Serum Levels of Some Electrolytes of Captive Sri Lankan Elephants

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Introduction

Baseline data on serum levels of electrolytes of the Sri Lankan elephant (*Elephas maximus maximus*), a subspecies of the Asian elephant (*E. maximus*), is extremely scarce: restricted to two studies from one laboratory (Silva & Kuruwita 1993a, 1993b). On the other hand, several workers belonging to different laboratories have documented serum electrolyte levels of the African elephant (*Loxodonta africana*) (Bartles *et al*.1963; Dillman & Carr 1970; Brown & White 1979) and the Indian elephant (*E. maximus indicus*) (Simon 1961; Brown & White 1980; Sreekumar & Nirmalan 1989; Sarmah *et al.* 1999). Since the Sri Lankan elephant is endemic and critically endangered it is important to confirm the only data available on its mineral status of blood (Silva & Kuruwita 1993a, 1993b) by other workers and also to extend the investigations to other electrolytes as well. In addition, such data are extremely useful directly in proper diagnosis and treatment, captive breeding and general welfare, and indirectly in the longterm conservation and efficient management of our majestic animal. This study was undertaken with these aims in mind.

Methods

A total of 21-48 adults (9-20 males and 13-28 females), apparently healthy elephants, who participated in the Navam Perehera (a cultural pagent) in 1997 and 1998, were the subjects of this study.

Blood samples (5-10 ml) were collected (in standing position) from a vein or artery on the posterior side of either ear (using aseptic precautions) without sedation (between 7.00 h – 12.00 h) using butterfly needles (18 gauge) connected to a plastic 10 ml syringe. The entire bleeding process lasted 1.0-1.5 min.

Blood was allowed to clot at room temperature (28-31°C) and the serum was separated within three hours of collection by centrifugation at 500 g for 20 min. The serum was stored at –70°C until the mineral levels were determined. All the assays were carried out using standard commercially available test kits (Randox, Ireland).

The potassium level in the serum was determined by a precipitation technique. The potassium ions are reacted with sodium tetraphenylboron in a protein free alkaline medium to produce a turbid suspension of potassium tetraphenylboron. The amount of turbidity produced is proportional to the potassium concentration. Initially, a precipitating reagent (trichloro acetic acid, 500 µl) was added to the serum sample (50 µl) and the mixture was centrifuged at high speed for 5- 10 min. The clear supernatant was separated. Then the working reagent (1000 µl, prepared by mixing equal volumes of solutions of sodium tetraphenylboron and sodium hydroxide) was added to the supernatant (100 µl). The solutions were mixed to produce a homogeneous turbidity and allowed to stand for ~5 min at 25°C. A standard solution of potassium ions was also treated in a similar manner. The absorbance of these solutions was measured at 578 nm using a Shimadsu double beam spectrophotometer (UV-21005, Schimadzu Corp., Kyoto, Japan), against a reagent blank. The potassium levels in the samples were calculated relative to the standard. The method is linear up to a potassium concentration of 10 mmol/l.

A colorimetric procedure was used in order to estimate the calcium level in the serum. Reaction principle: Calcium ions react with O-cresolphthalein complexone in an alkaline medium to produce a violet complex. The serum sample (25 µl) was mixed with the complexing reagent (0.5 ml) and buffer solution (0.5 ml, pH=10.7) provided in the test kit. The absorbance

Table 1. Serum potassium, calcium, magnesium and inorganic phosphorus levels of elephants.

Serum parameter		n	Mean ± SD	Range	Mode	Median	Variance
Potassium	Males	20	2.89 ± 0.89	1.28 – 5.05	2.54	2.73	0.80
	Females	28	2.95 ± 1.16	1.02 – 4.83	--	3.37	1.35
	Entire group	48	2.92 ± 1.05	1.02 – 5.05	2.54	2.86	1.10
Calcium	Males	10	2.37 ± 0.21	1.88 – 2.66	--	2.40	0.04
	Females	13	2.38 ± 0.27	2.06 – 2.80	2.39	2.39	0.07
	Entire group	23	2.38 ± 0.24	1.88 - 2.80	2.30	2.39	0.06
Magnesium	Males	14	0.90 ± 0.26	0.53 – 1.32	--	0.87	0.07
	Females	19	0.63 ± 0.23	0.27 – 0.99	--	0.60	0.05
	Entire group	33	0.75 ± 0.27	0.27 – 1.32	--	0.75	0.07
Phosphorus	Males	9	1.34 ± 0.29	0.97 – 1.64	1.55	1.55	0.09
	Females	12	1.27 ± 0.29	0.97 – 1.61	1.58	1.13	0.08
	Entire group	21	1.30 ± 0.29	0.97 – 1.64	1.03	1.13	0.08

of the sample was measured at 570 nm using a Shimadsu double beam spectrophotometer (UV-21005, Schimadzu Corp., Kyoto, Japan), against a reagent blank between 5-50 min. A similar procedure was followed with the standard calcium solution. The concentration of calcium (mmol/l) was calculated using the equation:

Calcium concentration = A_{sample}/A_{std} x 2.5

The calcium concentration is linear up to a value of 3.75 mmol/l.

