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Haematologic and Biochemical Parameters of Haemogregarine-infected and Non-infected African Hinge-Back Tortoises in Ibadan, Nigeria

***Adetunji, V. E. and Adeyemo, O. K.**

Department of Veterinary Public Health and Preventive Medicine, Aquatic and Wildlife Medicine Unit, University of Ibadan, Nigeria

* *Author for Correspondence: onize5@yahoo.com*

ABSTRACT

This study was conducted to evaluate haematological and biochemical parameters of haemogregarine-infected (h-infected) and non-infected African hinge-back tortoises in Ibadan, Nigeria. Blood samples were collected from 120 tortoises, of which 70 were *Kinixys belliana* and 50 were *K. homeana*. Stained thin smears were examined for haemogregarines using light microscope. Haematological and biochemical analyses were carried out following standard procedures. A total of 91(75.83 %) tortoises were positive for haemogregarines. Significantly ($P<0.05$) lower values of haematocrit (23.92 %), haemoglobin (5.21g/dl) and mean corpuscular haemoglobin concentration (MCHC) (21.78 %) were recorded for h-infected tortoises with haematocrit (33.29 %), haemoglobin (8.31g/dl) and MCHC (24.96 %). Higher values of white blood cells (WBC) (7.26 x 10^9/L) and lymphocytes (2.71x10^9/L), were observed in h-infected than non-infected with WBC (5.58 x 10^9/L) and lymphocytes (2.15x10^9/L). Higher values of haematocrit and haemaglobin were recorded for *K. Homeana.* Males had higher haematocrit (27.27 %) and WBC (7.09 x 10^9/L) than females with haematocrit (24.35 %) and WBC (6.93 x 10^9/L). Females had higher MCHC, haemoglobin and calcium values than males. The lower values of haematocrit, haemoglobin and MCHC obtained for h-infected tortoises were expected since haemogregarines are usually found intra-erythrocytic in their host thereby destroying affected erythrocytes and causing a decrease in haematocrit value. Higher WBC counts in h-infected tortoises is typical in diseased conditions. The higher level of calcium in female tortoises is due to their reproductive cycle especially vitellogenesis and egg formation. Hypo-proteinaemia recorded in h- infected tortoises was attributed to parasitism. It is concluded that majority of haematological and biochemical analytes showed considerable variations with level of infection status, species and gender.

Keywords: *Kinixys belliana; Kinixys homeana*; Haematology; Plasma biochemistry; Haemogregarine parasites

INTRODUCTION

The African hinge-back tortoises are one of the most vulnerable of all vertebrates, with about 61% reportedly endangered or extinct. This is because most conservationists do not consider turtle diversity of global importance, hence efforts at conserving them is not prioritized (Roll *et al*., 2017). Two species of the African hinge-back tortoises (*Kinixys belliana* and *K. homeana*) are common in Nigeria and are usually found in zoological gardens next to the reptiles mostly(snakes). They are common pets in Nigeria owned by both the rich (especially traditional rulers) and lower income individuals. They are endangered species due to over-exploitation by humans and ravaging by infectious diseases including those caused by haemogregarines (Murphy, 2016).

Haemogregarines are blood parasites affecting reptiles especially the *Kinixys* tortoises and are capable of

destroying affected Red blood Cells (RBCs), resulting in low haematocrit, low RBC counts, low haemoglobin concentration and consequently low oxygen-carrying capacity in affected host (Brown *et al*., 2006).

Haematology, plasma or serum biochemical analyses have become the basis for early disease diagnosis in all species of animals, including man (Hetenyi *et al*., 2016; Dissanayake *et al*., 2017). Haematological parameters are vital for health evaluation in reptiles, and particularly chelonians because they demonstrate few observable clinical signs (Lopez-Olvera *et al.*, 2003).

Haematological and biochemical parameters of chelonians are influenced by many factors, including season, gender, health status, geographical sites, physiological state and reproductive status of the animals (Javanbakht *et al.,* 2013).

