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Full Length Research Paper

Haematology and prevalence of blood parasites of the common frog (*Rana temporaria*) in the tropical environment

Omonona A. O.* and Ekpenko V.

Department of Wildlife and Fisheries Management, University of Ibadan, Nigeria

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A total of 116 adult frogs were captured from Moniya area of Ibadan and University of Ibadan fish farm, Oyo State of Nigeria between 31st March and 8th June 2009 and investigated for blood parasites by microscopic examination of stained blood films. Some haematological parameters were estimated. The mean packed cell volume, haemoglobin, red blood cells, white blood cells, platelet count, mean cell volume, mean cell haemoglobin concentration and mean cell haemoglobin of the samples from the low rain forest area of the south Western Nigeria were 26.90 and 29.67%, 8.85 and 9.83 g/dl, 2.71 and 2.73 × 10^6 μl^{-1}, 15618.97 and 16704.31 × 10^3 μl^{-1}, 173344.83 and 163124.14 ×10^9 μl^{-1}, 102.83 and 117.67 fl, 32.95 and 33.80 g/dl, and 33.87 and 38.77 pg. Only haemoflagellate from the genus *Trypanosoma* was identified with different morphological forms which are attributed to *Trypanosoma rotatorium* complex. Prevalence of infection in the two study sites were estimated as 32.8 and 29.3%, respectively. The infected frogs had double nucleated red blood cells.

Key words: Haematology, common frog, *Rana temporaria*, blood parasites, Ibadan.

INTRODUCTION

Frogs are transitional animals that live partly in water and on land, striking a balance between the two environments. They belong to the class of vertebrates known as amphibians. Amphibians are thought to be ancestors of all reptiles, birds and mammals. They are found throughout the world (Ajit, 2008) and disease has been implicated as a factor in the decline of amphibian populations worldwide (Blaustein et al., 1994b, Laurance et al., 1996, berger et al., 1998, Daszak, 2000, Kiesecker et al., 2001) as have other factors including habitat loss and fragmentation, che-mical pollution, climate change, introduction of exotic species, increased ultraviolet radiation, and natural pollution fluctuations (Pechmann et al., 1991, Blausstein et al., 1994a; Knapp and Matthews, 2000). Haematology plays an important role in the diagnostic evaluation of animals (Campbell, 1998). Various hematological studies have been carried out on various *Rana* species, and these are mostly performed on the blood cell counts (Kaplan 1951, 1952; Hutchinson and Szarski, 1965; Arıkan, 1989) and measurements (Atatür et al., 1999; Arıkan et al., 2001) and few researchers have examined hematological parameters such as the blood volume and the hematocrit value (Prosser and Weinstein, 1950).

Disease and health problems are one of the challenges confronting frog culture and these occur in different forms. Frogs are affected by bacteria, viral organisms, haemoparasites, protozoans and worms. Wide distribution of blood parasites hosted by amphibians has been reported (Bardsley and Harmsen, 1973; Levine and Nye 1977; Ray, 1983; Barta and Desser, 1984). *Karyolysus sonomae*, *Haemogregarina boyli,* and *Trypanosoma boyli* from *Rana boylii* was reported by Lehmann (1959 a, b, c); *Haemogregarina aurora,* was reported from *Rana aurora* (Lehmann, 1960); *Lankesterella* sp. and a *Trypanosoma* sp. were detected by Stenberg and Bowerma (2008) in *Rana pretiosa;* Žičkus (2002) reported the presence of *Trypanosoma rotatorium* in *Rana esculenta-lessonae, Rana temporaria, Bufo bufo,* and *Bufo viridis*. The aim of this study is to

*Corresponding author. E-mail: aomonona@yahoo.co.uk Tel: +2348037258481.

determine the haematological parameters and blood parasites of common frogs in some parts of Ibadan city, as a case study (Moniya and University of Ibadan fish farm), located in the south-western part of Nigeria.

METHODOLOGY

Study area

This study was carried out in Ibadan Metropolis cutting across two local Governments (Ibadan North, and Akinyele) of Oyo State. Ibadan located on latitude 7°21` and 3°54`E. This area is located within the tropical rainforest (high forest) (Wikipedia, 2010).

