ORIGINAL RESEARCH

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Heavy Metals in Horse Blood, Serum, and Feed in Minas Gerais, Brazil

Leandro Maia,[a] Maria Verônica de Souza, MV, MS, DS,[a] Raphael Bragança Alves Fernandes, BS, MS, DS,[b] Maurício Paulo Ferreira Fontes, BS, MS, PhD,[b] Matheus Werner de Souza Vianna,[a] and Walcrisley Vercelli Luz, BS, MS[b]

ABSTRACT

Pollution is a subject of worldwide concern and discussion and various areas of science are seeking to integrate their knowledge in an attempt to improve understanding of the harmful effects of modern human activities. Thus, the objective of the current study was to assess the potential for using the horse as a bio-indicator of environmental pollution. Blood and serum samples from 120 horses from locations with different degrees of urbanization and industrialization and the feeds used for these animals were analyzed to determine their heavy metal (Cd, Pb, Ni, Cu, and Zn) content. The data obtained were compared. The results did not show increases in heavy metal concentration in the blood and serum of the horses with the intensification of urbanization and industrialization around the farms. Only Cd, as determined in the blood of the animals, showed a certain potential for using horses as bio-indicators of environmental pollution. The data for sex, age, and chemical composition of the foodstuffs supplied to the animals were not determinants in explaining the variation in the concentrations of heavy metals observed.

Keywords: Environmental Monitoring; Pollution; Equine

INTRODUCTION

Contamination by heavy metals has attracted increasing attention from environmental authorities. These elements, although they occur naturally, because of anthropological action have presented relative accumulation in soils, plants, and sediments, and can reach the animal food chain with the possibility of producing serious consequences to ecosystems and especially human health. According to data from the Agency for Toxic Substances and Disease Registry (ATSDR),[1] 5 of the 20 most dangerous substances on the planet are heavy metals (As, Pb, Hg, Cd, and Cr).

The possibility of using animals as bio-indicators of environmental contamination is widespread in the literature, especially with aquatic or soil organisms. Conversely, the use of domestic species is less common and is a more recent field of research.

The proximity of living with human beings, the coincidence in habitats, and greater similarity in terms of organisms makes animals such as horses and cattle potentially useful as environmental contamination indicators.

Acute or chronic accidental heavy metals ingestion by horses can result in many afflictions that, on certain occasions, may not be perceptible clinically. Heavy metal intoxication in adult horses may be related to the use of pastures contaminated by atmospheric emissions, from metal smelters, various industries, and the urban environment.[2-4] A further source of contamination for horses and cattle may be the feed supplied by the owners, either concentrates or mineral supplements.[5-7]

The objective of this study was to assess the potential of using heavy metals concentrations in blood, serum, and feed offered to horses in different locations in the state of Minas Gerais (MG), Brazil, to verify the potential of this species as a bio-indicator of environmental pollution.

The hypothesis of this work is that the concentration of heavy metals in the blood and serum of horses could undergo an increase as a function of urban and industrial intensification in the environment surrounding the farms.

MATERIALS AND METHODS

Four sets of horses (90 crossbred and 30 Campolina breed), of both sexes (64 female and 56 male) and of different ages (2–24 years, x ≡10.44), were studied. Each set consisted of 30 clinically healthy animals (based on

From the Departments of Veterinary Medicine[a] and Soil[b], Federal University of Viçosa, Minas Gerais, Brazil.
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Reprint requests: Maria Verônica de Souza, MV, MS, DS, Departamento de Veterinária, Universidade Federal de Viçosa, Viçosa, MG 36570-000, Brazil.
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physical and routine laboratory examinations) that performed different activities and had been in the following locations for at least 1 year:

a) L1—set 1 (control): animals from rural farms in Viçosa, MG, Brazil. The farms were at least 3 km distant from the county center, and the animals were fed basically with Tifton grass (*Cynodon* spp.).

b) L2—set 2 (small town): cart horses that performed activities in the urban perimeter of the city of Viçosa, MG, Brazil and its micro region. The horses´ diet was forage and corn; mineral salt was not offered, only common salt.

c) L3—set 3 (large city): animals from the urban patrol (Military Police Cavalry—PMMG) in the city of Belo Horizonte, MG. The animals were fed four times per day with chopped elephant grass (*Pennisetum purpureum Schum.*), concentrate supplied twice per day, and 50 g mineral salt added to the concentrate.

d) L4—set 4 (industrial city): cart horses and horses for personal use that worked or were reared in the city of Ipatinga, MG. The horses were fed on elephant grass, brachaiaria (*Brachiaria decumbens*), or coast cross (*Cynodon dactylon*) hay, concentrate, and mineral salt (except for the cart horses).