The determination of baseline data of blood values in a species is critical in assessing and distinguishing healthy animals from diseased ones (Geffre *et al*., 2009; Nardini *et al*., 2013). The comparative studies of healthy and sick chelonians are germane to conservations and of immense help especially for critically endangered species

(Christopher *et al*., 2003; Diaz-Figueroa, 2005, Joyner *et al*., 2006; Omonona *et al*., 2011). Information on the effect of haemogregarines on the haematologic and biochemical parameters of affected *Kinixys tortoises* in Nigeria is scarce because of overt focus on parasites of domestic animals.

This study was therefore carried out to evaluate the haematological and biochemical parameters of haemogregarine-infected and non-infected African hinge-back tortoises and the effects of species and gender on these parameters in Ibadan, Nigeria.

MATERIALS AND METHODS

Source of Animals

This was a cross-sectional study conducted on 120 adult hinged back tortoises including 70 (35 males and 35 females) *K. belliana* and 50 (21 males and 29 females) *K. homeana* sourced from the central herb/wildlife market at Bode, Molete in Ibadan, Nigeria. The sampled tortoises were supplied by traders in batches of ten (10) per purchase every month. Sampling was done over a period of one year and six months. Only tortoises having a straight carapace length of > 10 cm were sampled in this study for ease of sex determination. Tortoises were housed in a vivarium and provided daily diet of vegetables; water leaves (*Talinum triangulare)* and fruits (mango, pawpaw and pineapples) and given free access to water and direct exposure to sunlight.

Sex Determination

Sexes of the tortoises were differentiated as described by Stuart *et al.* (2004). Adult male tortoise had longer tail, concave plastron and its vent located posterior to the junction of carapace and plastron while the female had relatively shorter tail, flat plastron and its vent located at the junction of carapace and plastron.

Sample Collection

Blood samples (2-mL) were drawn from the sub-carapacial sinus of each tortoise (*Kinixys belliana* and *Kinixys homeana*) according to McArthur *et al*., (2004), using a 25G needle and syringe. Approximately 1ml each of blood was collected into heparinised tubes for haematology and into plain tubes for biochemical studies. All blood samples were processed immediately after collection.

Haematology

Thin smears were prepared by staining according to standard methods (Houwen, 2000; Cook *et al*., 2014; 2015). Slides were stained by first fixing for 10 minutes in methanol and stained with Giemsa's stain (Sigma-Aldrich, Steinheim, Germany) for 30 minutes, rinsed under a running tap, drained, and arranged vertically on a slide rack to dry naturally. They were later screened for evidence of haemogregarine parasitic infection with oil immersion at x100 using Olympus BX41 microscope.

Leucocytes counts were enumerated by counting 200 different leucocytes on peripheral smear proportionately. Total erythrocytes and leukocytes counts were evaluated by the direct method using Natt Herrick's stain (Natt Henrick TICR 1:200 PlusGermany and a Neubauer haemocytometer (Canemco Inc. Canada) according to procedure described by Arikan and Cicek, (2011). Total leukocyte and differential leukocyte counts were evaluated using the techniques described by Stacy *et al*. (2011). Automated cell counts procedure was ruled out because of the nucleated structure of reptile's leucocytes and erythrocytes (Mader 1996).

The haematocrit (packed cell volume) was evaluated using the microhaematocrit method in capillary tubes (NRIS Vitrex Medical, Denmark) and centrifuged at 12,000g for 5 minutes using a haematocrit centrifuge (Surgifield Instrument SH120-1, England), according to? the standard method of International Council for Standardisation in Haematology (ICSH,2001). The PCV was read on a micro-capillary reader. Two measurements were taken and the average was computed for data analysis.

The erythrocyte indices were calculated mathematically with data on RBC count, PCV and haemoglobin concentration and using the following standard formulas for the estimation of mean corpuscular volume (MCV), mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration.

a. $\text{MCV (fL)} = \frac{\text{Packed cell volume}}{\text{Red blood cell counts}}$

b. $\text{MCH (g/dL)} = \frac{\text{Haemoglobin percentage}}{\text{Red cell percentage}}$

c. $\text{MCHC (\%)} = \frac{\text{Haemoglobin percentage}}{\text{PCV}} \times 100$

Biochemical Analyses

The plasma was separated from the whole blood by centrifugation at 10,000g for 5 minutes using **(**Bench top centrifuge Centurion Scientific ESD72A, United Kingdom). Biochemical protein (albumin, globulin and total protein), electrolytes (sodium, calcium, potassium, chloride and phosphorus) and enzymes (Aspartate aminotransferase (AST), Alanine transaminase (ALT) and Alkaline phosphtase (ALP) were evaluated using commercially supplied kits (Randox Laboratories Limited, Country Antrim, United Kingdom) according to manufacturer's instruction. The end products were analysed using spectrophotometer (Spectrumlab 23A, China) at various wavelengths.

