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Haematological and Blood Chemical Values from *Bothrops ammodytoides* (Ophidia–Crotalidae) in Captivity

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Abstract. In order to establish reference haematological and blood chemistry parameters, blood samples were obtained from 50 healthy specimens of *Bothrops ammodytoides* kept in captivity. The haematological parameters determined were: red blood cell count (RBC); total leucocyte (WBC) and differential leucocyte cell count; thrombocyte count; haematocrit (PCV); haemoglobin concentration; mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Blood chemistry parameters measured were: total protein; albumin; globulins; glucose; urea; uric acid; triglycerides; cholesterol; calcium; phosphorus; magnesium; sodium; potassium and chloride concentrations and the activities of aspartate amino transferase (AST) and alanine amino transferase (ALT). Compared to the values published for other ophidian species, RBC count, PCV and WBC count in *Bothrops ammodytoides* are lower than in most of the crotalids. Total protein and glucose concentrations are lower, whereas uric acid concentration, AST and ALT levels are higher than the values reported for other species.

Keywords: Biochemical values; Blood; *Bothrops ammodytoides*; Captivity; Haematology

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Introduction

Bothrops ammodytoides, the smallest representative of the *Bothrops* genus is found only in Argentina. It is one of the species of widest distribution in the country from the northwest, pre-Andean region to Patagonia in the south. It inhabits forests, mountains (up to 2000 m) and arid regions where temperature may oscillate between 40 °C during the day and 0 °C during the night. Most studies have focused on other, larger sized *Bothrops* species which appear to be more important as a public health problem. Therefore, no data on the biology of this widely distributed species have been, to our knowledge, previously published. In the present paper we report some haematological and blood chemistry parameters of specimens of *B. ammodytoides* kept in captivity.

Materials and Methods

A total 50 healthy specimens of *B. ammodytoides* of different ages and sex captured in Argentina (deparasitised on arrival at the snake farm and quarantined for one month) were kept in individual boxes in an environment of 22–28 °C and 40–60% humidity with natural light/dark periods as occur in the different seasons of the year. All the specimens fed spontaneously on living prey. Snakes were given one adult mouse (20–25 g) a week and received filtered water *ad libitum*. In order to take the blood samples, the snakes were removed from the boxes by means of a metallic hook, the head and the rest of the body was firmly restrained by two operators. The use of anaesthetics or sedative agents,

which are known to induce significant alterations in the haematological parameters (Custer and Bush 1980) was avoided. Blood (3.0 ml) was obtained by a third operator, by venepuncture of the caudal vein (Bush and Smeller, 1978) using disposable 21G-needles fitted to sterile 3 ml syringes. All the samples were obtained during 1997.

Immediately after collection of the sample, three blood smears were prepared, air dried and stained with a mixture of May–Grünwald and Giemsa stains in absolute methanol (Rosenfeld 1947). Part of the sample was transferred to a clean glass containing heparin (Sodium salt, 20 μl/ml of blood) as anticoagulant and was used to determine the haematological parameters. Haematocrit (PCV) was determined by the microhaematocrit method. Haemoglobin was measured by mixing 20 μl of blood with 2.5 ml of Drabkin solution (Wiener Laboratories, Argentina). Formation of cyanmethaemoglobin was determined from the absorbance at 540 nm and compared to that of a haemoglobin standard (Wiener Laboratories, Argentina). Red blood cells, total leucocyte and thrombocyte counts were performed in a 1:200 dilution of blood in a solution as described by Otis (1973), which allow erythrocytes, leucocytes and thrombocytes to be counted in the same standard Neubauer haemocytometer. Haematimetric indexes were calculated according to the original Wintrobe (1933) formulae.

For the differential leucocyte counts, the stained blood smears were examined with a optical microscope with a oil-immersion lens. At least 200 cells were counted in each slide. The leucocyte types are described according to the morphological nomenclature proposed by Hawkey and Dennet (1994), i.e. mononuclear cells (lymphocytes, monocytes and azurophiles) or granulocytes (heterophils, basophils and eosinophils).

The other part of the blood sample was transferred to a clean glass tube without anticoagulant and after coagulation, the serum was removed. In this serum sample, total protein was determined spectrophotometrically at 540 nm by the biuret method. Albumin was measured at 600 nm after complex formation with bromocresol purple. Globuin concentration was calculated as total protein concentration minus albumin concentration.

