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Full Length Article

Hematological and plasma chemistry values for the African rock python (*Python sebae*)Henry O. Jegede^{a,*}, Temidayo O. Omobowale^b, Babatunde S. Okediran^c, Afolabi A. Adegboye^d^a Veterinary Teaching Hospital, University of Ilorin, Nigeria^b Department of Veterinary Medicine, University of Ibadan, Nigeria^c Department of Veterinary Physiology and Biochemistry, University of Ilorin, Nigeria^d Department of Veterinary Pathology, University of Ilorin, Nigeria

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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.

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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 placed 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 (905g force) for 15 min and plasma was decanted from the supernatant. Before biochemical processing, 50 μ L 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

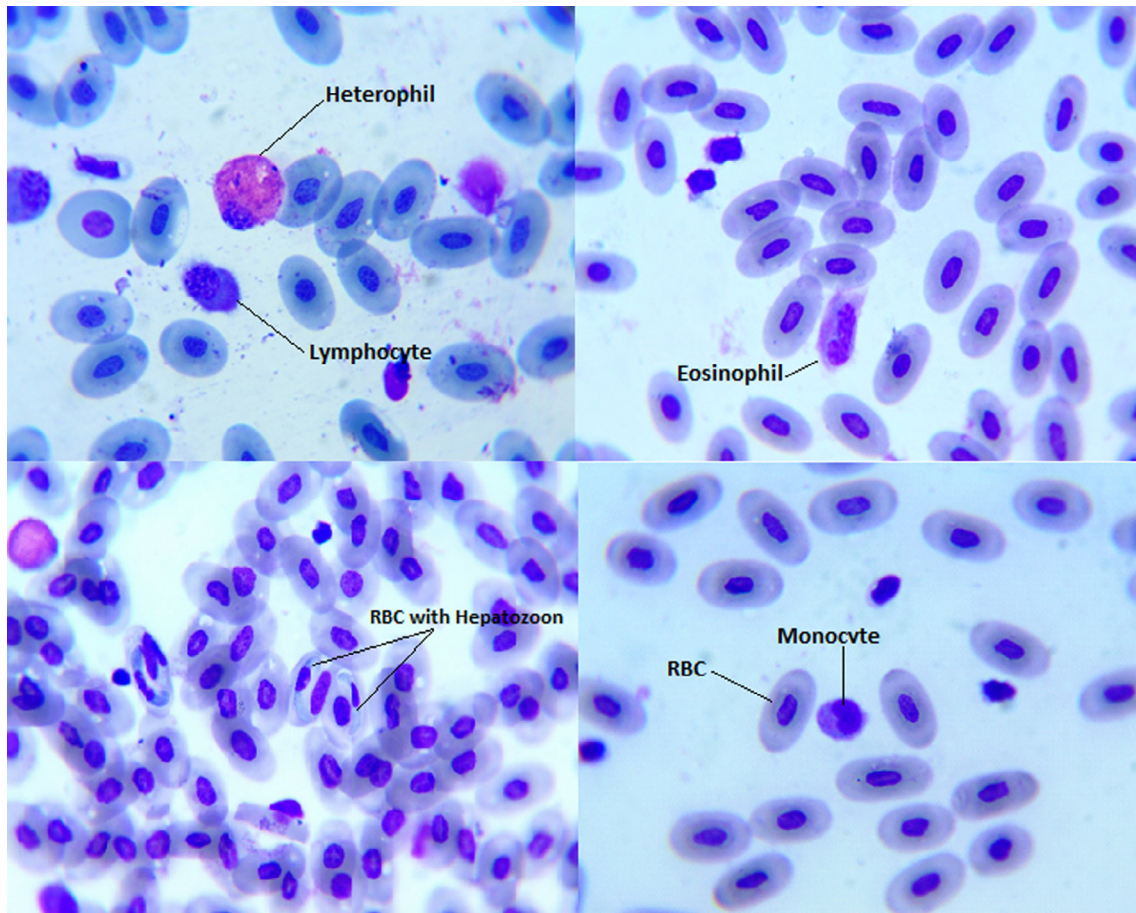


Fig. 1. Blood films of the African rock python showing morphology and staining characteristics of various cells labelled along with intra-erythrocytic *Hepatozoon* spp.

Table 1
Hematological and plasma chemistry reference values (RIs) for the African rock python ($P < .05$).