Data Analyses

Data was analysed using an online statistical tool, Social Science Statistics, (2020). Results were presented using descriptive statistics. Haematological parameters, plasma biochemical parameters and enzymes activities of haemogregarine infected and non-infected tortoises (*K. belliana* and *K. homeana*) were determined using the student t-test. The level of significance was taken as 5%.

Ethical Statement

The ethical approval for this study was obtained from the University of Ibadan Animal Care and Use Research Ethics Committee (UIACUREC) with approval number: UI ACUR EC/App/10/2016/01

RESULTS

Morphology of the Normal and Infected Erythrocytes

Normal erythrocytes were elliptical-shaped and their nuclei located near the centre of the cell, cytoplasm were light yellowish pink but infected erythrocytes demonstrate shape alteration with lengthening and various abnormal shapes and with sausage or banana-shaped intra-erythrocytic nucleated haemogregarine parasite gamonts seen in the cytoplasm. They had marginal and atrophic nucleus and were larger than non-parasitized erythrocytes. The cytoplasms of the infected erythrocytes were darker than the non-infected erythrocytes (Figure 1).

Out of 120 tortoises (*K. belliana* and *K. homeana*) sampled, 91 (58 *K. belliana* and 33 *K. homeana*) were infected for haemogregarines (h-infected) while 29 (12 *K. belliana* and 17 *K. homeana*) were non-infected. This gave overall infection rate of 75.83% and specie prevalence rates of 82.86 % and 66 % for *K. belliana* and *K. homeana*, respectively.

Haematological and Biochemical Values of Haemogregarine Infected and Non-infected African Hinge-back Tortoises

Significantly ($p < 0.05$) lower values of haematocrit (23.92%), haemoglobin (5.21g/dl), red blood cells ($0.98x10^{12}$L), MCV (244.08fl), MCH (53.16pg) and MCHC (21.78%) were recorded for h-infected tortoises (*K. belliana* and *K. homeana* combined) than noninfected tortoises with haematocrit (33.29%), haemoglobin (8.31g/dl) and red blood cells (1.10×10^{12}L) MCV (302.64fl), MCH (75.54pg) and MCHC(24.96%) (Table 1).

The h-infected tortoises also had significantly higher values for white blood cells (7.26×10^9/L), lymphocytes (2.71×10^9/L) and eosinophils (2.93×10^9/L) than non-infected tortoises with white blood cells ($5.58x\ 10^9$/L), lymphocytes (2.15×10^9/L) and eosinophils ($1.65\ x10^9$/L) (Table 1).

Significantly ($p < 0.05$) lower values of total protein (3.98g/dl), albumin (1.26 mg/dl), globulin (2.72 mg/dl) and AST (46.48 U/L) were recorded for h-infected tortoises than non-infected tortoises with total protein (5.32 g/dl), albumin (1.66 mg/dl), globulin (3.65 mg/dl) and AST (64.42 U/L). Significantly higher value of ALT (30.84 U/L) was recorded for h-infected tortoises than non-infected with 8.24 U/L(Table 2).

Haematological and Biochemical Values of Different species of Haemogregarine Infected and Non-infected African Hinge-back Tortoises

Of the two tortoises species examined in this study, significantly lower values of haematocrit (22.53 %), haemoglobin (5.86 g/dl), heterophils (1.34×10^9/L) and MCV (220.88fl), MCH (57.45pg) and MCHC (26.00 %) were recorded for *K. belliana* species than *K. homeana* with haematocrit (26.01 %), haemaglobin (6.38 g/dl), heterophils ($1.52x10^9$/L) and MCV (250.10fl), MCH (61.35pg) and MCHC (24.53%) (Table 3). The differences in values of other hematological parameters (RBCs, WBCs, lymphocytes and monocytes, between the two tortoise species were not significant.