Uric acid was determined after deproteinisation with tungstic acid and calcium carbonate by treatment with uricase and peroxidase, which results in quinoneimine dye. Glucose was measured by the enzymatic method of hexoquinase/glucose 6-phosphate dehydrogenase. Urea was determined spectrophotometrically at 505 nm after reaction with indophenol blue. Triglycerides were measured at 540 nm after reaction with quinoneimine dye. Cholesterol was determined spectrophotometrically by the 4p-benzoquinone reaction. Phosphorus was determined at 630 nm after formation of phosphomolybdate; calcium was measured at 570 nm after reaction with cresophthalein-complexone; chloride was measured at 460 nm after complex formation with sulphocyanide; magnesium was measured at 520 nm after complex formation with magon. Potassium and sodium were measured in plasma by flame spectrophotometry. Alanine aminotransferase (ALT) and asparate aminotransferase (AST) activities were determined kinetically by the decrease in absorbance at 340 nm resulting from oxidation of NADH by the coupling reaction catalysed by lactate dehydrogenase. The reactives were provided by Wiener Laboratories (Argentina). All parameters are expressed in SI Units (Doxey 1977).

Results

Haematological parameters in *B. ammodytoides* (mean (x) $\pm$ standard deviation (SD)) are presented in Table 1.

Blood chemistry parameters (mean (x) $\pm$ SD) are presented in Table 2.

Discussion and Conclusions

The red blood cell count in *B. ammodytoides* is within the range described for two other crotalids *Bothrops jararaca* (Valle and Leal Prado 1947; Sans-Martins 1978) and *Agkistrodon piscivorus* (Hutton 1958); the colubrid *Heterodon contortix* (Wintrobe 1933) and one Australian elapid *Notechis scutatus* (Board et al. 1977). On the other hand, the RBC count for *B. ammodytoides* is significantly lower than those described with other species of crotalids such as *Crotalus durisssus terrificus* (Troiano et al. 1997a), *Crotalus horridus* (Carmichael and Pretcher 1945), *Crotalus cerastes* (MacMahon and Hammer 1975), *Cerastes vipera* and *Cerastes cerastes* (Al-Badry and Nuzhy, 1983); colubrids like *Waglerophis merremii* (Sano-Martins 1978), *Pituophis sayii* (Ryerson 1949), *Natrix natrix* (Wintrobe 1933; Binyon and Twigg 1965) and *Lampropeltis gettulus* (Hutton 1958), the Australian elapids *Pseudonaja nuchalis*, *Pseudechis*

Table 1. Haematological values of *Bothrops ammodytoides* (n = 50)

Determination	Values (x) $\pm$ SD
RBC count (10^9/l)	489.4 ± 14.8
WBC count (10^9/l)	9.42 ± 5.30
Haemoglobin (g/dl)	8.2 ± 1.2
Haematocrit (%)	19.11 ± 1.7
MCV (fl)	391 ± 32
MCH (pg)	169.5 ± 22
MCHC (%)	43.22 ± 2.7
Lymphcoytes (%)	52.20 ± 6.87
Monocytes (%)	8.20 ± 0.91
Azurophils (%)	9.80 ± 1.21
Heterophils (%)	12.20 ± 1.34
Eosinophils (%)	16.30 ± 1.85
Basophils (%)	1 ± 0.3
Thrombocytes (10^9/l)	4.60 ± 0.53

RBC, red blood cells; WBC, white blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MHCH, mean corpuscular haemoglobin concentration.

Table 2. Blood chemical values of *Bothrops ammodytoides* (n = 50)

Determination	Values (x) ± SD
Total proteins (g/l)	40.84 ± 2.82
Albumin (g/l)[a]	29.69 ± 4.04
Globulin (g/dl)	11.15 ± 3.42
Albumin/Globulin	2.66
Glucose (mmol/l)	1.78 ± 0.26
Uric acid (mmol/l)	490 ± 111.80
Urea (mmol/l)	1.78 ± 0.18
Cholesterol (mmol/l)	4.49 ± 0.16
Triglycerides (mmol/l)	1.73 ± 0.19
Phosphorus (mmol/l)	1.88 ± 0.25
Magnesium (mmol/l)	2.58 ± 0.32
Calcium (mmol/l)	5.23 ± 0.28
Sodium (mmol/l)	149.12 ± 8.69
Potassium (mmol/l)	5.46 ± 0.36
Chloride (mmol/l)	112.12 ± 4.42
AST–GOT (IU/l)	33.34 ± 3.21
ALT–GPT (IU/l)	16.84 ± 1.71

AST–GOT, aspartate aminotransferase – glutamic oxaloacetic transaminase; ALT–GPT, alanine aminotransferase – glutamic pyruvic transaminase.
[a]grams of bovine albumin.

porphyriacus and *Austerelaps superbus* (Board et al. 1977) and boids like *Boa constrictor constrictor* and *Python regius* (Rosskopf et al. 1982). In addition, preliminary results (Troiano et al. 1997b) indicate that RBC for *B. ammodytoides* is 30–40% lower than those obtained with other *Bothrops* species (i.e. *B. alternatus*, *B. jararacussu* and *B. neuwiedii*) from Argentina.

