cd) as a result of their feeding on pastures during the summer season, as well as with preserved fodder, including cereals, during the winter season. Cadmium diffuses to plants from the soil and it also contaminates plant surfaces from atmospheric deposition, especially in industrial areas. In humans and animals, cadmium accumulates, in particular, in the liver and kidneys. Cadmium is primarily toxic for the kidney; it causes damage to the proximal tubules and failure of the tubular reabsorption leading to renal dysfunction (Lee et al. 2006). The exposure of humans to cadmium is also connected with neurotoxic, cancerogenic, genotoxic and teratogenic effects, as well as with the genesis of osteoporosis or osteomalacia, damage to the endocrine system and reproduction functions. Recent data on the exposure of humans to cadmium in the common population were statistically connected with an increased risk of lung, endometrium, urinary bladder, and breast cancer. According to an opinion of the Scientific Panel on Contaminants in the Food Chain (CONTAM), the food groups that contribute to a major part of the dietary exposure to cadmium were cereals and cereal products, vegetables, nuts and pulses, starchy roots, or potatoes, as well as meat and meat products. The highest concentrations of cadmium were detected in seaweed, fish

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and seafood, chocolate, and foods for special dietary uses, as well as in fungi, oil seeds and edible offal (EFSA 2009; EFSA 2011; EFSA 2012).

No beneficial biological function for human health is known for cadmium, neither for lead and mercury, so chronic human exposure to these elements, predominantly via food, is relatively serious (Sinicropi et al. 2010; Carocci et al. 2014; Carocci et al. 2016; Genchi et al. 2020).

The aim of this paper was to evaluate cadmium concentrations in the muscle, liver, and kidneys of three age categories of domestic cattle for the period of 2014–2019 and to determine the age limit at which the concentration of cadmium in a target organ presents an increased risk for consumers in comparison with the maximum limit (ML) – a critical limit. We assume that the accumulation of cadmium in the target organ is mainly dependent on the age of the cattle.

MATERIAL AND METHODS

Sampling of cattle

The results of the analyses of the cattle tissues were obtained within the implementation of the national programme for monitoring residues and contaminants pursuant to Council Directive 96/23/EC (Council of the European Union 1996) during the period 2014–2019. The selection of the slaughtered animals was performed on a random basis by veterinary inspectors at different slaughterhouses in the Czech Republic. Tissue samples were taken from 291 slaughtered domestic bovine animals (bred domestically) divided according to their age into three groups: 41 heads of "calves" from 1 to 8 months of age, 88 heads of "young bovine animals" under 2 years of age (excluding calves) and 162 heads of "cows" (bovine animals above 2 years of age). Muscle, liver and kidney samples were always taken from each animal. Samples of any low-fat muscle, any part of the liver (both at least 0.5 kg) and a whole kidney were taken from each animal.

Analytical methods

The analyses were performed at the laboratories of the State Veterinary Institutes in the Czech Republic. All three laboratories were accredited according to the EN ISO/IEC 17025:2005 standard (Conformity assessment – General requirements for the competence for testing and calibration laboratories) and regularly participate in inter-laboratory comparison tests. The methods used were validated, and the quality of the routine analyses was controlled by testing reference, duplicate and blank samples. Before processing, the samples were kept in a freezer (at a temperature ≤ –18 °C). Prior to analysis, the biological material samples were homogenised (GM200; Retsch, Haan, Germany; min. 10 s, min. 10 000 rpm) and mineralised in a microwave device (Ethos Plus; Milestone Srl, Sorisole, Italy; at least 35 min with 180 °C achieved) after the addition of 4–5 ml of concentrated nitric acid (65–69%) and 1–2 ml of hydrogen peroxide (≥ 30%). The homogenised biological material samples were mineralised in a microwave device after the addition of the concentrated nitric acid and hydrogen peroxide. The measurement of the cadmium concentration was performed using electrothermal atomic absorption spectrometry with a Zeeman correction (electrothermal atomic absorption spectroscopy – ET-AAS, or graphite furnace atomic absorption spectroscopy – GF-AAS), or, in certain cases, using inductively coupled plasma mass spectrometry (ICP-MS).

