

## Serum Concentrations of Strontium, Lead, Nickel, Vanadium and Aluminum in Horses

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### Abstract

Providing data on the serum concentration of some important heavy metals in horses of different age and sex is an indicator of short-term exposure and also help to understand influence of these factors in metals intoxication. We reported serum concentrations of some elements in healthy horses and assessed any relationship with age and sex. Fifty-three samples from horses were analyzed by atomic absorption. Mean serum concentrations ( $\mu\text{g/ml}$ )  $\pm$  SD values for strontium were ( $0.25 \pm 0.15$ ), vanadium ( $0.20 \pm 0.02$ ), aluminium ( $0.71 \pm 0.16$ ), nickel ( $0.10 \pm 0.08$ ) and lead ( $0.09 \pm 0.04$ )  $\mu\text{g/ml}$ . No significant correlations were noted between measured elements with age, however, a significant correlation was detected between age with concentration of lead ( $P < 0.05$ ). We concluded that older horses had more ability to absorb this element from their environment, or ability to detoxify lead decreased with increase in age.

**Keywords:** Strontium; Aluminum; Vanadium; Nickel; Lead; Horse

### Introduction

Heavy metals are widely spread in human and animal environments by sources like industries and can cause some biological malfunctions. For example, absorbed Ni changes membrane properties and balance of oxidation/reduction systems. It has great affinity for cellular structures like chromosomes and its toxicity is teratogenic and carcinogenic [1-3]. This element is excreted mainly via the urine and to a lower extent in breast milk. Milk and dairy products are one of the main contributors to the chronic dietary exposure to nickel (Ni) in humans [4]. Lead (Pb) has been one of the most common toxicants encountered in veterinary practice and peripheral neuropathy, intermittent colic, and mild anemia are common features of acute toxicosis with this element in general [5]. Equines are food and milk-producing animals in many countries, therefore determining status of toxicants in equine biological components is relevant to human health, and therefore should not be underestimated [6,7]. However, the toxic doses of elements in animals have not been known yet because there is disagreement between results of published studies, and still the physiological mechanisms involved in mineral poisoning are also not yet understood. Finding correlations between some physiological parameters such as age and level of elements accumulation in blood helped understanding this criteria.

Although there are several studies which revealed that quantities of metals in horse biological components were good bio-indicator for environmental pollution and nutritional status [8-11] there is still limited reference about influence of age and sex on blood elements concentrations in these animals. This study was conducted to evaluate the amounts of some important elements such as strontium (Sr), aluminum (Al), vanadium (V), nickel (Ni) and lead (Pb) in the blood of horses of different age and sex in Shiraz which could be used in disease assessment of human and equine and better understanding the physiological parameters attributed to these elements intoxication.

### Materials and Methods

This study was conducted on a group of 53 horses which belonged to two farms around Shiraz. The horses were considered healthy based on physical examination. There were 22 males, 31 females. Animals

were divided into 3 groups regarding age <5 years (n=16), 5-10 years (n=26), >10 years (n=11).

### Sampling

Blood samples were taken aseptically from jugular veins, placed into tubes without any anticoagulant and put in the water-bath at 37°C to coagulate. After clotting, the samples were centrifuged and sera were obtained. Samples were kept at -20°C until chemical analysis.

### Animal ethics

The experiment was performed under the approval of the state committee on animal ethics, Shiraz University, Shiraz, Iran (IACUC no: 4687/63). Also, the recommendations of European Council Directive (86/609/EC) of November 24, 1986, regarding the protection of animals used for experimental purposes were considered.

### Preparation of the samples

The serum samples were analyzed by 700  $\mu\text{l}$  hydroperchloric acid 98% and nitric acid 65% added to 300  $\mu\text{l}$  of serum and kept for 16-20 hours in 80°C water-baths. The prepared samples were analyzed by atomic absorption spectrometry (Shimadzu AA-670, Japan).

### Measurement of the elements

Trace elements were analyzed by flame and air-C<sub>2</sub>H<sub>2</sub> for Pb, Sr, Ni and N<sub>2</sub>O-C<sub>2</sub>H<sub>2</sub> for Al and V. Primarily, the atomic absorption was set to a special wavelength, 217 nm for Pb, 232 nm for Ni, 309.3 nm

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for Al, 460.7 for Sr and 318.4 nm for V; the machinery was calibrated by injecting 5-6 standard solutions with different concentrations in normal range of each element, solutions were injected to the machinery and element concentration was measured in µg/ml.