Experimental animals

Adult frogs (116) were captured within the months of March and June 2009, being the months with average rainfall in the rainy season, from Moniya area of Ibadan and University of Ibadan fish farm, in Oyo State of Nigeria.

Sample collection

Blood was collected from the heart ventricle of individual frog using 5 ml syringe. The blood samples were collected in test tubes containing 1 mg of the anticoagulant, disodium salt of EDTA (Ethylene diamine tetra acetatic acid).

Haematological examination

A drop of the blood was spread on a dry slide, air-dried and fixed in absolute methanol for 3 min before Giemsa –Wright's stain was applied and photomicrograph of the observed blood cells was then taken with camera under light microscope according to Siripan et al. (2008). The haematological parameters determined were haemoglobin (Hb) concentration, red blood cell count (RBC), white blood cell count (WBC), platelet counts and packed cell volume (PCV), while mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), mean cell volume (MCV) were calculated.

Red blood cell (RBC) and white blood cells (WBC) were counted using haemocytometer (MAFF, 1984). Platelet counts were done using Rees and Ecker's method (MAFF, 1984). Packed cell volume (PCV) was determined using the microhaematocrit centrifuge and the reader (MAFF, 1984). Haemoglobin (Hb) concentration was measured by the cyanmethaemoglobin method (Olayemi et al., 2006). From the values obtained, the haematometric indicies [mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)] were calculated (Jain, 1986).

The thin films of blood made on the slides were examined under the microscope for the presence of living trypanosomes (Southworth et al., 1968) 'SPSS' (version 10) statistical package programme was used to estimate averages, while parametric t-test (Arserim and Mermer, 2008) was used for comparism of means and significance ($P \leq 0.05$).

RESULTS

The mean packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), platelets count in University of Ibadan (UI) fish farm and Moniya Ibadan are shown on Table 1. No significant differences existed between the two locations in terms of PCV ($t = 2.102$, $df = 114$, $P = 0.038$); haemoglobin concentration ($t = 2.212$, $df = 114$, $P = 0.029$); RBC count ($t = 0.096$, $df = 114$, $P = 0.924$); WBC count ($t = 1.332$, $df = 114$, $P = 0.185$); and platelet count ($t = 0.737$, $df = 114$, $P = 0.463$). The mean cell volume (MCV) and mean corpuscular haemoglobin (MCH) were estimated to be (102.83 ± 2.44 and 117.67±4.09) fentolitres; and (33.87±0.81 and 38.77±1.27) picograms in UI farm and Moniya, respectively. There were significant differences in the MCV ($t = 3.114$, $df = 114$, $P = 0.002$) and MCH value ($t = 3.245$, $df = 114$, $P = 0.002$) between the two locations. The mean corpuscular haemoglobin concentration (MCHC) was (32.93 ± 0.12 and 33.08 ± 0.29) g/dl in UI farm and Moniya, respectively. No significant difference occurred between the two locations in terms of MCHC ($t = o.421$, $df = 114$, $P = 0.675$).

The lymphocytes are spherical cells; their cytoplasm is stained as light grey while the nucleus was stained dark purple with Giemsa stain. The nucleus is as large as to fill almost the whole cell whereas the cytoplasm was observed as thin ring. Although large lymphocytes look like monocytes in shape, they are distinguished easily due to their nuclei shape and staining properties. Monocytes are also spherical in shape and their cytoplasm is stained pinkish while the nuclei are dark purple. The chromatin structure is not as dense as that of the lymphocytes. The shape of the red blood cells (RBCs) of *R. temporaria* is ovoid, like other amphibians. The nuclei are also ovoid and oriented centrally. Their long axis is located in parallel to long cell axis. The stained red blood cells appeared light grey while the nuclei are dark purple with Giemsa's stain. Monocytes have kidney-shaped nuclei that are located on one side of the cell. Eosinophiles are also spherical and have some large pinkish and shiny granules in their cytoplasm that can be seen easily with Giemsa's stain. Their nuclei are segmented or lobed.