The following instruments were used for the cadmium determination: ICP-MS 8800 Triple Quad (Agilent Technologies, Tokyo, Japan); ICP-MS Elan DRC-e (PerkinElmer, Norwalk, CT, USA); ICP-MS Agilent 7500ce (Agilent Technologies, Tokyo, Japan); AAS AAnalyst 800 (PerkinElmer, Norwalk, CT, USA); AAS SpectrAA220Z (Varian, Melbourne, VIC, Australia). In the case of the ET-AAS method, the mineralised sample was atomised in an electrically heated atomiser (graphite cuvette). The cadmium absorbance was measured at a wavelength of 228.8 nm, which was directly proportional to the concentration of the cadmium in the sample. In the case of the ICP-MS method, the mineralised sample was nebulised with the help of a conical nebuliser and a cooling chamber and introduced into the plasma, where the elements were freed from their chemical bonds due to the temperature of the argon plasma (approx. 10 000 K), ionised and further transferred into the mass spectrometer (MS) equipped with a quadrupole analyser. The required level of quantification (LOQ) in the tissues for the methods used was of 0.01 mg/kg for the liver and kidney, and of 0.005 mg/kg for the muscle.

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Statistical analyses

The data on the cadmium concentration in the muscle, liver and kidney of the cattle divided into three groups according to their age were processed using statistical analyses. An exploratory analysis was performed to detect all the specific features, particularities, as well as to verify the assumption of normality. The basic statistical parameters were calculated for the particular independent data sets of the cadmium concentrations in the particular tissues: i.e., average and median, 95% confidence intervals (CI), and range (R). Furthermore, the measured cadmium concentrations were analysed statistically in detail depending on the age of the cattle. A correlation analysis was performed and the correlation coefficients were calculated; the assessment of the significance of the difference between the maximum limit (critical limit), ML = 1 mg/kg, for the cadmium concentration in the kidney, as laid down in Commission Regulation (EU) No. 488/2014 (European Commission 2014) and the average of the measured cadmium concentration using the *t*-test ($\alpha = 0.05$), were performed. The results of the quantitative chemical analyses, which were lower than or equal to the LOQ (limit of quantification), were replaced by a constant equal to ½ of the LOQ value. The statistical analysis was performed by an interactive procedure using the statistical software Statistica v13.1 (StatSoft, USA), and QC-Expert v3.3 (TriloByte, Czech Republic).

RESULTS

In the performed survey data analysis, a deviation from normality (an asymmetric distribution) was detected in the vast majority of the assessed random selections. The data showed systematic asymmetry. Tables 1, 2, and 3 contain the results of the tests for the detection of the cadmium concentrations in the muscle (meat), liver, and kidney of the cattle

Table 1. Cadmium concentration in calf tissue (1–8 months of age) in the Czech Republic for the period 2014–2019 (mg/kg wet weight)

Tissue	*N*	Average	95% CI (L-bound)	95% CI (U-bound)	Median	Range (min.)	Range (max.)
Muscle	41	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Liver	41	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Kidney	41	0.06	0.05	0.08	0.05	0.01	0.32

CI = confidence interval; L-bound = lower bound; LOQ = limit of quantification; U-bound = upper bound

Table 2. Cadmium concentration in young cattle tissue (under 2 years of age) in the Czech Republic for the period 2014–2019 (mg/kg wet weight)

Tissue	*N*	Average	95% CI (L-bound)	95% CI (U-bound)	Median	Range (min.)	Range (max.)
Muscle	88	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Liver	88	0.06	0.05	0.07	0.05	0.01	0.17
Kidney	88	0.24	0.21	0.28	0.18	0.03	0.83

CI = confidence interval; L-bound = lower bound; LOQ = limit of quantification; U-bound = upper bound

Table 3. Cadmium concentration in cow tissue (above 2 years of age) in the Czech Republic for the period 2014–2019 (mg/kg wet weight)

Tissue	*N*	Average	95% CI (L-bound)	95% CI (U-bound)	Median	Range (min.)	Range (max.)
Muscle	162	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Liver	162	0.10	0.09	0.12	0.08	0.01	0.50
Kidney	162	0.62	0.55	0.69	0.49	0.04	2.78

CI = confidence interval; L-bound = lower bound; LOQ = limit of quantification; U-bound = upper bound

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divided into three age categories in the form of basic statistical parameters. The cadmium level in cattle tissues can be – with respect to health safety – assessed pursuant to the maximum limits (ML) laid down in Commission Regulation (EU) No. 488/2014 (European Commission 2014), i.e., 0.050 mg/kg wet weight for the meat, 0.50 mg/kg wet weight for the liver, and 1.0 mg/kg wet weight for the kidney.