The haemoglobin concentration of *Bothrops ammodytoides* is higher than those reported with *Heterodon contortix*, *Natrix natrix* and *Thammophis sirtalis* (Wintrobe 1933). However, it is within the range described for crotalids like *Bothrops jararaca* (Valle and Leal Prado 1947; Sano-Martins 1978); *Crotalus cerastes* (MacMahon and Hammer 1975); *Crotalus horridus* (Carmichael and Pretcher 1945); *Crotalus durisssus terrificus* (Troiano et al. 1997a), viperids like *Cerastes vipera* and *Cerastes cerastes* (Al-Badry and Nuzhy 1983); colubrids like *Waglerophis merremii* (Sano-Martins 1978), *Pituophis sayii* (Ryerson 1949) and elapids *Naja haje haje* (Al-Badry et al. 1992), *Noetechis scutatus*, *Pseudonaja nuchalis*, *Pseudechis porphyriacus* and *Austerelaps superbus* (Board et al. 1977). Haemoglobin concentration in *B. ammodytoides* is 15%–20% lower than the values obtained in other *Bothrops* species (Troiano et al. 1997b).

The PCV values in *Bothrops ammodytoides* is higher than that reported for *Heterodon contortix* (Wintrobe 1933); within the range of those described for *Agkistrodon piscivorus* (Hutton 1958); *Crotalus durissus terrificus* (Troiano et al. 1997a) and *Bitis arietans* (Hattings and Willemse 1976), but lower than those reported for other crotalid species such as *Bothrops jararaca* (Valle and Leal Prado 1947), *Crotalus cerastes* (MacMahon and Hammer 1975), *Crotalus horridus* (Carmichael and Pretcher 1945); Australian elapids like *Notechis scutatus*, *Pseudonaja nuchalis*, *Pseudechis porphyriacus* and *Austerelaps superbus* (Board et al. 1977); colubrids such as *Thammophis sirtalis* (Wintrobe 1933), *Natrix natrix* (Hutton 1958), *Pituophis sayii* (Ryerson 1949) and in boids like *Boa constrictor constrictor* and *Python regius* (Rosskopf 1982),

The WBC count ($9.42 \pm 5.30 \times 10^9$/l) is within the range reported with *Boa constrictor constrictor* and *Phyton regius* (Rosskopf et al. 1982), but is significantly lower than those reported in *Bitis arietans* (Otis 1973), *Crotalus cerastes* (Board et al. 1977); *Crotalus durissus terrificus* (Troiano et al. 1997a) and *Eunectes murinus* (Calle et al. 1994).

In agreement with results reported in *Bitis arietans* (Otis 1973); *crotalus cerastes* (MacMahon and Hammer 1975); *Crotalus durissus terrificus* (Troiano et al. 1997a), *Boa constrictor constrictor* (Rosskopf et al. 1982), *Python regius* (Rosskopf et al. 1982), *Ablabophis rufulus*, *Crotaphopeltis hotamboeia* (Pienaar 1962) and *Naja haje haje* (Al-Badry et al. 1992), the differential leucocyte count shows a high percentage of lymphocytes (52.5%). However, this percentage is higher than that reported in *Bothrops jararaca* (Sano-Martins 1978). In this regard, some authors reported that the most common leucocyte type in the rat snake is the azurophil, whereas in other snakes it is the heterophil (Dotson et al. 1994; Bounous et al. 1996).

The lower percentage of basophils in the differential leucocyte count is in accordance with reports in *Bothrops jararaca* (Sano-Martins 1978), *Bitis arietans* (Otis 1973), *Eunectes murinus* (Calle et al. 1994) and *Crotalus durissus terrificus* (Troiano et al. 1997a). On the other hand, high percentages of this type of leucocyte were reported in South African snakes as *Ablabophis rufulus*, *Causus rombheatus*, *Crotaphopeltis hotamboeia*, *Psammophis subtaenitus subtaenitus* and *Naja nigricollis*, with a maximum of 22.3% in *Psammophis subtaenitus subtaenitus* (Pienaar 1962).