### Statistical analysis

Statistical analysis was performed using SPSS software (version 18). The effect of age on the concentration of the analyzed elements in the serum of horses was determined by Kruskal-Wallis H except for Ni, which was analyzed by one-way ANOVA. The effect of sex on the concentration of the analyzed elements in the serum of horses was determined by Mann-Whitney U test except for Ni, which was analyzed by Independent-Samples t-test. P<0.05 was considered as statistically significant. The relationship between the amount of serum concentrations of individual elements and different age and sex was calculated using Spearman correlation analysis.

### Results and Discussion

Serum concentration of analyzed elements (µg. mL<sup>-1</sup>) in different categories with regard to sex and age are presented in Tables 1 and 2. All heavy metals evaluated were present in the blood of the horses and serum concentration of the analyzed elements followed the order Al>Sr>V>Ni>Pb. The different age and sex studied here did not influence the serum contents of Ni, Al and V (P>0.05) although significant correlation between age and concentration of Pb was detected (P<0.05).

#### Strontium (Sr)

In our study Sr concentration in females (0.3 ± 0.02 µg/ml) was more than in males (0.18 ± 0.03 µg/ml) which was not consistent with a study done by Pablack et al. [12] that showed no gender differences in Sr concentration existed in liver and kidney of horses. The reason for this difference is not clear and no information is available in this regard. So, more investigations are required for better understanding of this finding. According to Table 2, No significant correlation between concentrations of Strontium with age was seen which was in agreement with a study carried out by Pablack et al. [12] which showed no age-dependent differences in Strontium concentration in liver and kidney of horses. Although Sr concentration in animals with age more than 10 years old (0.20 ± 0.07 µg/ml) was lower than horses with age 5-10 years (0.27 ± 0.02 µg/ml) and <5 years old (0.25 ± 0.02 µg/ml) (P<0.05). This is probably because of higher absorption of strontium from the intestines of younger horses than adults. In the body, strontium behaves very much like calcium and will accumulate in bones. Excess stable strontium causes problems with growing bone. For this reason,

children are more susceptible to the effects of stable strontium than adults who have mature bone [13]. Studies in humans suggested that the elimination rate of strontium is strongly affected by age and sex, due to differences in bone metabolism [14].

#### Lead (Pb)

As lead is an important environmental contaminant, in this context, reports on lead poisoning exist for horses and other livestock in contaminated areas [15-17]. Pourjafar and his coworkers in 2008 confirmed usability of horse hair as a test bio-indicator for Pb environmental pollution [9]. However, there was no decisive agreement on intoxication of horses by this heavy metal. For example, in a study carried out in India with 288 horses from three different areas (industrial, highway adjacent and rural zone) no clinical signs associated with intoxication by lead were detected in the studied animals [18]. On the other hand, a study conducted with horses living on farmland in the vicinity of non-ferrous metal smelters in China showed signs of intoxication by lead in horses [19]. Lead concentrations in the blood and organs of horses indicated lead concentrations in equine by-products. The lead toxicity values in the blood for farm animals have been always lower than those in humans [20] and in horses, the concentration of 0.25 µg mL<sup>-1</sup> is accepted as maximum limit [21]. According to our results, contents of Pb in 100% of samples analyzed present levels below the detection limits. As shown in Table 1, in males, Pb concentration (0.1 ± 0.00 µg/ml) was significantly higher than females (0.08 ± 0.01 µg/ml). As shown in Table 2, although all of the horses used here were in the same environment, there is significant increase in Pb concentration in group with more than 10 year-old horses (0.1 ± 0.00 µg/ml) compared to the other two groups. Also, Pb concentration increased with increasing in age, so there is a correlation between age with concentration of Pb (P<0.05). It seemed that ability to detoxify lead decreased with increase in age or older horses had more ability to absorb this element from their environment, however, further investigations are needed to confirm this hypothesis. Present data was in agreement with a research done by Rudy which indicated that contamination of meat of cattle by Pb clearly depends on the age of these animals and older cattles had more Pb concentrations [22]. But our finding is not in agreement with Asano et al. [23] who reported that there was no age-dependent correlation in Pb concentration of mane hair in male and female racing horses. In present study, the mean serum Pb concentration in 53 horses was (0.09 ± 0.04) µg/ml which was lower than the values reported by De Souza et al. [24] (1.058 µg/ml).

