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Blood Parasites of Toads (*Amietophrynus regularis*) in Al-Sorojia and Jebel Aulia, Khartoum State, Sudan

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طفيليات الدم من الضفادع (*Amietophrynus regularis*) في السروجية وجبل أولياء، ولاية الخرطوم، السودان

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Abstract

The present study was conducted to detect and identify blood parasites infecting toads *Amietophrynus* (*Bufo*) *regulariss* and find out the association between the infection of the parasites and host factors sex, length, body weight, and age. One hundred and twenty *Amietophyrnus* (*Bufo*) *regularis* (99 males and 21 females) were collected from May to August 2016 in two localities in Khartoum State (Al-Sorojia and Jebel Aulia). They were examined for blood parasites using blood films stained in Giemsa's. A total of 77 (64.16%) of specimens were infected by 880 parasitic protozoa made-up of 879 (99.89%). Apicomplexa composed of *Haemogregina* spp. (89.66%), *Hepatozoon* spp. (10.22%), and 1 (0.11%) Kinteoplastides composed of *Trypanosoma* spp., while 43 (35.83%) were uninfected. No nematodes were recovered. The infection rate based on sex shows that females significantly (χ^2 =12.520, p≤0.05) have a higher rate of infection than males. For the toad maturity, there was a significant difference in the infection rate between the mature group and the immature (χ^2 =19.471, p=0.003). The infection rate increases with decreasing of weight and length with a negative correlation (r_s = –0.022, p = 0.814 and r_s = –0.004, p = 0.966, respectively). This study showed a high level of parasitic infection in African common toads in Khartoum State which contributed to the decline in amphibian populations. Further research on amphibian parasitism and its threats to human health is warranted.

Keywords: *Amietophrynus regularis, Al-Sorojia, Blood Parasites, Jebel Aulia, Toads, Sudan.*

الملخص

أجريت هذه الدراسة للكشف والتعرف على طفيليات الدم التي تصيب الضفادع *Amietophyrnus* (*Bufo*) *regularis* ومعرفة العلاقة بين الإصابة بالطفيليات وعوامل المضيف الجنس والعمر ووزن الجسم والطول. تم جمع مائة وعشرين من الضفادع (99 ذكور و21 إناث) خلال الفترة من مايو إلى أغسطس 2016 من منطقتين في ولاية الخرطوم (السروجية وجبل أولياء) وفُحصت طفيليات الدم بإستخدام مسحة الدمّ وصبغها بصبغة جيمسا. تبيّن أن 77 عُلجم مصاب (64.16٪) من قبل 880 طفيلي، الطفيلية المكونة من 879 (99.89٪) معقدات القمة *Apicomplexa* تتألف من *Haemogregina* spp. (89.66%) و *Hepatozoon* spp. (10.22%) و1 (0.11%) ذوات منشأ الحركة Kinetoplastida تتألف من *Trypanosoma* spp، في حين أن 43 (35.83٪) لم يصابوا. لم تظهر أي اصابة بالديدان الخيطية. يظهر معدل الإصابة على أساس الجنس أن الإناث لديها معدل إصابة أعلى من الذكور (χ^2 = 12.520، p≤0.05) وبالنسبة لنضج الضفدع، كان هناك فرق معنوي في معدل الإصابة بين

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المجموعة الناضجة وغير الناضجة (χ^2 = 19.471، p = 0.003). يزداد معدل الإصابة مع تناقص الوزن والطول مع وجود ارتباط سلبي (r_s = -0.022، p = 0.814 و r_s = -0.004، p = 0.966) على التوالي. وأظهرت هذه الدراسة ارتفاع مستوى الإصابة الطفيلية في الضفادع الإفريقية في ولاية الخرطوم مما ساهمت في انخفاض اعداد البرمائيات. وهناك ما يبرر إجراء مزيد من البحوث حول التطفل البرمائي وتهديداته على صحة الإنسان.

الكلمات الدالة: *Amietophrynus regularis*، السروجية، طفيليات الدم، جبل أولياء، الضفادع، السودان.

1. Introduction

The African common toad *Amietophrynus* (*Bufo*) *regularis* also known as a square-marked toad, Egyptian toad, African bouncing toad and Reus's toad is a member of the family *Bufonidae*, containing more than 300 species. Toads are fat-bodied amphibians, having warts and are widespread in savannah regions south of the Sahara (Rödel, 2000), but are most abundant in the tropical regions. Toad has been reported in various countries around the world including Africa. Typical toads are found in a region stretching from Senegal through West Africa to Central Africa and through North Africa to Egypt (IUCN SSC Amphibian Specialist Group, 2014). Toads represent as important animal models in science, and are commonly used in many disciplines of biomedical research and teaching, including toxicology, physiology, limb regeneration, evolutionary biology, and reproductive biology (Baker, 2007).

Toads are an integral part of the food web, an important source to diverse array of predator upon a variety of insects, including disease vectors that can transmit fatal illness to human (Crossland,1998) as Salmonellosis and tuberculosis. Also, amphibian can harbor some protozoan organisms capable of causing diseases such as leishmaniasis which is caused by a *Sauroleishmania* (Hassan *et al*., 2015).

