Research article

Lead and cadmium blood levels and transfer to milk in cattle reared in a mining area

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ABSTRACT

The presence of heavy metals in milk is a public health problem that negatively affects human health, especially infant health. This study evaluated the concentration levels of lead (Pb) and cadmium (Cd) in blood and its transfer to the milk of 20 cows in production in a rural community near the La Oroya Metallurgical Complex in Peru, which has emitted fine particulate matter for more than 90 years. Validated protocols were used for sample collection. The samples were analyzed by atomic absorption spectrophotometry. The results of the analysis indicated that the levels, in mg/kg, of Pb in blood and milk were 0.38 ± 0.041 and 0.58 ± 0.018, respectively; Pb in milk was 54% higher than that in blood (P < 0.01). Cd levels, in mg/kg, in blood and milk were 0.016 ± 0.002 and 0.02 ± 0.007, respectively; milk had 28% more Cd than did blood (P < 0.05). The results for Pb in milk were compared with the Codex Alimentarius standard (0.002 mg/kg); the mean concentration of Pb in milk was 29 times higher than the acceptable limit, and the mean concentration of Cd was 2 times higher than the acceptable limit of the Romanian standard (0.01 mg/kg). The result could be attributed to the impact of environmental pollution by mining waste. In Peru, there are no norms for maximum Pb and Cd values, and the establishment of maximum value norms for these metals in milk is suggested.

1. Introduction

Air pollution by fine particulate matter (PM) is a risk factor for morbidity and mortality around the world (Gakidou et al., 2017). PM emitted by the metallurgical industry, due to its strong adsorption capacity, can adsorb to, combine with and transport heavy metals and other toxic substances (Dergham et al., 2012). PM-bound heavy metals are mobilized by air and deposited in irrigation water and in agricultural/grazing soils, and the heavy metals are transferred to plants and other parts of the ecosystem, entering the trophic chain (Yilmaz et al., 2009; Alloway, 2013) and affecting animal and human health due to their high toxic potential (Lokeshwari and Chandrappa, 2006; Castro et al., 2013, 2016).

In the ecosystem, heavy metals can geocoaccumulate, bioaccumulate or biomagnify (Lokeshwari and Chandrappa, 2006; Beltrán and Gómez, 2014). The bioaccumulation of Pb and Cd and their transfer to the food chain affects food safety and innocuousness (Nava-Ruiz and Méndez-Armenta, 2011; Tchounwou et al., 2012; Castro et al., 2013), and their consumption puts human health at risk (Nava-Ruiz and Méndez-Armenta, 2011; CDC, 2012; Counter et al., 2012; Castro et al., 2016; Tepanosyan et al., 2017).

In Peru, mining-metallurgical activity generates 20% of all tax revenue and is located mainly in the Andean highlands and midlands (Delgado, 2016), where livestock farming is also conducted, thus exposing livestock to the impact of mining-metallurgical activity (Alvarez-Berríos et al., 2016). In communities near the La Oroya Metallurgical Complex, Andean livestock is produced and sustained in pastures exposed to contamination by heavy metals, especially Pb and Cd, which are dynamically transferred to milk (Skipin et al., 2016; González-Montaño, 2009), and thus, their quantification and impact on biological systems is important (Léopold et al., 2016).

The objective of this study was to evaluate the Pb and Cd concentrations in blood samples from cows raised at the Paccha-La Oroya Rural Community (PRC) and the transfer of Pb and Cd to milk. These data can provide scientific evidence of the suitability of milk produced under these conditions for human consumption.

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2. Materials and methods

2.1. Study site and period

This study was conducted in May 2018 in the barn of the PRC, 11°31'03" South latitude and 75°53'58" West longitude, encompassing an area of 323.7 km², at an altitude of 3742 m and with minimum and maximum temperatures of -3.1 and 18.2 °C, respectively. The PRC has natural pastures composed mainly of grasses of low nutritional value of the genera Festuca, Piptochaetium, Bromus and Calamagrostis, where cattle and sheep are raised.