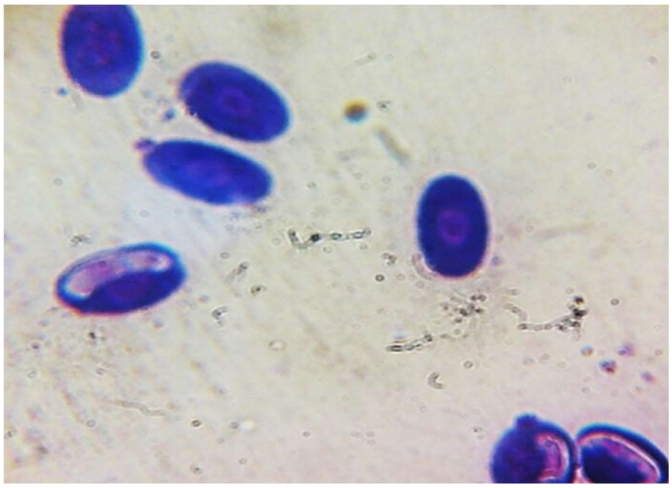

Figure 1: Banana shaped haemogregarine lying within the cytoplasm of infected erythrocytes obtained from *Kinixys belliana*. Giemsa stain x100

Significantly ($p < 0.05$) higher values of sodium (128.21mmol/l), ALT (30.18U/L) and ALP (208U/L) were recorded for *K. belliana* than for *K. homeana with* sodium (121.66mmol/l), ALT (24.24U/L) and ALP (116.08U/L), respectively (Table 4). The differences between total proteins, albumin, globulin, potassium, phosphorus, chloride and AST between *K. belliana* and *K. homeana* were not significant.

Haematological and Biochemical Values of Different Genders of Haemogregarine Infected and Non-infected African Hinge-back Tortoises

Significantly ($p < 0.05$) higher values of haematocrit (25.31% versus 34.00%) were obtained for the males than the females (22.57% versus 32.85%) in both infected and non-infected respectively. Significantly Higher values were obtained for WBC (7.31×10^9/L) in h-infected females than h-infected males (7.21×10^9/L) However, these were significantly higher in noninfected males (6.30×10^9/L) than females (5.13×10^9/L). (Table 1).

Significantly higher values of calcium (2.26 mmol/l) were obtained for the females as compared to males with 1.77 mmol/l). The value of AST (54.38 U/L) was significantly higher in the males than females 47.69 (U/L). The values of total protein, albumin, other plasma electrolytes (sodium, phosphorus, chloride), ALT and ALP were not significant (Table 5).

Table 1: Haematological Parameters of Haemogregarine Infected and Non-infected African Hinge-back Tortoises

Parameters	h-infected			Non-infected		
	Male (N=45)	**Female** (n=46)	**Total (91)**	**Male** (N=11)	**Female** (n=18)	Total (29)
Haematocrit(%) [b]	25.31±5.64	22.57±6.71	23.92±5.98	34.00±6.57	32.85±5.68	33.29±6.20
Haemoglobing/dl [b]	5.30±4.31	5.21±4.80	5.21±4.65	8.45±4.35	8.23±4.52	8.31±4.41
Red blood cells 10^{12}/L) [b]	1.00±0.80	0.96±0.55	0.98±0.66	1.15±0.65	1.07±0.63	1.10±0.60
MCV (fL)[b]	239.2±14.64	235.10±16.68	244.08±15.22	235.65±11.24	307.01±14.28	302.64±12.80
MCH (pg)[b]	53.00±5.60	54.27±6.89	53.16±5.99	73.48±4.56	76.92±6.34	75.54±5.48
MCHC (%) [b]	22.16±2.22	23.18±4.46	21.78±3.86	24.85±3.24	25.05±4.80	24.96±4.20
White blood cell(10^9/L) [a]	7.21±1.09	7.31±0.90	7.26±0.99	6.30±1.01	5.13±1.02	5.58+1.18
Lymphocytes (10^9/L) [a]	2.72±0.46	2.71±0.37	2.71±0.42	2.46±.56	1.97±0.49	2.15±0.56
Eosinophils (10^9/L) [a]	2.93± 0.47	2.94±0.36	2.93±0.42	1.90±0.42	1.50±0.23	1.65±0.37
Monocytes (10^9/L)	0.07±0.02	0.08±0.03	0.08±0.03	0.12±0.06	0.07±0.03	0.09±0.05
Heterophils (10^9/L)	1.45±0.28	1.54±0.34	1.50±0.32	1.77±0.47	1.56±0.45	1.64±0.46
Basophils (10^9/L)	0.04±0.03	0.05±0.04	0.04±0.03	0.05±0.04	0.04±0.03	0.04±0.03

[a]Significantly ($P < 0.05$) higher values obtained for haemogregarine-infected tortoises than non-infected, [b]Significantly lower values obtained for haemogregarine-infected tortoises than non-infected.