The cadmium concentrations in the muscle were < LOQ in all three age groups. It means that the cadmium concentration was not dependent on the age of the cattle, or, it could not be assessed statistically in our case, respectively. With respect to health safety, it is worth dealing only with the concentration of cadmium in liver and kidney of young bovine animals (excluding calves) and cows. The average and median of the cadmium concentration in the cows was higher in the liver (0.10 mg/kg, 0.08 mg/kg) and in the kidney (0.62 mg/kg, 0.49 mg/kg), when compared with the cadmium concentration in the liver (0.06 mg/kg, 0.05 mg/kg) and in the kidney (0.24 mg/kg, 0.18 mg/kg) of the young bovine animals. The rate between the cadmium value in the liver and kidney of the young bovine animals was 1 : 4 for the average and 1 : 3.6 for the median when the value in the liver was taken to be equal to 1. The same rate was recorded for the cows, namely 1 : 6.2 for average and 1 : 6.1 for median. A wider rate between the average and median and a higher range in the cadmium concentration in the kidney in the group of cows (R = 2.74), when compared with the young bovine animals (R = 0.8) indicated a higher cadmium cumulation in the kidney depending on the age of cattle. However, it must be recognised that the values of the cadmium in the liver and kidney in the group of cows were affected by a higher number of samples, as well as by a wider age interval of the cows from 2 years to almost 13 years (i.e., from 24 months to 155 months), when compared with the group of young bovine animals where the group consisted of animals with a narrower interval from 8 months to 2 years. A graphical expression of the average and standard deviation (SD) documents that the cadmium concentration and the range of the cadmium values was, in all age groups, the highest in the kidney (Figure 1).

With respect to exceeding of the critical limit of the cadmium concentration for the kidney

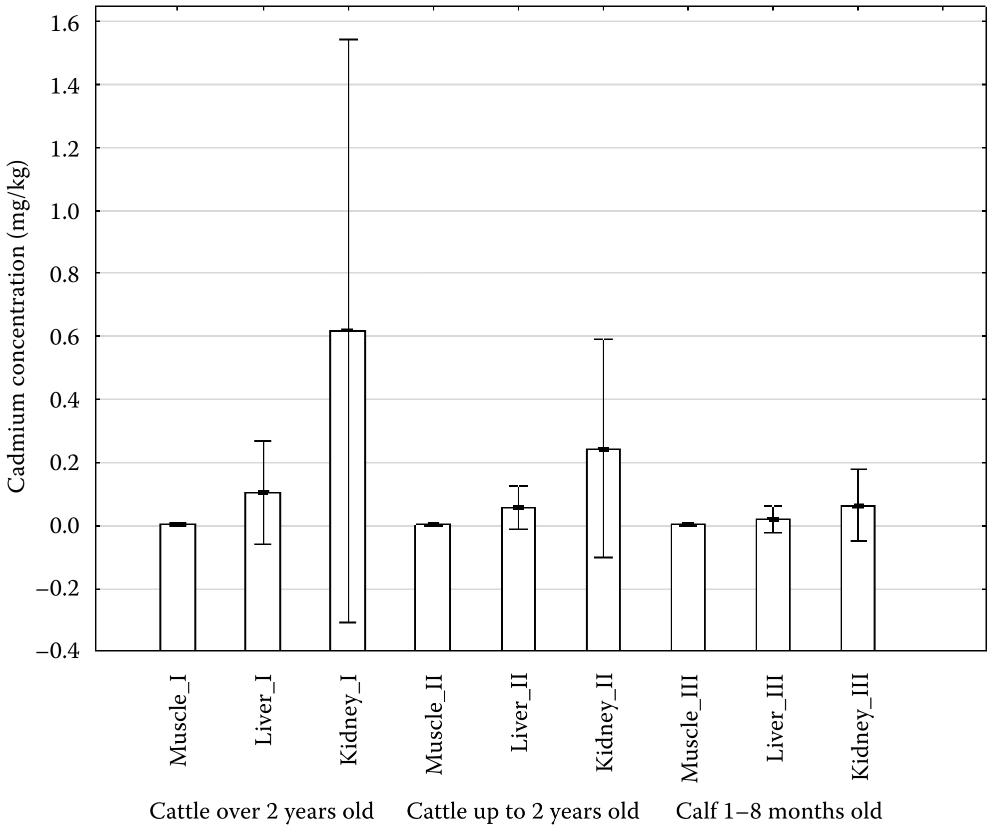

Figure 1. Comparison of the cadmium concentration in the cattle tissue in the Czech Republic for the period 2014–2019 (average ± SD)