The PRC is located 10.2 km from the largest metallurgical complex in Peru, where copper, zinc, silver, lead, indium, bismuth, gold, selenium, tellurium and antimony are mined and processed and where, for more than 90 years, smoke and PM-bound heavy metals have been emitted, contaminating ecosystems (Barrios-Napuri, 2008; Alvarez-Berrios et al., 2016). This fine PM, rich in Pb, Cd and other heavy metals, is deposited in the soil and in irrigation water and transferred to plants (Peláez-Peláez et al., 2016; Olayinka et al., 2017) that are the source of food for cattle raised in the High Andean Zone, close to the metallurgical complex.

The chemical analyses were performed at Baltic Control SAC, a laboratory accredited by the Accreditation Directorate of the National Institute of Quality (Instituto Nacional de Calidad – INACAL), Peru.

2.2. Population and biological samples

The population consisted of dairy cows from the PRC. The sample consisted of 20 lactating Criollo cows, aged 4–6 years (first or second lactation), accounting for 74% of the cows available at the PRC. Older animals were excluded. A total of 10 ml of blood was collected from the coccygeal vein into heparinized vacutainer tubes, following the procedure of Zambrano and Díaz (2014). Additionally, 500 ml of milk per cow was manually extracted during the morning milking and stored in labeled never-used polyethylene bottles previously washed with bidistilled water, following the protocol of Rodríguez et al. (2005). The samples were placed in styrofoam boxes at -4 °C and transported to the laboratory for analysis.

2.3. Chemical analysis

For the analysis of Pb and Cd in blood and milk, a flame atomic absorption spectrophotometer (NAMBEI AA320N) was used according to the reference standard (Latimer, 2016) and to the laboratory's internal methodology. For the digestion of the blood and milk samples, specific procedures were followed. For blood, 10 μl of the sample was digested with repeated additions of nitric acid (0.5% HNO₃) at a drying temperature, and subsequently, hydrogen peroxide (H₂O₂) attack was performed. Hot digestion was performed, the solution was filtered with Whatman No. 40 filter paper, and the volume was adjusted with bidistilled water.

For milk, 50 g of raw milk was placed in 100-ml porcelain crucibles for drying in an oven at 100 °C to constant weight and then placed in a muffle furnace at 450 °C for 16 h. The incinerated samples were cooled and bleached with 2 ml of 2N HNO₃ and dried on a thermostatic plate. The acid was evaporated, and the samples were reincinerated in a muffle furnace at 450 °C for 1 h. For ash recovery, 5 ml of 2N HNO₃ and 20 ml of 0.1N HNO₃ were used. The samples were filtered using Whatman No. 40 filter paper and stored in polypropylene tubes under refrigeration.

The wavelengths for the quantification of Pb and Cd were 283.3 nm and 228.8 nm, with detection limits of 0.045 and 0.002 mg/kg, respectively. Standards of 1000 mg/kg were used to generate the calibration curves. The data from the standard solutions for Pb and Cd in the blood samples were 100 ± 0.01 and 100 ± 0.02 mg/kg, respectively, and those for the milk samples were 155 ± 0.04 and 150 ± 0.05 mg/kg, respectively. All standards were acquired from Sigma-Aldrich. The precision of the instrumental methods and analytical procedures was verified by running the samples in duplicate. The high and low range for the blank (BK), duplicate sample (DS) and control standard (CS) were determined for every 15 samples. The concentrations of each element are expressed in mg/kg.

2.4. Statistical analysis

The results are expressed as the mean ± standard deviation (n = 20). To determine statistically significant differences in the concentration of Pb and Cd in blood and milk, samples were compared using the t test for paired samples. P < 0.05 was adopted as the significance level. Regression analysis was performed for Pb and Cd concentrations in blood and milk. All analyses were performed in SPSS v.23.

2.5. Bioethical aspects

The animals were treated responsibly and respectfully during the study. Blood sampling was performed by qualified and experienced staff, avoiding unnecessary injury and stress to the animals. The study was reviewed and approved by the Specialized Research Institute of the School of Zootechnics of the National University of the Center of Peru (Universidad Nacional del Centro del Perú) and was authorized by the PRC's Assembly.

3. Results

3.1. Pb concentration in the blood and milk of cows raised at the PRC

The mean concentration of Pb in the analyzed milk samples was significantly (p < 0.01) higher than the levels determined in the blood (Table 1, Figure 1).