Only haemoflagellates from the genus *Trypanosoma* were observed. Different morphological forms of trypanosome are shown in Figure 1; this was attributed to the *Trypanosoma rotatorium* Mayer, 1843 complex. The RBCs of infected frogs have double nuclei (Figure 2). The prevalence of haemoparasite (*T. rotatorium*) infection in *R. temporaria* in UI fish farm and Moniya Ibadan is presented on Table 2. Frogs from UI fish farm had a higher percentage prevalence of infection (32.8%) than frogs from Moniya (29.3%). Most of the frogs from both sites are free from the parasite.

The comparison of mean values of the haematological parameters of frogs that were positive and negative for parasitaemia in UI fish farm is shown on Table 3. The mean PCV values, haemoglobin, red blood cells, white blood cells, and platelets count of frogs that were positive and negative for parasitaemia were found to be 26.53 ± 1.92 and 27.08 ± 1.15%; 8.63 ± 0.61 and 8.96 ± 0.38 g/dl;

Table 1. Haematological parameters of *R. temporaria* in UI fish farm and Moniya, Ibadan,

Parameter	Provenances of frog	N	Minimum	Maximum	Mean ± SEM
PCV (%)	UI fish farm	58	13.00	43.00	26.90 ± 0.99
	Moniya	58	14.00	44.00	29.67 ± 0.88
Hb (g/dl)	UI fish farm	58	4.40	14.40	8.85 ± 0.32
	Moniya	58	3.70	14.60	9.83 ± 0.30
RBCs(× 10^6 cell/µl)	UI fish farm	58	1.03	4.31	2.71 ±0.12
	Moniya	58	1.29	4.66	2.73 ± 0.13
WBCs (× 10^3 cell/µl)	UI fish farm	58	5950.00	26400.00	15618.97± 618.31
	Moniya	58	5500.00	25600.00	16704.31± 530.23
Platelets count (× 10^9 cell/µl)	UI fish farm	58	78000.00	336000.00	173344.83± 9004.35
	Moniya	58	28200.00	380000.00	163124.14 ± 10557.17
MCV (fl)	UI fish farm	58	71.60	174.80	102.83 ± 2.44
	Moniya	58	70.80	214.30	117.67 ± 4.09
MCHC (g/dl)	UI fish farm	58	30.50	35.30	32.95 ± 0.12
	Moniya	58	26.40	42.70	33.80 ± 0.2
MCH (pg)	UI fish farm	58	23.10	55.30	33.87 ± 0.81
	Moniya	58	20.90	67.10	38.77 ± 1.27

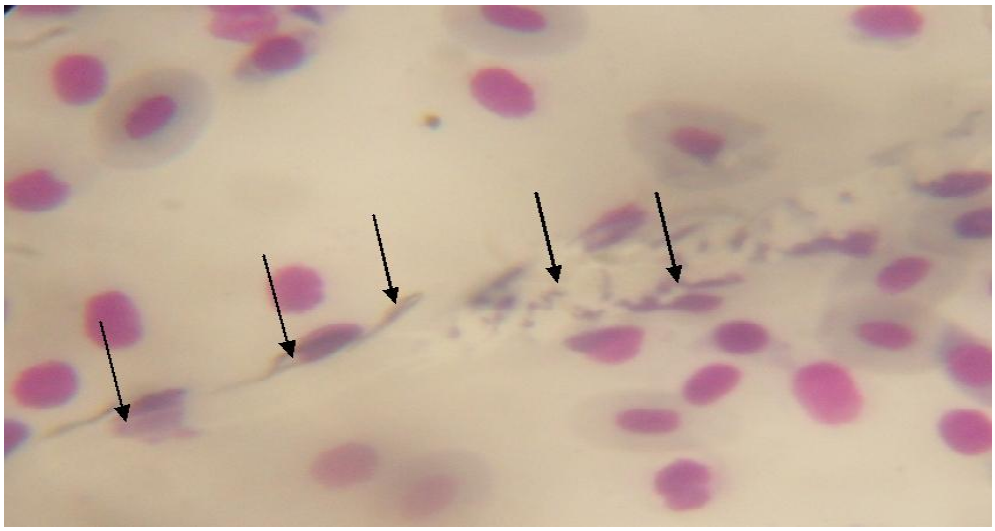

Figure 1. Photomicrograph of blood smear showing inter erythrocytic trypanosomes at different stages of development (Giemsa × 1600)