Table 2: Biochemical Parameters of Haemogregarine Infected and Non-infected African Hinge-back Tortoises

Parameters	h-infected		Non-infected	
	Male (N=45)	**Female** (n=46)	**Male** (N=11)	**Female** (n=18)
Total protein (g/dl) [b]	3.95±0.64	4.00±0.71	4.54±0.57	5.80±0.68
Albumin (mg/dl) [b]	1.28 ±0.31	1.25±0.38	1.36±0.35	1.79±0.52
Globulin (mg/dl) [b]	2.68 ±0.57	2.75±0.55	3.18±0.35	4.01±0.63
Sodium (mmol/l)	125.84 ±26.63	125.04±15.18	125.80±21.79	125.17 ±20.07
Calcium (mmol/l)	2.02±0.91	2.22±0.82	2.11±0.06	2.13±0.14
Potassium (mmol/l)	5.17 ± 1.11	5.19 ±1.03	4.91±2.08	4.96±2.14
Chloride (mmol/l)	100.45±13.69	105.81±15.65	95.42±16.61	107.22±20.58
Phosphorus (mmol/l)	7.32 ±2.78	6.47±2.22	5.46±2.07	7.14±3.75
Aspartate aminotransferase (AST) (U/L) [b]	45.38±11.84	47.55±16.82	91.22±20.34	48.05±18.07
Alanine transaminase (ALT) (U/L) [a]	26.56±10.70	35.02±16.55	9.44±20.28	7.50±18.92
Alkaline phosphatase (ALP) (U/L)	148.86±32.67	155.92±32.50	152.89±16.54	145.06±14.93

[a]Significantly ($P < 0.05$) higher values obtained for haemogregarine-infected tortoises than non-infected; [b]Significantly lower values obtained for haemogregarine-infected tortoises than non-infected.

DISCUSSION

Morphology of infected erythrocytes reported in this study, is similar to observations in a previous study (Knotkova *et al.,* 2005). The lower values of haematocrit, haemoglobin and erythrocyte indices (MCV, MCH and MCHC) obtained in infected tortoises in this study closely mirrored the findings of Knotkova *et al.* (2005) which reported lower haemoglobin in Bornean river turtles (*Orlitia borneensis*) that were infected with haemogregarine parasites than the non-infected. Aikindi and Mahmoud, (2002) also reported similar lower values of haemoglobin and MCHC in a group of density green turtle that were exposed to petroleum hydrocarbon pollution. Thrall *et al*. (2004) and Stacy *et al*. (2011) also reported lower haematocrit, haemoglobin concentration and RBC counts in reptiles infected with *Haemogregarine stepnowi* than the non-infected. A reduction in MCHC below 30% has been identified as an indication of anaemia in majority of cases (Bomford *et al*., 1974). Knotkova *et al.* (2005) reported that intra-erythrocytic haemogregarines were linked to anaemia, basophilia and eosinophilia in affected reptiles.

This is to be expected since haemogregarine parasites infect and impaired the erythrocytes and consequently decreased their oxygen-carrying capacities.

The higher value of white blood cells of haemogregarine infected tortoises obtained in this study is also similar to the findings of Knotkova *et al.* (2005) who observed increased values of white blood cells and lymphocytes in infected *Orlitia borneesis.* These parameters reduced significantly after treatment.

The white blood cells including lymphocytes, monocytes and heterophils are cells of the immune system (Arikan and Cicek, 2014) which increased when an animal body is challenged by a disease hence higher values were obtained in infected tortoises.