Parameter	Mean \pm SD (rainy)	Range	Mean \pm SD (dry)	Range	P-value
WBC ($10^9/L$)	17.18 \pm 6.86	7.10–29.40	38.80 \pm 19.11	15.20–69.00	.007
Heterophils ($10^9/L$)	11.38 \pm 5.67	4.26–21.76	26.39 \pm 15.28	7.60–58.60	.016
Lymphocytes ($10^9/L$)	5.39 \pm 2.23	2.50–8.20	12.06 \pm 9.74	4.15–33.00	.066
Mono/Azuro ($10^9/L$)	0.20 \pm 0.18	0.00–0.44	0.18 \pm 0.32	0.00–0.00	.882
Eosinophils ($10^9/L$)	0.18 \pm 0.23	0.00–0.59	0.06 \pm 0.15	0.00–2.16	.236
Basophils ($10^9/L$)	0.016 \pm 0.05	0.00–0.16	0.00 \pm 0.00	0.00–1.08	.396
RBC ($10^{12}/L$)	3.03 \pm 0.57	2.16–4.17	1.79 \pm 0.36	1.11–2.61	<.001
HGB (g/dL)	6.85 \pm 1.26	5.30–9.40	6.61 \pm 1.39	3.30–8.70	.730
PCV (%)	20.44 \pm 3.09	16.00–26.00	19.86 \pm 4.22	10.00–26.00	.752
MCV (fl)	66.93 \pm 5.60	57.00–74.10	111.84 \pm 17.39	57.80–142.90	.000
MCH (pg)	22.71 \pm 1.29	21.10–24.50	37.27 \pm 5.80	19.10–47.60	.000
MCHC (g/dL)	33.91 \pm 1.36	33.20–37.30	33.31 \pm 0.13	33.00–33.50	.269
ALT (IU/L)	24.72 \pm 16.60	10.80–64.50	17.67 \pm 8.28	10.10–30.40	.323
AST (IU/L)	31.96 \pm 18.84	10.60–61.20	54.17 \pm 15.29	37.00–76.90	.024
ALP (IU/L)	95.70 \pm 36.27	38.20–169.60	76.21 \pm 24.13	46.90–112.00	.242
Uric Acid (mmol/L)	1.24 \pm 0.69	0.18–2.60	0.64 \pm 0.66	0.18–1.91	.102
Urea (mmol/L)	1.90 \pm 0.62	1.32–3.34	1.58 \pm 0.77	0.70–2.96	.375
Glucose (mmol/L)	3.03 \pm 0.58	2.20–4.40	1.36 \pm 0.44	1.00–2.20	.000
Calcium (mmol/L)	1.30 \pm 0.33	0.72–1.84	1.27 \pm 0.32	0.96–1.87	.857
Total Protein (g/L)	70.67 \pm 12.45	54.00–90.00	67.86 \pm 5.79	62.00–80.00	.034
Albumin (g/L)	49.44 \pm 10.86	29.00–63.00	37.43 \pm 9.05	30.00–56.00	.591
Creatinine (μ mol/L)	ND	ND	32.56 \pm 9.55	17.80–42.40	–

MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; ND, not determined; PCV, packed cell volume; RBC, red blood cell count; RI, reference interval; SD, standard deviation; WBC white blood cell count.

signs go unnoticed making physical diagnosis of clinical disease difficult. Hematology and plasma chemistry in reptiles are helpful in bridging this gap. To distinguish pathologic and physiologic alterations in different analytes, reference values for normal ani-

mals are needed [21]. Hematological and plasma chemistry values may vary depending on various factors (exercise, weather, disease, etc.) [22], even within captivity as measured in this study. Therefore, the values were separated based on season, sex, weight/age,

Table 2

Comparative hematology and plasma chemistry values (mean \pm SD) between adult and juvenile African rock pythons in rainy season ($P < .05$).

Parameters	Adult	Juvenile	P-value
Number	14	5	
WBC ($10^9/L$)	23.04 \pm 9.48	13.94 \pm 7.52	.071
Heterophils ($10^9/L$)	14.54 \pm 7.12	10.10 \pm 5.81	.230
Lymphocytes ($10^9/L$)	7.94 \pm 3.35	3.64 \pm 2.43	.018
Mono/Azuro ($10^9/L$)	0.38 \pm 0.33	0.04 \pm 0.05	.034
Eosinophils ($10^9/L$)	0.16 \pm 0.19	0.16 \pm 0.24	.969
Basophils ($10^9/L$)	0.02 \pm 0.05	0.00 \pm 0.00	.403
RBC ($10^{12}/L$)	3.07 \pm 0.73	3.05 \pm 0.70	.957
HGB (g/dL)	7.40 \pm 1.54	6.46 \pm 0.71	.213
PCV (%)	21.79 \pm 4.06	19.40 \pm 2.07	.232
MCV (fl)	71.97 \pm 10.35	65.18 \pm 8.69	.209
MCH (pg)	24.51 \pm 2.75	21.68 \pm 2.82	.066
MCHC (g/dL)	33.80 \pm 1.09	33.32 \pm 0.11	.349
ALT (IU/L)	20.54 \pm 13.08	19.78 \pm 10.16	.908
AST (IU/L)	29.16 \pm 17.12	26.64 \pm 16.50	.779
ALP (IU/L)	81.33 \pm 29.59	109.96 \pm 39.35	.106
Uric Acid (mmol/L)	1.14 \pm 0.45	1.54 \pm 0.70	.164
Urea (mmol/L)	1.50 \pm 0.33	2.52 \pm 0.63	.000
Glucose (mmol/L)	2.96 \pm 0.37	2.90 \pm 0.88	.841
Calcium (mmol/L)	1.33 \pm 0.27	1.12 \pm 0.24	.133
Total Protein (g/L)	70.07 \pm 10.51	71.00 \pm 9.35	.864
Albumin (g/L)	45.64 \pm 10.95	50.00 \pm 9.03	.438