The magnesium levels were estimated using a colorimetric assay. The method involves the reaction of magnesium ions with the metallochrome dye calmagite in an alkaline medium. A chromophore which absorbs at 520 nm is formed (Calcium is excluded from the reaction by complexing with EGTA). Initially, a working reagent was prepared by mixing equal volumes of the dye reagent (contains calmagite and EGTA) and buffer solution (pH=12.5). Then, the serum sample (10 µl) was mixed with the working reagent (1.0 ml) and incubated at 25°C for 60 sec. A similar procedure was carried out on the standard solution. The absorbance of the two solutions was measured at 520 nm using a Shimadsu double beam spectrophotometer (UV-21005, Schimadzu Corp., Kyoto, Japan), against a reagent blank within 30 min. and the magnesium concentration was calculated:

Magnesium concentration = A_{sample}/A_{std} x 1.0

This method is linear up to 2.67 mmol/l.

The inorganic phosphorus levels in the serum were determined colorimetrically. The inorganic phosphate is reacted with molybdic acid to form a phosphomolybdic acid complex, which is reduced by ammonium ion (II) sulphate to molybdenum blue, which is measured at 690 nm. Initially, a working reagent was prepared by mixing equal volumes of the molybdate reagent and the reductant. The serum sample (30 µl) was mixed with the working reagent (1.0 ml) and allowed to stand for 10 min at 25°C. The same procedure was carried out using the standard phosphorus solution. The concentration of inorganic phosphorus in the serum samples was determined relative to the standard. This method is linear to 8.0 mmol/l. Two pre-assayed quality control sera (Randox, Ireland) were used as positive controls to monitor accuracy. The results are represented as means ± SD. Statistical analyses were made using Mann-Whitney U-test. Significance was set at P<0.05.

Results

All the serum samples were slightly yellowish in colour. The results obtained are summarized in Table 1. Of the electrolytes monitored, irrespective of the gender, potassium had the highest concentration and magnesium the lowest. Further, there was no significant difference (P>0.05) in the levels of different electrolytes between males and females.

Discussion

There are nine physiologically important electrolytes in mammalian blood serum (Carola et al. 1990). Of these, this study monitored the levels of four electrolytes (K^+, Ca^{2+}, Mg^{2+} and

inorganic phosphorus) in the blood of adult captive Sri Lankan elephant. What is more is that this is the first study to determine the serum magnesium level of the Sri Lankan elephant. Blood samples were collected from 21-48 apparently healthy captive animals, which is a sizable number to provide meaningful data: the present number of captive elephants in Sri Lanka is reported to be 186 (Kurt & Mar 2003). Further, the electrolyte profiles were determined using procedures, which are widely used and claimed to be reliable and sensitive. Because of these facts the data obtained can be considered as representative and regarded as reference baseline data for captive adult Sri Lankan elephants.

The results show that serum calcium, potassium and inorganic phosphorus levels are in agreement to what has been previously reported for domesticated (Silva & Kuruwita 1993b) and free-ranging (Silva & Kuruwita 1993a) adult Sri Lankan elephants, thus confirming their data. Since this is the first study to report the serum magnesium level of Sri Lankan elephants comparison cannot be made with other Sri Lankan workers.

Calcium and inorganic phosphorus levels reported in this study are also comparable to what has been reported for Indian (Simon 1961; Brown & White 1980; Sreekumar & Nirmalan 1989; Sarmah *et al.* 1999) and African (Bartles *et al.* 1963; Dillman & Carr 1970; Brown & White 1979) elephants. Interestingly, the serum potassium level of Indian elephants (by 40%) (Simon 1961; Brown & White 1980; Sreekumar & Nirmalan 1989; Sarmah *et al.*, 1999) and African elephants (by 47-51%) (Bartles *et al.* 1963; Dillman & Carr 1970; Brown & White 1979) were markedly higher than in the Sri Lankan elephant. There could be several reasons for this discrepancy. Differences in the composition of the diet may be one possibility: Sri Lankan captive elephants are given a fairly fixed menu consisting of three main items, namely, kitul (*Caryota urenus*), logs, coconut (*Cocus nucifera*) fronds and jak (*Artocarpus nucifera*) branches and leaves (Godagama *et al*. 1999). Species and subspecies difference may be another possibility: striking differences are reported with some physiological parameters in the Sri Lankan

elephant with the other subspecies of the Asian elephant (Ratnasooriya *et al*. 1992, 1995, 1999; Lincoln & Ratnasooriya 1996). Alternatively, the low serum potassium level in the Sri Lankan elephant may result from low resorption and/or high secretion of K^+ in the uriniferous tubules of the kidney as kidneys excrete 80-90% of serum potassium (Carola *et al*. 1990). Decrease in serum potassium level occurs in diarrhoea, vomiting, diabetic acidosis and chronic kidney diseases (Carola *et al*. 1990). But, such events cannot account for the low serum potassium levels of healthy Sri Lankan elephant.

In this study, gender differences between serum electrolytes determined was not evident as previously reported (Ratnasooriya *et al*. 2006) for lipid profile of Sri Lankan elephants. However, gender differences in serum glucose (Ratnasooriya *et al*. 1999) and cholesterol (Ratnasooriya *et al*. 1995) are reported in the Sri Lankan elephants.

In conclusion, this study, reports for the first time, the serum magnesium level of Sri Lankan captive adult elephants. It also confirms the previously reported serum levels of potassium, calcium and inorganic phosphorus of Sri Lankan elephants.

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Elephant herd at Buttuwa plain, Yala National Park, Sri Lanka
Photo by Prithiviraj Fernando