Pb in milk was 154% higher than that in blood, equivalent to a transfer factor (TF) of 1.54 ± 0.189. The correlation between the Pb content in blood and milk was 0.193 (p = 0.416), which indicated that the higher the concentration of Pb in the blood, the lower the concentration in the milk, which would be an indication that the mammary gland functions as a barrier to the transfer of Pb from blood to milk, which warrants further study.

3.2. Cd concentration in the blood and milk of cows raised at the PRC

The mean Cd concentration in the milk samples was higher (p < 0.05) than that in blood (Table 2, Figure 2).

Cd in milk samples was 128% higher than that in blood, equivalent to a TF of 1.283 ± 0.553. The correlation between Cd concentration in blood and milk was -0.249 (p = 0.290), also indicating the probable barrier action of the mammary complex.

Figure 3 shows the box plot for the TFs of Cd and Pb from the blood to the milk of cattle raised near a metal smelting zone. A less variable distribution was observed for Pb than for Cd, for which the maximum and minimum values were more dispersed.

4. Discussion

This study was conducted because of the lack of scientific evidence on the concentrations of Pb and Cd in cow milk in areas close to metal smelting activities. It is therefore important for public health, and the results could serve as an indicator of the quality of milk for human consumption.

The reference values for mean Pb and Cd concentrations in the blood of cows in production are, respectively, 0.5 and 0.05 mg/kg (Codex Standard, 2010; CEE, 2006), and in the present study, these concentrations were 0.38 and 0.016 mg/kg. We found TFs of 1.53 and 1.25 for Pb and Cd, respectively.

Comparing the mean concentrations of Pb and Cd with the limits allowed for cow milk according to the international standards of the European Union (CEE, 2006), Romania (Boru et al., 1985), Codex...
Table 1. Concentration of Pb in the blood and milk of lactating cows reared near a metal smelting zone (n = 20).

| Variable           | Mean   | SD    | CV (%) | Minimum  | Maximum |
|--------------------|--------|-------|--------|----------|---------|
| Pb in blood, mg/kg | 0.38b  | 0.041 | 0.20   | 0.302    | 0.453   |
| Pb in milk, mg/kg  | 0.58a  | 0.018 | 0.00   | 0.540    | 0.600   |

a,bMeans followed by different letters differ significantly (p ≤ 0.01).

Figure 1. Scatter plot of Pb concentrations in blood and milk of lactating cows reared near a metal smelting zone (n = 20).

Table 2. Cd content in blood and milk of cattle raised near a metal smelting zone (n = 20).

| Variable           | Mean   | SD    | CV (%) | Minimum  | Maximum |
|--------------------|--------|-------|--------|----------|---------|
| Cd in blood, mg/kg | 0.0157b| 0.0019| 0.00   | 0.0122   | 0.0191  |
| Cd in milk, mg/kg  | 0.0197a| 0.0073| 0.00   | 0.0110   | 0.0320  |

a,bMeans followed by different letters differ significantly (p ≤ 0.01).

Figure 2. Scatter plot of Cd concentrations in blood and milk of cows raised near a metal smelting zone (n = 20).
Alimentarius (FAO and OMS, 2015) and the Ecuadorian Technical Standard NTE INEN 9: 2012 (Pernía et al., 2015), it was observed that the mean concentration of Pb was 29 times higher than the value allowed for human consumption (0.02 mg/kg). For Cd, the Romanian norm (Banu, 2010) establishes a maximum value in milk of 0.01 mg/kg; the CD level in milk produced at the PRC was thus 2 times higher than the allowed value. This situation puts consumers at risk, especially children.

The present results are similar to those from studies conducted in highly polluted areas in other countries, such as Mexico, Egypt, Italy and Pakistan (Licata et al., 2004; Rodríguez et al., 2005; Enb et al., 2009). In 5 dairy barns in the municipalities of General Zuazua and Marín in Mexico, the mean concentrations of Pb and Cd were reported to be 0.74 and 0.30 mg/kg (Rodríguez et al., 2005), values slightly higher than those found in the present study. In barns in the province of León, Mexico, a mean Pb concentration of 0.0043 mg/kg was reported, a much lower value than that recorded in our study, as it is a rural area far from industrial activity (González-Montaña, 2009).