Table 3: Haematological Parameters of Haemogregarine Infected and Non-infected African Hinge-back Tortoises of Different Species

Parameters	***K. belliana***		***K. homeana***	
	H-infected (N=58)	**Non-infected** (n=12)	**H-infected** (n=33)	**Non-infected** (n=17)
Haematocrit (%) [b]	22.40±6.91	32.84±7.72	22.52±6.65	32.77±5.80
Heamoglobin (g/dl) [b]	5.35±2.56	8.35±2.71	5.48±1.32	8.13±1.50
Red blood Cell (10^{12}/L)	1.00±0.65	1.09±0.64	0.97±0.89	1.17±0.70
MCV (fl) [b]	224.00±14.42	310.46±12.42	232.16±18.64	280.09±14.86
MCH (pg) [b]	53.50±6.34	76.61±8.45	56.49±6.68	69.49±7.24
MCHC (%) [a]	23.88±4.02	24.67±6.00	24.33±4.46	24.81±5.62
White blood cell (10^{9}/L) [a]	7.33±1.03	5.61±1.09	7.14±0.93	5.55±1.27
Lymphocytes (10^{9}/L) [a]	2.76±0.45	1.74±0.36	2.63±0.34	2.26±0.65
Eosinophils (10^{9}/L) [a]	2.97±0.45	0.64±0.83	2.86±0.34	1.68±0.42
Monocytes (10^{9}/L)	0.08±0.02	1.24±0.90	0.08±0.04	0.07±0.04
Heterophils (10^{9}/L)	1.48±0.22	0.67±0.95	1.52±0.40	1.51±0.43
Basophils (10^{9}/L)	0.05±0.04	1.06±0.77	0.04±0.03	0.04±0.03

[a]Significantly ($P < 0.05$) higher values obtained for *K. belliana* than *K. homeana*, [b]Significant lower values obtained for *K. belliana* than *K. homeana.*

The hypo-proteinaemia (Table 3) recorded in this study in haemogregarine infected tortoises than non-infected, is similar to the observation by Knotkova *et al.,* 2005 in *Orlitia borneesis*. This was attributed to the effects of parasitism among others.

The differences observed in haematological and biochemica l values of *K. belliana* and *K. homeana* is similar to the differences observed in tortoises of the same genus but belonging to different species such as desert tortoise(*Gopher us agassizii*) and gopherus tortoise (*Gopherus polyphemus*) (Dickinson *et al.,* 2002) and Spur-thigh tortoise (*Testudo graeca*) and hermann's tortoise (*Testudo hermanni*) (Tosunoglu *et al.*, 2005). This could be classified as specie differences and which could also be due to such other factors as gender, age, pregnancy, physical exercise, weather, stress, altitude and captivity. Tosunoglu *et al*, (2005) attributed the different sizes of erythrocytes and haematological values in different reptile species to evolutionary history.

Higher values of PCV and RBCs as obtained in the males as compared with females in this study, is similar to what obtains in Aldabra giant tortoises (*Geochelone gigantea*) (Hart *et al.,* 1991), in Desert tortoise (*Gopherus agassizii*) (Dickinson *et al.,* 2002), in Geometric tortoise (*Psammobates geometricus*) in summer and autumn (Walton, 2002) and in radiated tortoise (*Geochelone radiata*) (Zaias *et al.*, 2006). This has been attributed to the erythropoietic stimulating effects of androgens on the bone marrow in the males (Walton, 2002). In contrast, Hamooda *et al*., 2014 reported no significant differences in the values of erythrocytic indices between male and female of adult *Testudo graeca* from Benghazi Province of Libya.

The higher values obtained for calcium in female tortoises than males in this study (Table 5) is similar to the observations of elevated Ca levels in females of free-range desert tortoise (*Gopherus agassizii*) (Dickinson *et al.*, 2002) and in hermann's tortoise (*Testudo hermanni*) (Andreani *et al.,* 2014). This has been attributed to the reproductive cycle especially vitellogenesis and egg formation in female tortoises (Dickinson *et al.*, 2002).

