Published in final edited form as:

J Zoo Wildl Med. 2011 June ; 42(2): 360–362.

BASELINE LEVELS OF TRACE METALS IN BLOOD OF CAPTIVE ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*)

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Abstract

Whole blood from 33 healthy captive Asian elephants (*Elephas maximus*) was analyzed for 12 trace elements: aluminum, chromium, manganese, cobalt, nickel, copper, zinc, arsenic, selenium, cadmium, mercury, and lead for the purpose of estimating preliminary baseline population parameters for these minerals. Metals were quantified by inductively coupled plasma mass spectroscopy. Baseline ranges for all animals and for all trace elements were comparable to normal concentrations reported in other species. This is the first report of normal trace element levels in the blood of captive elephants.

Keywords

Asian elephant; *Elephas maximus*; trace elements; trace metals; reference values; blood

BRIEF COMMUNICATION

Trace elements are metals necessary for health, (chromium, cobalt, copper, magnesium, manganese, nickel, selenium, and zinc) that occur in the body in minute quantities.^{6,10} Changes in their levels can be indicative of exposure, and measurement of their concentrations in body fluids can provide useful information about diet and environmental exposure, as well as identify toxicoses or deficiency syndromes.

No data exist documenting concentrations of trace elements in the blood of either species of elephant, although they have been measured in a wide variety of other species, including domestic animals,^{9,14,15,18} marine mammals,^{4,7,19} reptiles,⁵ and birds.^{3,17} The purpose of this study was to establish baseline levels of both biologically important and toxic trace elements in Asian elephants (*Elephas maximus*).

This study included 33 captive Asian elephants (30 females and three males). Fifteen were juveniles between 0 and 20 yr old; six were adults between 21 and 40 yr old and 14 were geriatric (>41 yr old). All elephants were in good health as evidenced by physical examination, complete blood counts, and serum biochemical profiles. Elephants were kept at one of two private facilities in central Florida or travelled 11 mo of each year with a circus.

Elephants were fed ad libitum grass and orchard hay, supplemental bread, fresh fruits and vegetables, and Mazuri elephant pellets (Mazuri Elephant Supplement TPGS, PMI Nutrition International, LLC, Brentwood, Missouri 63144, USA). Whole blood was collected from an ear vein of each elephant directly into a 7-ml royal blue top Monoject tube with K2 EDTA (BD Vacutainer, Becton Dickinson & Co., Roswell, Georgia 30076, USA). Samples were kept on ice in the field and stored at 4°C before analysis. For determination of trace elements, a 1-ml aliquot of whole blood was placed in polypropylene tubes (CPI International, Santa Rosa, California 95403, USA) for determination of aluminum or in borosilicate glass tubes (Fisherbrand, Thermo Fisher Scientific, Waltham Massachusetts 02451, USA) for determination of all other elements, and then the sample was digested with nitric acid (Optima grade, Fisher Scientific) and hydrogen peroxide (Ultrex II ultrapure reagent, Mallinckrodt Baker, Inc., Phillipsburg, New Jersey 08865, USA) by using a graphite digestion block as described previously.¹ Samples were reconstituted to 3 ml by using purified and deionized water (MilliQ, Millipore Corporation, Billerica, Massachusetts 01821, USA) or plasma grade water for aluminium determination. Each sample was then filtered using 0.22-mm nylon syringe filter (Fisherbrand, Fisher Scientific) and placed in a 15-ml metal-free polystyrene tube (Fisherbrand, Fisher Scientific) for analysis. Metals were quantified by inductively coupled plasma mass spectrometry (Thermo Electron X-Series ICP-MS, Thermo Fisher Scientific). Each sample was analyzed for 27Aluminum (Al), 52Chromium (Cr), 55Manganese (Mn), 59Cobalt (Co), 60Nickel (Ni), 65Copper (Cu), 66Zinc (Zn), 75Arsenic (As), 82Selenium (Se), 111Cadmium (Cd), 202Mercury (Hg), and 208Lead (Pb) using indium as an internal standard (Table 1).

Statistical analyses were performed using SigmaPlot (Version 11, Systat Software, Inc., San Jose, California 95110, USA). Trace metal concentrations are summarized in Table 1. All elephants had values considered to be within normal limits in other species.⁶ Zn was found to have the highest mean concentration. As and Pb had the lowest reported concentrations, near their lower limits of quantification. Descriptive statistics for Al, Mn, Co, Ni, Cd, and Hg were not calculated because their concentrations were very low.