Table 3

Comparative hematology and plasma chemistry values (mean \pm SD) between hepatozoon-negative and positive African rock pythons ($P < .05$).

Parameters	Hepatozoon negative	Hepatozoon positive	P-value
Number	9	10	
Prevalence (%)	47.4%	52.6%	
WBC ($10^9/L$)	17.18 \pm 6.86	23.77 \pm 11.14	.144
Heterophils ($10^9/L$)	11.38 \pm 5.67	15.16 \pm 7.76	.247
Lymphocytes ($10^9/L$)	5.39 \pm 2.23	8.08 \pm 4.26	.109
Mono/Azuro ($10^9/L$)	0.20 \pm 0.18	0.37 \pm 0.40	.259
Eosinophils ($10^9/L$)	0.18 \pm 0.23	0.14 \pm 0.16	.650
Basophils ($10^9/L$)	0.02 \pm 0.05	0.01 \pm 0.04	.828
RBC ($10^{12}/L$)	3.03 \pm 0.57	3.10 \pm 0.83	.841
HGB (g/dL)	6.85 \pm 1.26	7.42 \pm 1.55	.393
PCV (%)	20.44 \pm 3.09	21.80 \pm 4.32	.447
MCV (fl)	66.93 \pm 5.60	73.11 \pm 12.62	.194
MCH (pg)	22.71 \pm 1.29	24.72 \pm 3.76	.147
MCHC (g/dL)	33.91 \pm 1.36	33.46 \pm 0.28	.317
ALT (IU/L)	24.72 \pm 16.60	16.40 \pm 3.47	.139
AST (IU/L)	31.96 \pm 18.84	25.39 \pm 14.45	.403
ALP (IU/L)	95.70 \pm 36.27	82.72 \pm 32.10	.419
Uric Acid (mmol/L)	1.24 \pm 0.69	1.26 \pm 0.40	.943
Urea (mmol/L)	1.90 \pm 0.62	1.66 \pm 0.62	.408
Glucose (mmol/L)	3.03 \pm 0.58	2.86 \pm 0.48	.488
Calcium (mmol/L)	1.30 \pm 0.33	1.26 \pm 0.24	.782
Total Protein (g/L)	70.67 \pm 12.45	70.00 \pm 7.79	.889
Albumin (g/L)	49.44 \pm 10.86	44.40 \pm 9.95	.305

hepatozoon presence as shown in Tables 1–3 above. All animals used in this study were captive but wild caught (i.e. none were bred in captivity). This study establishes the first hematological and plasma biochemistry values for the African rock python. Differences in means were assessed for season, hepatozoon infection, age and sex.

Since, it is difficult to apply broad patterns of changes across species, and any inferences drawn should be limited to a particular species and geographic area [23], two major seasons (rainy and dry) in tropical West Africa were assessed for the African rock python. There was no significant statistical difference ($P < .05$) between males and females to any of the parameters measured except calcium 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 while there were significantly higher values obtained for RBC, uric acid, glucose and albumin. These results allow us to conclude that it is important to consider the season of the year when interpreting the reference values in African rock pythons.

Higher statistically significant lymphocytes in the younger snakes could be as a result of more frequent ecdysis which has been assessed by Wallach [24], as younger snakes in this specie molt 2–3 times more often than older snakes. Kakizoe [25] also reported higher lymphocyte values in juvenile loggerhead turtles as compared with adults. Azurophils in this study were also reported as monocytes since Campbell [26], stated that monocytes which have an azurophilic appearance to the cytoplasm are often referred to as azurophils in the literature, the cytochemical and ultrastructural characteristics are often similar to monocytes and therefore should be reported as monocytes rather than as a separate cell type. Therefore leading to our reporting as such. The PCV values (both rainy and dry seasons) of the African rock python in this study were within the normal range reported in most studies ranging between 20% and 40% [24–27] although the mean value was around the lower limit.