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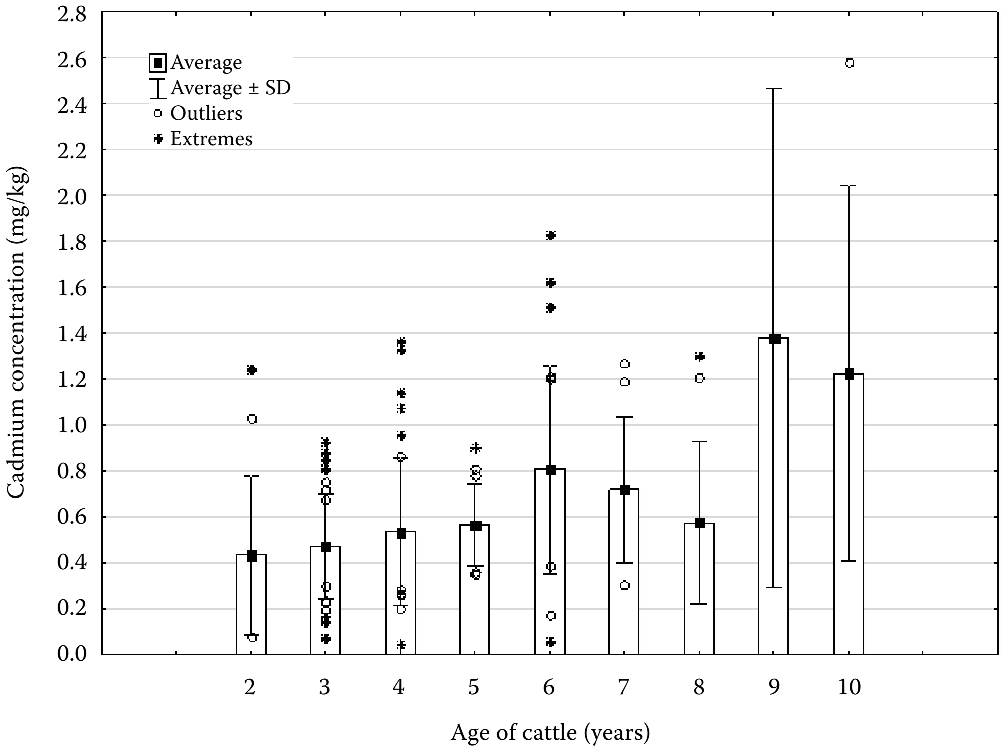

Figure 2. Relationship between the cadmium concentration in the kidney and the age of the cattle in the Czech Republic for the period 2014–2019

(ML = 1.0 mg/kg wet weight), only the values in the cows' kidneys are relevant. Of the total number of the 162 cow kidney samples, 22 samples (13.6%) had a cadmium concentration above the ML. The values in the kidneys of young bovines (≤ 2 years old) were not above the ML. From this reason, the measured cadmium concentrations in the kidney were further analysed in the group of animals above 2 years of age. The dependence of the cadmium concentration on the age of cattle was tested. The calculated paired correlation coefficient was r = 0.825 8 (P < 0.006) and Spearman's rank-correlation coefficient was ρ = 0.92 (P < 0.000 1). The correlation analysis demonstrated a significant positive correlation between the cadmium concentration in the kidney and the age of cattle. A statistically non-significant difference between the ML and the average cadmium concentration in the kidney (P = 0.029 for the age of cattle of 6 years) was demonstrated using the t-test. It means that an assumed higher cadmium concentration in the kidney, when compared with reaching the critical limit, was *de facto* similar in the cows above 6 years of age. However, extreme and outlying values of the cadmium concentration in the kidney above the critical limit were not exceptional in the cattle under 6 years of age as well (Figure 2). These outlying values were probably a result of a higher cadmium intake in areas with a higher environmental cadmium load, but this relation was not monitored in our work.

