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Hematological and plasma chemistry values for the African rock python (*Python sebae*)

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ABSTRACT

Hematology and plasma biochemistry parameters are useful in the assessment and management of threatened and endangered species. Although reference values are readily available for many mammalian species, reference values for snakes are lacking for most species. We determined hematology and plasma biochemistry values for captive African rock pythons (*Python sebae*) and studied the effects of age, sex, season and hemoparasites on these values. Blood (5 mL) was collected by venipuncture of ventral coccygeal vein from 19 African rock pythons in rainy season and 14 snakes from the same population in dry season. There was no significant statistical difference ($P < .05$) between males and females to any of the parameters measured except total calcium in the rainy season. Significantly higher values were obtained ($P < .05$) for the white blood cells (WBC), heterophils, lymphocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and aspartate aminotransferase (AST) in the dry versus the rainy seasons while there were significantly lower values obtained for red blood cells (RBC), glucose and total protein. Statistically significant differences in lymphocyte and monocyte counts were however found between adult and juvenile snakes. Differences in parameters for hepatozoon positive and hepatozoon negative snakes were not statistically significant although parameters like the total WBC count, heterophils and lymphocytes were markedly higher for hepatozoon positive snakes while packed cell volume (PCV) was slightly lower. This is the first study on blood parameters of the African rock python and serves as first pilot values for clinical assessments and future studies of this species.

1. Introduction

Hematological and serum biochemical evaluations are important tools in the evaluation of the health status of reptiles whether kept in captivity or in the wild [1–3]. In addition, blood evaluation can also help in the diagnosis of stress and other health problems in reptilians [4].

Blood parameters in reptiles therefore have to be assessed so as to guide the evaluation of physiological and health conditions of these species and used as an indicator in determining environmental conditions, since species appear to be very sensitive to habitat changes [5–8].

The wholesome knowledge of snake physiology is becoming increasingly imperative due to need for veterinarians to diagnose, increasing economic importance, conservation studies and their role as exotic pets. This stimulated the authors to carry out field work on a member of the Ophidia group. Blood can be collected from the caudal tail vein (coccygeal vein) or heart [9], although the latter can cause heart muscle laceration, haemorrhage, pericarditis and death and is generally only recommended in anaesthetised snakes [10–12].

African rock pythons are no longer as widespread as they once were. *Python sebae* is now restricted mainly to hunting reserves, national parks and secluded sections of the African savannah. Larger individuals are increasingly rare in many areas. African rock pythons have been placed on Appendix II of CITES and are legally protected in certain countries where populations have become increasingly vulnerable [13,14].

Most of the earlier works describing cell composition and blood chemistry rarely explored African species. African species, mainly

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tropical Africa, have been sparsely studied. Without the development of accurate species specific reference values, taking into consideration species and habitat [15], gross diagnostic misinterpretations can be made. We therefore carried out this study to determine the normal hematology and plasma biochemistry values of the African rock python as regards seasons (wet and dry), age, sex and effect of hemoparasites.

2. Materials and methods

2.1. Experimental animals

Nineteen (19) African rock pythons (*Python sebae*) were sampled in the rainy season and 14 snakes from the same population were sampled in the dry season from various captive animal collections in Nigeria (including zoos, private snake owners and snake charmers). Study area was North Central and Western parts of Nigeria cutting across eight states. Study period were the months of August 2016 and January 2017 which are within the rainy and dry seasons respectively in Nigeria. Snakes in these collections were housed in wire mesh cages and provided a water body and fed with poultry (Chickens and chicks) weekly.

2.2. Health assessment, biological and morphometric data collection

Each snake was subjected to physical and clinical examination, morphometric measurement and weighed via a secure cotton bag place on a scale. Sex of each snake was also determined using a probe. Each snake was photographed for identification and individually restrained for examination. Morphometric measurements included snout-vent length (SVL), total length (TL) and head width. Environmental conditions (temperature and humidity) were measured using a digital thermohygrometer (WINCOM HTC-2, China).