Maximum concentrations of Pb in milk have been reported. In Ecuador, Egypt, Italy, Mexico and Pakistan, they were 7.77, 0.96, 1.32, 0.74 and 0.058 ppm, respectively; the maximum concentrations of Cd in milk reported in Ecuador, Egypt, Italy, Mexico, Pakistan and Romania were 0.46, 0.11, 0.02, 0.29, 0.06 and 0.01 ppm, respectively (Pernía et al., 2015). Gonzales-Montaña (2009) reported a maximum Pb content of 0.02 mg/kg fresh weight for raw cow milk.

In Peru, Chata (2015) conducted a study at Coata-Puno River basin and reported 0.21 mg/l of Pb, a value that exceeds the maximum limit established by the Codex Alimentarius and European Union. The Cd content was 0.0037 mg/l, a value that does not exceed the maximum limit of the Technical Norm of Romania. In the present study, the mean Pb and Cd values were determined to be 2.8 and 5.4 times higher than those recorded at Coata-Puno, which may be due to the high air, water and soil contamination by emissions from the La Oroya Metallurgical Complex, which has been in operation for more than 90 years and is located just 10.2 km away from the highland Andean community of Paccha.

Milk produced at the PRC, due to its high concentration of Pb and Cd, can cause health problems, especially in children (WHO, 2016). Pb bioaccumulates in different tissues, mainly bone (Castro et al., 2013), inhibits hemoglobin synthesis, causing anemia (Counter et al., 2012; Castro et al., 2016), increases blood pressure, damages renal tissue and alters the central nervous system (Nava-Ruíz and Méndez-Armenta, 2011), causing delayed mental development and decreased cognitive function (Lassiter et al., 2015), aggressive behaviors and a tendency to violence (Needleman et al., 2002) and can cause stomach and lung cancer (Mushak, 2011).

Cd, due to its capacity for bioaccumulation and bioamplification in the food chain (Pernía et al., 2008), is associated with renal diseases, hypertension, anemia, osteoporosis, osteomalacia, diabetes, anemia, chronic rhinitis and eosinophilia (Åkesson, 2012; Gallagher and Meliker, 2010; Schwartz et al., 2003; Henson and Chedrese, 2004). It is a carcinogen that causes leukemia and pancreatic, lung, breast and prostate cancer (Henson and Chedrese, 2004; McElroy et al., 2006; Julin et al., 2010).

Under the study conditions, the main source of Pb and Cd contamination is PM emitted by metal smelting, which reaches the water and grazing fields, mainly in the rainy season, and is transferred to pastures, then to the blood and milk of cows, binding with fat and proteins such as casein and whey proteins (Magarinos, 2006; Alais, 2003). Cd in milk is transferred to the cream, rennet and milk curd. A concentration of Cd 3 times higher in the milk of sheep fed diets with Cd compared to those fed a Cd-free diet have been reported (Milhau et al., 1998).

This study shows that Pb and Cd are easily transferred to milk, being a risk factor for health, especially in children. Daily consumption of 200 ml of this milk would provide 0.12 mg of Pb and 0.004 mg of Cd, which after 180 days would represent a cumulative intake of 216 and 0.72 mg of Pb and Cd, respectively. The problem is more concrete when considering that the mean per capita milk consumption in Peru is 87 kg/person/year (MINAGRI, 2018). Therefore, the consumption of milk produced at Paccha-La Oroya, containing 0.58 mg/kg of Pb, would result in an ingestion of Pb per capita of 50.46 mg.

5. Conclusion

All blood samples from the evaluated cows contained Pb and Cd; the concentration intervals for Pb and Cd ranged from 0.40 to 0.36 mg/kg and 0.017 and 0.015 mg/kg, respectively.

All evaluated milk samples contained Pb and Cd; the concentration intervals for Pb and Cd ranged from 0.59 and 0.57 mg/kg and 0.023 and 0.016 mg/kg, respectively, exceeding the limits allowed by international standards for raw cow milk.

The TFs of Pb and Cd from blood to milk were 1.53 and 1.25, respectively.

The milk produced at the PRC, due to its high level of contamination by Pb and Cd, is not suitable for human consumption. Pb and Cd can bioaccumulate, which represents a serious risk to public health.

Declarations

Author contribution statement

Doris M. C. Peinado: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Jorge I. C. Bedriñana: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.
Additional information

No additional information is available for this paper.

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