2.76 ± 0.23 and 2.69 ± 0.14 × 10^6 μl^{-1}; 14610.53 ± 1240.98 and 16110.26 ± 691.41 × 10^3 μl^{-1}; 187368.42 ± 15614.36 and 166512.82 ± 10993.37 × 10^9 μl^{-1} respectively (Table 3). There are no significant differences between parasitized and non-parasitized frogs in terms of PCV (t = 0.260, df = 56, P = 0.796); haemoglobin concentration (t = 0.486, df = 56, P = 0.807); red blood cells count (t = 0.283, df = 56, P = 0.778); white blood cells count (t = 1.141, df = 56, P = 0.259); and platelet count (t = 1.089, df = 56, P = 0.281).

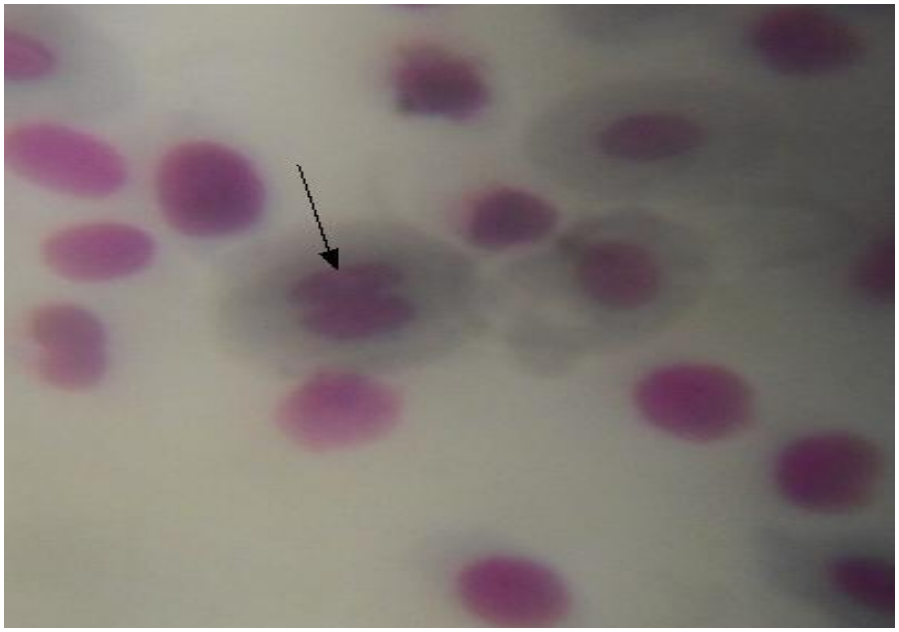

Figure 2. The photomicrograph of the blood smears of infected frog with a double nucleated red blood cell (Giemsa × 1600)

Table 2. Prevalence of *Trypanosoma rotatorium* in *R. temporaria* in both locations.

Location	No. of frogs examined	No. of frogs infected	Percentage prevalence (%)
UI fish farm	58	19	32.8
Moniya	58	17	29.3

The mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) estimated in parasitized and non parasitized frogs were 99.35 ± 3.93 and 104.52 ± 3.09 fl; 32.65 ± 0.23 and 33.10 ± 0.14 g/dl; and 32.46 ± 1.34 and 34.56 ± 1.00 pg, respectively (Table 3). There were no significant differences between them in terms of MCV (t = 0.993, df = 56, P = 0.325); MCHC (t = 1.788, df = 56, P = 0.079); and MCH (t = 1.220, df = 56, P = 0.228).