The concentration of total proteins, albumin and globulins in *B. ammodytoides* are within the range of variation described for *Agkistrodon intermedius*, *Bothrops schlegelii* and *Trimeresurus arbolaris* (De Smet 1978a), higher than those described for *Vipera lebetina*, *Vipera ruselli*, *Xenodon* spp. and *Baedon fuliginossus* (De Smet 1978b) and lower than those reported in *Ptyas mucosus*, *Echis carinatus*, *Vipera ammodytes*, *Vipera berus*, *Naja haje*, *Naja nigricollis* (De Smet 1982a), *Bitis arietans* (Otis 1973), *Boa constrictor constrictor* (Chiodini and Sundberg, 1982; Rosskopf et al., 1982), *Python regius* (Rosskopf et al. 1982), *Eunectes murinus* (Calle et al. 1994) and *Eunectes notaeus* (Troiano et al. 1995).

Serum glucose concentrations (1.78 mmol/l) are similar than those described in *Bitis arietans* (Otis 1973), *Boa constrictor constrictor* (Chiodini and Sundberg 1982) and *Python regius* (Rosskopf et al. 1982), but lower than those reported in *Agkistrodon piscivorus*, *Coluber constrictor*, *Lampropeltis gettulus*,

Natrix natrix (Hutton 1958), *Bothrops jararaca*, *Phylodrias olfersii* (Leal Prado 1946) and *Eunectes murinus* (Calle et al. 1994).

The urea concentration found in *Bothrops ammodytoides* is within the range described for other ophidians such as *Agkistrodon piscivorus*, *Coluber constrictor* (Hutton 1958), *Crotalus horridus* (Carmichael and Pretcher 1945), *Vipera aspis* (Izard et al. 1961), *Boa constrictor constrictor* and *Python regius* (Rosskopf et al. 1982); higher than those observed in *Eunects notaeus* (Troiano et al. 1995) as well as in some Crocodylia (Dessauer 1982), The uric acid concentration is a more interesting parameter, since in ophidians, nitrogen metabolism is directed to purine biosynthesis resulting in uric acid, and this metabolic pathway appears to have a critical function in water balance. In addition, measurement of uric acid concentration allows early detection of hyperuricaemia, a metabolic disorder to which some crotalid species are particularly susceptible (e.g. *Crotalus durissus terrificus*, Machado 1964). In fact, when associated with dehydration, hyperuricaemia promotes urate crystal deposition in kidneys (renal gout). This decreases uric acid excretion and results in urate crystals deposits in the pericardial sac, liver, spleen, lungs, subcutaneous and other soft tissues (visceral gout) and eventually death.

Uric acid concentration in *Bothrops ammodytoides* is significantly higher than those described in *Agkistrodon piscivorus*, *Coluber constrictor*, *Lampropeltis gettulus* and *Natrix natrix* (Hutton 1958), *Crotalus horridus* (Carmichael and Pretcher 1945), *Vipera aspis* (Izard et al. 1961), *Boa constrictor constrictor* (Chiodini and Sundberg 1982; Roskopf et al. 1982), *Eunectes notaeus* (Troiano et al. 1995), *Eunectes murinus* (Calle et al.1994), *Python regius* (Rosskopf et al. 1982) and other *Bothrops* species (Troiano et al. 1997b).

Interestingly, a similarly high uric acid concentration was reported for *Cerastes cerastes* and *Cerastes vipera* (Al-Badry and Nuzhy 1983), two viperid species which also inhabit dry regions with a high daily temperature oscillation. However, a high uric acid concentration may not represent an adaptive response to a ‘dry habitat’, since the *B. ammodytoides* specimens used in this study were kept in an environment of controlled temperature, received food monthly and had free access to water for, at least 6 months. Furthermore, as indicated by RBC count, PCV values and ion compositon, no apparent signs of haemoconcentration were observed in any of the specimens studied.

Cholesterol and triglycerides are within the range of variation described for other crotalids such as *Agkistrodon piscivorus* (Hutton 1958), *Crotalus horridus* (Carmichael and Pretcher 1945) and some colubrids such as *Coluber constrictor* and *Natrix natrix* (Hutton 1958). However, cholesterol levels in *B. ammodytoides* were 10%–30% higher than those in other *Bothrops* species (Troiano et al. 1997b).