Normal blood glucose concentration of most reptiles reported ranges between 3.33 and 5.55 mmol/L [28]. Our findings in this study fall within this range although the mean value is slightly below the lower limit which is subject to marked physiologic variation. Glucose concentrations have been reported to decrease significantly over time in heparinized blood samples; therefore immediate separation of blood cells from the plasma is important [29], our samples were separated almost immediately after collection. Uric acid represents 80%–90% of the total nitrogen excreted by the kidneys [27] and normal blood uric acid concentration in most reptiles is less than 10 mg/dL (594.8 μ mol/L) [26] which is lower than the mean value in this study. This can be due to the carnivorous nature of this reptile as carnivorous reptiles have been reported to have a 1.5- to 2.0-fold increase in uric acid values [27] especially if samples were taken a day after meals [30].

High ALT and alkaline phosphatase (ALP) activity has been reported in the reptilian kidney [31] although significant increases in the plasma activities of these enzymes do not occur with renal disease because most of the enzymes released from damaged renal cells are released in urine, not in plasma [31,32]. The plasma ALP, AST and ALT activity is not considered to be organ specific because activities for these enzymes can be found in many tissues (e.g. liver, skeletal muscles, kidneys, etc.), although could be very helpful in detecting tissue damage especially vital organs [26]. Normal plasma AST activity reported for reptiles is less than 250 IU/L [26] while ALT activity is usually less than 20 IU/L [26], which agrees with the mean values in this study.

The plasma total protein concentration reported for healthy reptiles generally ranges between 30 and 70 g/L [28] which is in agreement with this study although most samples were on the higher limit. Donoghue and Langenberg [33] stated that captive reptiles may exhibit greater plasma total protein concentrations when compared with the same free-living species because of prolonged high-protein diets. Creatinine concentration reported in this study is much lower than reports from [34] who reported normal concentration as 88.4 μ mol/L. Blood creatinine concentration is generally considered to be of poor diagnostic value in the detection of renal disease in reptiles. Although a statistically significant difference was observed between adult and juvenile snakes, with the former having a marked high value.

The study also established classical hematological and plasma chemistry parameters of hepatozoon positive and hepatozoon negative pythons. It was demonstrated that the hemogregarine infection did not statistically change erythrocytic and plasma values; however, considerable change in certain values were detected.

The total WBC count, heterophils and lymphocytes were markedly higher for hepatozoon positive snakes while mean PCV was slightly lower.

Lymphocyte and heterophil counts are cells that have been reported to be associated with parasitic infections and stimulation of the immune system and also suggesting phagocytic response of these cells [35,36], although increases were not statistically significant. Bonadiman et al. [37] reported marked monocytosis along with increased lymphocytes, heterophils and eosinophils in a reptile, the lizard *Ameiva ameiva* naturally infected with hemogregarines, which is in agreement with the findings in this study. Plasma chemistry values were however unaffected by the presence of hemogregarines.

Brown et al. [38] described the parasite-snake association as 'surprisingly benign' and 'sometimes may have only trivial consequences for host fitness in natural populations', adding that 'coevolution weakens or eliminates fitness costs of parasitism'. On the other hand, Madsen et al. [39] concluded that 'only snakes harbouring lower levels of parasitemia were able to survive to old age'. These are conflicting results although the parasite was seen to elicit immune responses and caused adverse hematologic effects in this study although the level of pathogenicity was not ascertained.

Hemoparasites in reptiles are considered apathogenic. Nevertheless, anemia is reported in severe infestation by the malarial hemoparasites (*Plasmodium*), *Haemoproteus*, and *Saurocytozoon* [40], which is in agreement with the present findings with Hemogregarines in this study. Salakij et al. [41] observed the hemolysis of erythrocytes of snakes heavily infested with Hemogregarines and reported associated hemolytic anemia.

Eosinophils and basophils were not increased as reported before in parasitic infections [12]. This may be due to the minute numbers of the leukocytes found on the smears.

Further studies should be conducted in the molecular identification of the hemoparasites found in the African rock python for better identification of these agents.

5. Conclusions

To the authors' knowledge this is the first hematological and plasma chemistry values reported for the African rock python, therefore pilot values reported here can be used to assess baseline health of African rock python in Nigeria and compare these to populations in other parts of Africa. This study also indicates that there is seasonal and hepatozoon infection influence on the hematological and plasma chemistry parameters.

Competing interests

The authors declare no conflict of interests.

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