The comparison of mean values of haematological parameter of frogs positive and negative for parasitaemia in Moniya, Ibadan is shown on Table 4. The mean PCV values, haemoglobin, red blood cells, white blood cells, and platelets count, for frogs positive and negative for parasitaemia in Moniya Ibadan were found to be 31.82 ± 1.80 and 28.78 ±. 98%; 10.64 ± 0.60 and 9.49 ± 0.34) g/dl; (2.83 ± 0.27 and 2.68 ± 0.15) × 10^6 μl^{-1}; 16558.82 ± 1168.51 and 16764.63 ± 583.08 × 10^3 μl^{-1}; (152894.12 ±.21550.03 and 167365.85 ±. 12087.88) × 10^9 μl^{-1}, respectively. There were no significant differences between the parasitized and non-parasitized frogs in Moniya in terms of PCV (t = 1.598, df = 56, P = 0.116); haemoglobin (t = 1.766, df = 56, P = 0.083); RBC (t = 0.507, df = 56, P = 0.614); WBC (t = 0.175, df = 56, P = 0.862); Platelet (t = 0.621, df = 56, P = 0.537); MCV (t = 0.868, df = 56, P = 0.389); MCHC (t = 0.880, df = 56, P = 0.293); and MCH (t = 1.063, df = 56, P = 0.293) respectively.

DISCUSSIONS

Ischenko (1978) in his study on the lake Sevan population of *R. macrocnemis* reported the mean packed cell volume as 53% in individuals with vertebral stripe and 43% in individuals without the vertebral stripe. This value was 26.94% in *Glyphogloosus molossus* (Siripan, 2007). These values varied from those reported in this study.

The haemoglobin value in *G. molossus* was reported to be 8.62 g/dl (Siripan, 2007), in *Rana pipiens*, it was reported to be 6.75 g/dl (Rouf, 1969). These values are not so close to the values recorded in this study for *R. temporaria* in University of Ibadan Fish Farm (8.85 g/dl) and Moniya, Ibadan (9.83 g/dl) this variation might be due the differences in species, nutritional conditions, location and some physiological conditions (Arıkan, 1989, Rouf, 1969, Wojtaszek and Adamowicz, 2003).

Table 3. Haematological parameters of *R. temporaria* positive and negative for parasitaemia in UI fish farm.

Parameter	Grouping variable	N	Mean ± SEM
PCV (%)	Positive	19	26.53± 1.92
	Negative	39	27.08±.15
Hb (g/dl)	Positive	19	8.63±0.61
	Negative	39	8.96 ± 0.38
RBCs (× 10^6 cell/µl)	Positive	19	2.76 ± 0.23
	Negative	39	2.69 ± 0.14
WBCs (× 10^3 cell/µl)	Positive	19	14610.53± 1240.98
	Negative	39	16110.26 ± 691.41
Platelets count (× 10^9 cell/µl)	Positive	19	187368.42± 15614.36
	Negative	39	166512.82± 10993.37
MCV (fl)	Positive	19	99.35± 3.93
	Negative	39	104.52 ± 3.09
MCHC (g/dl)	Positive	19	32.65± 0.23
	Negative	39	33.10± 0.14
MCH (pg)	Positive	19	32.46± 1.34
	Negative	39	34.56± 1.00

The red blood cells in 1 mm^3 of blood varies between 500,000 and 1,500,000 on average in anurans (Glomski et al., 1997). Alder and Huber (1923) reported the RBC count in *R. temporaria*. The mean RBC was found to be 1,040,000 in *G. molossus* (Siripan, 2007). In this study, mean RBC count was calculated to be 2.71 × 10^6 μl^{-1} in UI farm and 2.73 × 10^6 μl^{-1} in Moniya, Ibadan. There was no significant difference in the RBC of frogs in this study. This finding is not in accordance with Hutchison and Szarski (1965) who noted that the differences in the RBC counts are due to the varied geographical locations.

The white blood cells (WBCs) count varies depending on species, season, sex, nutritional conditions and some physiological conditions (e.g. diseases and breeding) (Wojtaszek and Adamowiez, 2003; Arikan, 1989; Rouf, 1969). This value was 1,370 in Balloon frogs (*G. molossus*) (Siripan, 2007). According to Suha and Ahmet (2008), WBC count was found to be 2600 to 5200 in *Rana macrocnemis,* specie of frog. This range is quite low when compared to those recorded from the two locations in this study.