Table 4: Biochemical Parameters of Haemogregarine Infected and Non-infected African Hinge-back Tortoises of Different Species

Parameters	***K. belliana***		***K. homeana***	
	H-infected (N=58)	**Non-infected** (n=12)	**H-infected** (n=33)	**Non-infected** (n=17)
Total protein (g/dl)	4.14±2.55	5.37±2.81	3.69±0.64	5.28±0.87
Albumin (mg/dl)	1.31±0.76	1.65±0.77	1.18±0.82	1.67±0.90
Globulin (mg/dl)	2.83±0.43	3.73±0.56	2.51±0.54	3.61±0.70
Sodium (mmol/l) [a]	128.88±24.60	124.99±25.89	119.39±42.40	126.08±34.80
Calcium (mmol/l)	2.01±0.73	2.10±0.90	1.96±0.55	2.10±0.80
Potassium (mmol/l)	5.12±2.00	4.94±2.40	5.23±2.43	4.94±2.80
Chloride (mmol/l)	93.63±15.70	105.96±16.40	95.74±12.80	100.48±14.68
Phosphorus (mmol/l)	6.93±2.40	6.30±4.00	6.82±3.78	6.65±4.80
Aspartate aminotransferase (AST) (U/L)	47.45±8.80	66.37±10.40	44.75±12.10	63.06±13.45
Alanine transaminase (ALT) (U/L) [a]	34.50±8.80	9.32±4.10	32.88±8.60	7.47±10.80
Alkaline phosphatase (ALP) (U/L) [a]	221.28±40.80	148.07±30.80	99.64±25.90	148.00±30.46

[a]Significantly ($P < 0.05$) higher values obtained for *K. belliana* than *K. homeana.*

Table 5: Biochemical Parameters of Haemogregarine Infected and Non-infected African Hinge-back Tortoises of Different Genders

Parameters	Males		Females	
	H-infected (n=45)	**Non-infected** (n=11)	**H-infected** (n=46)	**Non-infected** (n=18)
Total protein (g/dl)	3.95±1.64	4.54±2.97	4.00±2.37	5.80±3.20
Albumin (mg/dl)	1.28±0.31	1.46±0.97	1.25±1.00	1.79±0.88
Globulin (mg/dl)	2.67±0.58	3.08±0.13	2.75±0.19	4.01±0.09
Sodium (mmol/l)	125.84±26.63	125.80±30.79	125.04±30.93	125.17±28.13
Calcium (mmol/l) [b]	1.76±0.91	1.81±0.34	2.22±0.83	2.38±0.95
Potassium (mmol/l)	3.10±1.11	4.91±2.16	3.14±1.64	4.96±1.67
Chloride (mmol/l)	97.37±13.69	95.43±15.45	91.09±31.89	107.22±22.74
Phosphorus (mmol/l)	7.32±2.78	5.46±2.42	6.47±3.89	7.14±1.46
Aspartate aminotransferase (AST) (U/L) [a]	45.37±11.62	91.22±32.48	47.55±13.44	48.06±11.46
Alanine transaminase (ALT) (U/L)	32.78±10.23	9.44±7.45	35.02±11.25	7.50±1.60
Alkaline phosphatase (ALP) (U/L)	160.65±35.11	152.89±25.06	165.92±24.92	145.06±23.02

[a]Significantly ($P < 0.05$) higher values obtained for male tortoises than females. [b]Significantly lower values obtained for male tortoises than females.

The higher values AST in males than females obtained in this study is similar to the reports in male desert tortoises (*Gopherus agassizii*) (Dickinson *et al.*, 2002) and in hermanni tortoises (*Testudo hermanni*) (Andreani *et al.,* 2014). This has been attributed to increased activity and aggressive behaviour associated with mating or fighting which is very common in the males (Dickinson *et al.*, 2002). AST has been observed to be non- organ-specific and elevated AST may be associated with tissue damage, toxin injection, inflammation and various metabolic disorders (Wilkinson, 2004).

Conclusion

The results of this study showed that majority of haematological and biochemical analytes show considerable variations with level of infection status, species and gender. The reference ranges defined in the present study for haematologic and biochemical blood values can be considered a useful tool for evaluating tortoise health status. It is concluded that routine screening for haemogregarines and evaluation of their effects on the haematological and biochemical parameters in *Kinixys* tortoises is therefore recommended to promote their conservation.

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Conflict of Interest

The authors declare that they do not have any conflict of interest.

Authors Contributions

AVE designed the research. AVE and AOK carried out the research. Both authors were involved in the analysis of samples, and in writing and proof reading of the manuscript.

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