Blood samples were collected from the animals by puncturing the jugular vein and collecting blood in glass tubes with and without an anticoagulant (sodium heparin). To determine the Ni, Cd, and Pb concentration, 10 ml blood was previously digested in 0.01% nitric acid. To determine Cu and Zn, 5 ml serum was diluted 5 times in Milli-Q water.[8] After dilution and homogenization, the concentration of heavy metals was determined by flame atomic absorption spectrophotometry. The results were expressed in μg/ml. The detection limits of analytical methods were 0.030 μg/ml for Cu and 0.010 μg/ml for Cd, Pb, Ni, and Zn.

Samples of the roughage, concentrate, and mineral salt used in the animals' feed were collected in all sets except for set L2 (small town). The samples were placed in plastic bags, labeled, sealed, and refrigerated. The roughage, concentrate, and mineral salt samples were dried in a forced air chamber at 65°C for 72 hours and then ground in a Willey-type grinder. Next, the samples were submitted to nitroperchloric digestion (3:1, v/v) at 150°C. The concentration of heavy metals in the extracts was determined by flame atomic absorption spectrophotometry and the results expressed in mg/kg.

Data obtained were submitted to statistical analysis using the 9.0 SAEG[9] software. A descriptive statistical analysis of the data was conducted, followed by the analysis of variance (ANOVA). The effects of the set and the animal sex and age in relation to the heavy metals content determined in the blood (Pb, Cd, and Zn), the serum (Cu and Zn), and the feed (Pb, Cd, Ni, Cu, and Zn) were assessed by the Tukey test ($P < .05$). The animals were divided into three groups to assess the influence of age regarding concentration of heavy metals in the blood and serum: <7 years, between 7 and 14 years, and >14 years.

RESULTS

Table 1 shows the mean concentration of heavy metals in serum and blood of the horses in the four sets studied. The elements quantified in the serum presented more expressive contents than those identified in the blood that were in line with the chemical composition of these components of the blood tissue.

The mean Cu concentrations in serum from the animals in sets L2, L3, and L4 were similar and statistically superior to those from horses from the rural area (L1). In the case of Zn, the animals in set L3 (large city) presented the lowest concentrations compared with the other locations.

In the blood, the elements Pb and Ni were not identified in the samples from the animals in L2 (small town) but were detected in the other sets, where significant differences among the concentrations could not be ascertained. Cd was the heavy metal least identified in the samples collected, and it was quantified in only 12 animals in L4.

The analysis of data obtained as a function of sex (Table 2) indicated no influence of this characteristic on the concentration of heavy metals quantified in the blood and serum. In the case of the analysis as a function of animal age (Table 3), statistical similarity was verified between the mean Cu and Zn (serum) and Pb and Ni (blood) concentrations for the three groups of animals considered. For Cd, the analysis demonstrated that the younger (7 years old) and older (14 years old) animals presented higher mean concentrations than the animals in the intermediate age group (7–14 years old).

Bearing in mind that nutrition is an important source of heavy metal contamination, samples of the main feed used by the horses was collected in some sets, which were evaluated for their chemical composition. In spite of the small number of samples analyzed, the data obtained (Table 4) indicated similarity in mean concentration of heavy metals among the forage plants supplied to the animals in sets L1, L3, and L4 and among the concentrate supplied to sets L3 and L4. In the case of mineral salt, similarity was also detected in the concentration of heavy metals among the samples of the different sets, except for Pb, for which the samples in L1 showed significantly greater mean concentrations than those in the other sets.

DISCUSSION

Heavy Metals in the Blood and Serum

Effect of location on the concentration of heavy metals. The lower Cu concentration ($P < .05$) in the serum of animals in set L1 compared with the other sets may have been associated with the feed supplied to the animals, consisting only of forage crops. This may have been the reason the Cu concentration in the serum ob-