The platelet count has been examined by few researchers. The platelet count was found to be 31,069 in *R. macrocnemis* (Suha, 2007) and 880,000 in *R. pipiens* (Kaplan, 1951, 1952). The value found by Kaplan (1951, 1952) is 5 times higher than those detected in this work, which might be as a result of species and geographical location differences because no location-based difference was observed in the platelet count in *R. temporaria* in the two sites studied in this work.

The mean corpuscular volume (MCV) was calculated to be 409.29 fl in *G. molossus* (Siripan, 2007); 694.54 fl in *R. macrocnemis* (Suha, 2007). These values are far greater than those determined in this work. The mean corpuscular haemoglobin concentration (MCHC) was calculated to be 32.96% in *G. molossus* (Siripan, 2007) and 251.43% in *R. macrocnemis* (Suha, 2007). In *Bombina bombina*, the MCHC was given as 36.78% (Wojtaszek and Adamowicz, 2003). The value found by Siripan (2007) is in accordance with the values calculated in *R. temporaria* in the two locations under this work. The mean corpuscular haemoglobin (MCH) was 167.88 pg in *R. macronemis* (Suha, 2007) and 142.11 pg in *G. Molossus* (Siripan, 2007). These values are higher than those found in UI fish farm and Moniya, Ibadan.

According to Desser (1993), sporozoan haemoparasites from the families Lankesterellidae and Haemogregarina were reported in amphibians. The species *haemogregarina, hepatozoon, lankesterella and schellackia* were found in frogs (Desser, 1993). Barta and Desser (1989) recorded dactylosomatids (Dactylosomatidae) in frogs in North America. During this study, other haemoparasites discovered by other researchers were not found in this study because only

Table 4. Haematological parameters of *R. temporaria* positive and negative for parasitaemia in Moniya, Ibadan.

Parameter	Grouping variable	N	Mean ± SEM
PCV (%)	Positive	17	31.82 ± 1.80
	Negative	41	28.78 ± 0.98
Hb (g/dl)	Positive	17	10.64 ± 0.60
	Negative	41	9.49 ± 0.34
RBCs ($\times 10^6$ cell/µl)	Positive	17	2.83 ± 0.27
	Negative	41	2.68 ± 0.15
WBCs($\times 10^3$ cell/µl)	Positive	17	16558.82± 1168.51
	Negative	41	16764.63 ± 583.08
Platelets count ($\times 10^9$ cell/µl)	Positive	17	152894.12± 21550.03
	Negative	41	167365.85± 12087.88
MCV (fl)	Positive	17	123.19 ± 8.49
	Negative	41	115.38 ± 4.62
MCHC (g/dl)	Positive	17	33.48 ± 0.68
	Negative	41	32.92 ±0.31
MCH (pg)	Positive	17	40.86 ± 2.49
	Negative	41	37.90 ± 1.47

Trypanosomes were found. The double nucleated red blood cells observed in frogs infected might be due to the effect and action of the parasites on the blood cells.

The percentage prevalence of infection was found to be higher in UI farm (32.8%) than in Moniya Ibadan (29.3%). This could be attributed to the difference in environmental conditions of the two locations. Frogs in UI farm were captured from ponds containing high level of water while those from Moniya were taken from muddy spots containing little water and from their hiding sites that are moist.

In conclusion, haematological parameters and blood parasites of *R. temporaria* observed in UI fish farm and Moniya area of Ibadan appear to be similar to those of other anuran species from the temperate zones of the world and other parts of Africa. No location-based differences were observed in terms of the mean RBC, WBC, platelet count and MCHC. The slight differences in the prevalence of blood parasites between UI fish farm and Moniya area may be due to differences in environmental conditions, nutritional patterns and also their niche in each of the two locations. Although small differences were observed between parasitized and non-parasitized frogs in both locations in terms of haematological values, they were found to be statistically insignificant.

This study provides information on the blood picture of the anurans especially *R. temporaria* in wet zones of the tropics, which can serve as a means of comparison for other studies on this species in the aquatic ecosystem.

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