DISCUSSION

The issue of cadmium concentrations, as well as those of other "heavy metals", in the tissues of farm animals was dealt with, e.g., by Doganoc (1996), Pechova et al. (1998). The authors demonstrated a higher cadmium cumulation in the kidney and liver dependent on the age of the cattle. Our results were quite consistent with their findings. Zasadowski et al. (1999) measured the cadmium concentration in the liver and kidney of cattle from two areas of Poland in 1998. The average cadmium concentration was, in forty-four bovines under 2 years of age, 0.425 mg/kg (0.104–0.937 mg/kg) in the kidney, and 0.159 mg/kg (0.060–0.487 mg/kg) in the liver. In fifteen bovine animals above 2 years of age, the cadmium concentrations were significantly higher. The average in the kidney was 1.703 mg/kg (0.590–4.275 mg/kg), and the average in the liver

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was 0.263 mg/kg (0.081–0.672 mg/kg). Our results were consistent with the results of the authors of the study, as well as with the finding that the age of the animals is one of factors that increases the cadmium concentration in the liver and, in particular, in the kidney of the animals.

Brereton et al. (2014) analysed 441 samples of muscle, liver, and kidney from 147 bovine animals. Samples were taken directly at slaughterhouses over the entire United Kingdom during the period from December 2013 to March 2014. The sampling covered bovine animals from three age groups: 48 animals under 30 months of age, 51 animals from 30 to 72 months of age, and 48 animals above 72 months of age. The highest cadmium levels were detected in the samples from the kidneys from the oldest age group (above 72 months of age) where seventeen of these samples contained more than 1.0 mg/kg of cadmium with an average level, for this group, of 0.89 mg/kg. Bovine animals from the youngest age group (under 30 months of age) showed the lowest cadmium levels, i.e., 0.19 mg/kg on average; only one sample contained more than 1 mg/kg of cadmium. The results of our study were consistent with the evidence on the cumulation of cadmium in the liver and, in particular, in the kidneys of bovine animals as a function of age. Also, Canty et al. (2014) identified the cadmium concentration (as well as other heavy metals) in cattle kidneys with respect to the cadmium levels in the soil in all the counties of Ireland. The key factors of high cadmium concentrations in the kidney were cadmium levels in the soil and feeds, and the age of the animals. In areas with high cadmium levels in the soil, the cadmium concentration in cattle kidneys could exceed the current maximum limit in older animals; the kidneys of most cattle under three years of age complied with the maximum limit for cadmium. Ruprich et al. (2015) evaluated the cadmium content in cattle tissues as a source of consumer exposure in the Czech Republic for the period 1993 to 2010. The arithmetic averages of the cadmium content in the kidney and liver of the cows were higher than those in the kidney and liver of young bovine animals ($P < 0.000\,1$), but not in the muscle ($P = 0.227$). Similar results were published by Colic et al. (2017) from the area of Central Bosnia for the period from December 2013 to June 2014. The measurement of cadmium content comprised fifty meat samples, twenty liver samples, and twenty kidney samples; the cattle age was in an interval between 1 to 12 years. The lowest cadmium concentrations were in the muscle tissue; the values of the cadmium in all the muscle samples were lower than 0.008 mg/kg. The cadmium concentrations in the kidneys were between 0.088–4.493 mg/kg, and between 0.016 and 0.206 mg/kg in the liver. The average cadmium value in kidney was 0.750 ± 0.99 mg/kg and 0.076 ± 0.05 mg/kg in the liver. The statistical analysis showed an important correlation between the cadmium concentration in the kidney and the age of the cattle ($r = 0.98$, $P < 0.000\,1$), as well as between the cadmium concentration in the liver and the age of the cattle ($r = 0.77$, $P < 0.000\,1$). The results of cited studies and our results were consistent as for the detection of a significant cumulation of cadmium in the kidney.

Our work proved a statistically significantly higher average cadmium concentration in the kidney and liver of bovine animals over two years of age, when compared with the average cadmium concentration in the kidney and liver of young bovine animals under two years of age. Furthermore, a correlation analysis proved a statistically significant positive correlation between the cadmium concentration in the kidney and the age of the cattle. Using the *t*-test, a statistically non-significant difference between the maximum limit (ML = 1 mg/kg wet weight) and the average cadmium concentration in the kidney of the bovine animals from the age of six and more years was proven. However, extreme and outlying cadmium concentration values in the kidney above the critical limit were not exceptional in cattle under 6 years of age as well. The cadmium concentration in the muscle was, when compared with its content in the liver and kidney, low and was not statistically significantly influenced by the age of the cattle.

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Conflict of interest

The authors declare no conflict of interest.

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