This is the first report describing trace element reference ranges in elephants. All elephants in this study had reference values within normal limits for other species, and this study was intended as a clinical screening of elephants in a single collection. Absolute toxicity thresholds for trace metals are not well established for any zoological species. In addition, these thresholds are affected by numerous factors, including the genetic makeup of the individual, the mode of exposure (e.g., inhalation or ingestion), concurrent health issues, and simultaneous toxicities.⁶ Nevertheless, reference ranges have tremendous potential for monitoring exposure to a particular metal, as well as in making a diagnosis of intoxication.

Elephants in both Asia and North America live in a variety of environments in which exposure to environmental trace elements is highly variable. In addition, they are a long-lived species with a propensity for geophagy. The deliberate ingestion of Co-, Mn-, and Serich deposits at salt licks is well documented in African elephants (*Loxodonta africana*).^{8,13} Herbivores typically acquire necessary trace elements from the plants they eat. This, in turn, is affected by the regional geology, or by the presence of pollutants.² Trace elements occur only in minute amounts in the body; thus, by definition, they do not include metals such as iron and magnesium, which are present in larger quantities.⁶ Trace element requirements are often unknown in zoologic species. Even in well-studied species, it is sometimes unclear whether certain elements are even toxic. For example, some evidence exists in both humans and livestock that As, generally considered toxic in any amount, may in fact be required for certain physiologic functions.^{10,14}

Trace elements are routinely measured in whole blood, serum, and urine. Whole blood was used to measure trace element levels in this study, as it is a good indicator of recent metal exposures. In addition, elements, such as cadmium, cobalt, and lead, concentrate in the red cell fraction⁶, thus making whole blood a useful medium for measuring trace elements in an animal. Whole blood also requires less handling time, as centrifugation is not necessary, and can be easily obtained in most captive elephants.

Body fluid analysis of trace elements enables environmental monitoring, identification of trends, and recognition of acute toxicoses. Body fluids, however, which have rapid turnover, generally do not reflect long-term accumulation of trace elements. This measure is better determined by their quantification in organ tissues or in slow-growing and long-lasting body tissues, such as hair and teeth.⁷ In elephants, magnesium, Co, and Zn concentrations have been measured in ivory from deceased animals and have been correlated with the grazing environments of individual animals.¹⁶

Although trace element toxicoses have not been reported in elephants, pica is a frequently described sign in these species, in association with specific diseases, colic, and antituberculosis treatment.^{11,12} Because the potential for intoxication exists, the reference ranges reported here have use as a diagnostic guide in suspected cases of trace element toxicity in Asian elephants.

Acknowledgments

The project described was supported by Award Number KL2RR029888 from The National Center for Research Resources.

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Table 1

Baseline reference ranges of trace elements in captive Asian elephants.

Trace mineral	No. assayed	No. detected	Mean $\pm$ 1 SD ($\mu\text{g/g}$)	Median ($\mu\text{g/g}$)	Range ($\mu\text{g/g}$)	95% confidence limit of the mean ($\mu\text{g/g}$)	Limits of quantification ($\mu\text{g/g}$)
Chromium	33	32	0.044 $\pm$ 0.029	0.032	0.016–0.119	0.033–0.054	0.01
Copper	33	33	0.766 $\pm$ 0.103	0.761	0.582–0.991	0.730–0.803	0.01
Zinc	33	33	6.136 $\pm$ 4.534	4.078	3.065–19.84	4.528–7.744	0.01
Arsenic	33	33	0.019 $\pm$ 0.005	0.018	0.010–0.037	0.017–0.020	0.01
Selenium	33	33	0.382 $\pm$ 0.091	0.376	0.227–0.604	0.349–0.414	0.01
Lead	33	11	0.017 $\pm$ 0.007	0.015	0.011–0.033	0.013–0.022	0.01
Manganese	33	0	N/A ^a				0.01
Cobalt	33	0	N/A				0.01
Nickel	33	0	N/A				0.01
Aluminum	33	2	N/A				0.25
Cadmium	33	0	N/A				0.01
Mercury	33	0	N/A				0.01

^a N/A, descriptive statistics not calculated because more than 70% of the samples were either nondetectable or below the limits of quantification and therefore at very low concentrations.