2.3. Blood collection

Blood was collected by venipuncture of the ventral coccygeal vein using a 23-gauge needle attached to a 5 mL syringe. Each snake was restrained with the tail lower than the head in order to promote blood pooling in the tail, as described by Lock [16]. For larger snakes (>1 kg), 21-gauge needles were used. Blood samples were stored in heparinized tubes (Silver Health Diagnostics, Nigeria) and labelled appropriately.

2.4. Hematology/hemoparasite screening

Blood samples were analysed within one hour (1 h) after sampling. Three blood smears per snake were made using the heparinized blood. Smears were air dried and two best slides were selected and stained with Wrights–Giemsa stain (Guangzhou Fischer Chemical Co., Ltd., China) on a manual slide stainer. Packed cell volume was determined using a Micro hematocrit reader after the capillary tubes were then spun in a microhematocrit centrifuge for five minutes at 12,000 rpm. The total red blood cell count (RBC), was determined manually with the improved Neubauer counting chamber after the blood was diluted 200 times (1:200) with the Natt and Herrick's solution [17]. Leukocyte estimation, differential and thrombocyte estimation was based on examination of the blood smear that is considered to have the most regular distribution of white cells across the blood smears [18]. Parasite identification and evaluation were determined according to Telford [19] using stained smears (Fig. 1).

2.5. Plasma biochemistry

The remaining blood was spun in a centrifuge at 3000 rpm (905*g* force) for 15 min and plasma was decanted from the supernatant. Before biochemical processing, 50 μL of plasma was aliquoted and analysed for total protein concentration (FDTP), albumin Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Aspartate transaminase (AST), total calcium, glucose, urea and uric acid and creatinine using RT-9200 Rayto Chemistry Analyser and UV spectrophotometer (Rayto Life and Analytical Sciences Co., Ltd, China).

2.6. Data analysis

First pilot data on hematologic and blood chemistry parameters for the African rock python were established with the use of parameters assessed from all snakes. For each parameter obtained, data from season, hematozoa positive or negative, age range and sex were calculated for means and standard deviation. Hematological and plasma biochemistry data resulting from our study were analysed using the software SPSS 16.0 for Windows. Significant differences between means were determined using an independent sample *t*-test model. Results were considered significant at $P < .05$ [20].

3. Results

All snakes appeared healthy at the time of blood collection based on physical examination and absence of any external parasites or lesions. No drugs were administered to the animals before blood collection. Weights of snakes ranged from 0.21 to 8.44 kg spanning across different age ranges (snakes <1 kg were classified as juveniles while those >1 kg were classified as adults).

Temperature and humidity of the two seasons as measured using the thermohygrometer ranged from 26.1 to 27.9 °C/45 to 77% for rainy season (August) and 27.5 to 32.6 °C/13 to 32% for dry season (January).

The results of the hematologic and plasma biochemical measurements are presented in Table 1. There was no significant statistical difference ($P < .05$) between males and females to any of the parameters measured except total calcium which was higher in females in the rainy season. Significantly higher values were obtained ($P < .05$) for the WBC, heterophils, MCV, MCH and AST in the dry versus the rainy season while there were significantly lower values obtained for RBC, glucose and total protein. The mean values for lymphocytes were higher (over twice the value) for the dry season against the rainy season although not statistically significant. Statistically significant differences in lymphocyte and monocyte counts were found among juveniles and adults (Table 2). Creatinine was not measured in the rainy season.

Ten (10) out of 19 (52.6%) of the samples had hepatozoon in the rainy season and 8 out of 14 (57.1%) had hepatozoon in dry season. Cytological identification of the species was concordant with genus *Hepatozoon*, according to their disposition, aspect, shape and morphology (Fig. 1).

Change in parameters was not statistically significant although parameters like the total WBC count, heterophils and lymphocytes were markedly higher for hepatozoon positive snakes while PCV was slightly lower (Table 3).

4. Discussion

Diagnosis of disease in reptiles, especially snakes has been quite challenging as they exhibit a stoic nature due to evolution and adaptation in avoiding predators. During clinical disease, most

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