Phosphorus values are similar to thse reported for *Agkistrodon piscivorus*, *Natrix natrix* (Hutton 1958), *Crotalus horridus* (Carmichael and Pretcher, 1945), *Eunectes murinus* (Calle et al. 1994), *Eunectes notaeus* (Troiano et al. 1995) and lower than those in *Lampropeltis gettulus*, *Coluber constrictor* (Hutton 1958), *Vipera aspis* (Izard et al. 1961) and *Boa constrictor constrictor* (Chiodini and Sundberg 1982).

The concentration of magnesium in serum appears to be similar to that reported in *Notechis scutatus*, *Pseudonaja nuchalis*, *Pseudechis porphyriacus* and *Austerelaps superbus* (Board et al. 1977) and higher than that in Agkistrodon piscivorus, *Coluber constrictor*, *Lampropeltis gettulus*, *Natrix natrix* (Hutton 1958), *Crotalus horridus* (Carmichael and Pretcher 1945), *Vipera aspis* (Izard et al. 1961), *Cerastes cerastes*, *Cerastes vipera* (Al-Badry and Nuzhy 1983) and *Eunectes notaeus* (Troiano et al. 1995). Calcium concentration is in the same range as reported for Australian elapids such as *Notechis scutatus*, *Pseudonaja nuchalis*, *Pseudechis prophyriacus* and *Austerelaps superbus* (Board et al. 1958) and *Crotalus horridus* (Carmichael and Pretcher 1945) and higher than those reported for *Agkistrodon piscivorus*, *Coluber constrictor*, *Lampropeltis gettulus* and *Natrix natrix* (Hutton 1958), *Vipera aspis* (Izard et al. 1961), *Boa constrictor constrictor* (Chiodini and Sundberg, 1982; Rosskopf et al. 1982), *Python regius* (Rosskopf et al. 1982), *Eunectes murinus* (Calle et al. 1994) and *Eunectes notaeus* (Troiano et al. 1995).

With the exception of *Lampropeltis gettulus* and *Natrix natrix* (Hutton 1958), the serum concentration of sodium is similar to those reported in free-ranging and captive boids (Calle et al. 1994; Troiano et al. 1995), colubrids such as *Natrix natrix* (Binyon and Twigg 1965), the Australian elapids *Notechis scutatus*, *Pseudonaja nuchalis*, *Pseudechis porphyriacus* and *Austerelaps superbus* (Board et al. 1958), *Cerastes cerastes*, *Cerastes vipera* (Al-Badry and Nuzhy 1983), *Vipera aspis* (Izard et al. 1961), *Bitis arietans* (Otis 1973) and *Crotalus horridus* and *Crotalus atrox* (Carmichael and Pretcher 1945). Potassium concentration is within the range described for *Vipera aspis* (Izard et al. 1961), *Cerastes cerastes*, *Cerastes vipera* (Al-Badry and Nuzhy 1983), *Boa constrictor constrictor* (Chiodini and Sundberg 1982; Roskopf et al. 1982), *Eunectes murinus* (Calle et al. 1994), *Eunectes notaeus* (Troiano et al. 1995), *Python regius* (Rosskopf et al. 1982) and some Australian elapids as *Notechis scutatus* and *Pseudonaja nuchalis* (Board et al. 1977).

Chloride concentration is within the range described in *Vipera aspis* (Izard et al. 1961), *Eunectes murinus* (Calle et al. 1994) and *Eunectes notaeus* (Troiano et al. 1995), but slightly higher that those reported from *Agkistrodon piscivorus*, *Coluber constrictor*, *Lampropeltis gettulus* and *Natrix natrix* (Hutton 1958).

Finally, the concentrations of the serum enzymes AST and ALT in *B. ammodytoides* appear to be 3-fold higher than those reported in *Bitis arietans* (Otis 1973) and *Eunectes murinus* (Calle et al. 1994). On the other hand, these values cannot be compared to those obtained with *Boa constrictor constrictor* (Chiodini and Sundberg

1982; Rosskopf et al. 1982) and *Python regius* (Rosskopf et al. 1982) because of the wide ranges of variation of the reported values.

Further studies are required in order to ascertain whether factors like age and sex as well as seasonal variations produce statistically significant variations in the haematological parameters, particularly RBC count, PCV, haemoglobin concentration, WBC and thrombocyte counts as well as in the chemical parameters (uric acid, cholesterol concentration, etc.) in this autoctonous crotalid.

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