#### Nickel (Ni)

If we consider presence of high amount of Ni in legumes such as

Sex	Ni (µg mL <sup>-1</sup> )	Pb (µg mL <sup>-1</sup> )	Sr (µg mL <sup>-1</sup> )	Al (µg mL <sup>-1</sup> )	Va (µg mL <sup>-1</sup> )
Male	0.096 ± 0.01	0.1 ± 0.00 <sup>a</sup>	0.18 ± 0.03 <sup>a</sup>	0.72 ± 0.03	0.20 ± 0.00
Female	0.115 ± 0.01	0.08 ± 0.01 <sup>b</sup>	0.30 ± 0.02 <sup>b</sup>	0.70 ± 0.02	0.20 ± 0.00

Values that have a different superscript (a, b) differ significantly from each other (P<0.05).

**Table 1:** Concentrations (µg/ml) of strontium (Sr), aluminum (Al), vanadium (Va), nickel (Ni) and lead (Pb) in the serum of horses, depending on the sex of the animals (Male: n=22; Female: n=31).

Age	No	Ni (µg mL <sup>-1</sup> )	Pb (µg mL <sup>-1</sup> )	Sr (µg mL <sup>-1</sup> )	Al (µg mL <sup>-1</sup> )	Va (µg mL <sup>-1</sup> )
<5	16	0.09 ± 0.02	0.08 ± 0.02 <sup>a</sup>	0.25 ± 0.02 <sup>a</sup>	0.71 ± 0.04	0.2 ± 0.00
5-10	26	0.11 ± 0.01	0.09 ± 0.00 <sup>b</sup>	0.27 ± 0.02 <sup>a</sup>	0.69 ± 0.03	0.2 ± 0.00
>10	11	0.1 ± 0.01	0.1 ± 0.00 <sup>c</sup>	0.20 ± 0.07 <sup>b</sup>	0.75 ± 0.04	0.2 ± 0.00

Values that have a different superscript (a, b, c) differ significantly from each other (P<0.05).

**Table 2:** Concentrations (µg/ml) of strontium (Sr), aluminum (Al), vanadium (Va), nickel (Ni) and lead (Pb) in the serum of horses, depending on the age.

alfalfa which is in equine diet the importance of evaluating this element in these animals is evident. However, reference values of blood Ni concentrations were not common in the literature and no toxicity studies were identified for horses. Besides, according to the European Food Safety Authority, contribution of foods of animal origin with high exposure to Ni to human diet is not insignificant [25]. In the present study, there is no significant difference in Ni concentration in all of the groups and the different age and sex studied here did not influence the contents of this element ( $P>0.05$ ). This was the same as the findings revealed by Asano et al. [23] who reported that there was no age dependent correlation in Ni concentration of mane hair in male and female racing horses. In present study, the mean serum Ni concentration in 53 horses was  $(0.10 \pm 0.08) \mu\text{g/ml}$  which was higher than the values reported by De Souza et al. [24]  $(0.006 \mu\text{g/ml})$ .

### Vanadium (V)

Vanadium and related compounds are known to exert potent toxic effects on a wide variety of biological systems mediated by oxygen-derived free radicals [26]. Concentration of this element in blood was the most suitable indicator of the body burden and was better tolerated by small animals including rats and mice than by larger animals, such as horses [27]. Acute toxicity value for vanadium is considered highly species-dependent [28,29]. Reference value of blood V concentration was not common in the literature. Based on Tables 1 and 2, difference in age and sex had no significant influence on vanadium status and there was no age-related correlation in vanadium concentration between groups ( $P>0.05$ ).

### Aluminum (Al)

Aluminium was classified as metalloestrogens because it increased estrogen-related gene expression in human breast cancer cells cultured in the laboratory [30]. In our investigation the contents of this toxic metal in the blood analyzed was not influenced by sex and age, therefore, there was no age-related correlation in Al concentration ( $P>0.05$ ) which was in agreement with an investigation performed by Asano et al. [23] who reported that there was no age-dependent correlation in Al concentration of mane hair in male and female racing horses. In present study, the mean serum Al concentration in 53 horses was  $(0.71 \pm 0.16) \mu\text{g/ml}$  which was lower than the values reported by Asano et al. [23]  $(64.5 \pm 77.0) \mu\text{g/g}$  in the mane hair of horses.

### Conclusion

We concluded that contamination of blood of horse by lead depends on the age of these animals although further investigations are needed to confirm this hypothesis.

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### Conflict of Interest

The authors declare that they have no conflicts of interest.

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