Therefore, the objective of this work was to detect blood parasites infecting toads *Amietophrynus* (*Bufo*) *regularis* in Al-Sorojia and Jebel Aulia areas in Khartoum State, and to find out the association between the infection of the parasites according to toad sex, age, body weight, and length.

2. Materials and Methods

One hundred and twenty (120) toads (*Amietophrynus regularis*) were collected manually from the farms in Al-Sorojia area is a relatively old village, 5 km north of Khartoum and on the eastern side of the river Nile. L15.47.5- 32.33.5°N. The average of rainfall, approximate 13 mm, however the mean annual rainfall is 177 mm. The area is characterized by the maximum temperature in May (48°C) when the sun is overhead. But generally it ranges between 32-38°C throughout the year. It may also reach 48°C in June. The rainy season is short (July-September). At the beginning of the summer season (March-April) the relative humidity is very low and may be unstable especially at the lowest layers of the atmosphere, as a result of dust storm becomes wide spread during the summer season. The soil is dry cracking soil, and dry savannah and areas around the dam in Jebel Aulia is a village, 40 km south of Khartoum. L15.14.2- 32.29.5°N. The rainfall here averages 154 mm. The driest month is January with 0 mm of rainfall, although the greatest amount of precipitation occurs in August with, an

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average of 66 mm. The annual temperature is 29.1°C. The warmest month is May, with an average 33.3°C, the lowest average 23.4°C occur in January. The soil in the area is high in vertisol cracking clay soils, Sudan between May to August 2016. The specimens were transferred in plastic baskets with covers and then transported to the physiology laboratory in Department of Zoology, Faculty of Science, and sacrificed using chloroform. The snout-vent length (SVL) and gender were recorded for each individual. The hosts were grouped into two age groups on the basis of their SVL (immature: < 40 mm, and mature: 40-80 mm). The sex of host was determined by two methods: Color of the throat; male's throat is dark or green while female's is white, and reproductive system; females have two ovaries and two oviducts.

2.1. Preparation and Screening of Toad Blood

Blood samples were taken by removal of the tip of one toe from each toad and directly obtained via cardiac venipuncture with a heparinized insulin syringe. Thick and thin blood smear were prepared, air-dried and then fixed immediately in absolute methanol for 3 minutes, re-dried in the air and later stained in Giemsa's stain 10% for 15 minutes. The slides were removed and rinsed in distilled water, and then left to drain and dry. They were screened using a 100×immersion oil objective on a light microscope, and images were captured with an attached Canon digital camera (7.1 Mega Pixels). Parasites were identified according to Gardiner *et al*. (1988) and Bell (1986). The identification for microfilaria nematodes were done according to the characteristics described by Esslinger (1986) and Bain *et al*. (1992).

2.2. Statistical Analysis

Descriptive statistics of parasitism were calculated for the different parasite species recovered from each *Amietophrynus regularis*. The ecological terms were analyzed according to Bush *et al*. (1997):

$$Prevalence = \frac{The\ number\ of\ toad\ infected}{Number\ of\ toad\ examined} \quad \ldots\ldots (1)$$

$$Mean\ intensity = \frac{Total\ number\ of\ parasite\ of\ particular\ species\ found\ in\ a\ sample}{Number\ of\ hosts\ infected\ with\ that\ parasite} \quad \ldots\ldots (2)$$

$$Mean\ abundance = \frac{Total\ number\ of\ individuals\ of\ a\ particular\ parasite\ species\ in\ a\ sample}{Number\ of\ toad\ examined} \quad \ldots (3)$$

The relationship between the host factors such as sex, weight, length, age, and the parasite infection were examined from data collected from the two sampled localities in Khartoum. The Chi-square test for independence was used at 95% level of significance to compare differences in prevalence of parasite among host sex or age. Correlations of parasite abundance with host weight and length were tested using Spearman rank correlation coefficient (r_s). Three-way ANOVA was used to compare among the areas of study, host sex and host age (Sokal and Rohlf, 1995). All statistical analysis were done using statistical software SPSS 21 for windows.

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3. Results

Seventy-seven (64.16%) (60 males and 17 females) out of 120 *Amietophrynus regularis* were infected with blood parasites. The microscopic diagnosis detected 99.89% Apicomplexa and 0.11% Kinteoplastides. No nematodes were recovered. These three species collected include, Apicomplexa: *Haemogregina* sp. (89.66%), *Hepatozoan* sp. (10.22%); Kinteoplastides: *Trypanosoma* sp. (0.11%). The overall parasitic infection of *A. regularis* is shown in Table (1) and their morphological features are shown in Plates (1-6).

Table 1. Overall parasitic infection of *Amietophyrnus regularis* examined.

Parasite species	Number of infected	Prevalence %	Mean intensity	Mean abundance
Haemogregina sp	65	54.16	11.83	6.40
Hepatozoon sp	9	7.5	10	0.75
Trypanosoma sp	1	0.83	1	0.01
U.P.	2	1.66	10	0.166

U.P. = Unknown protozoan parasite.

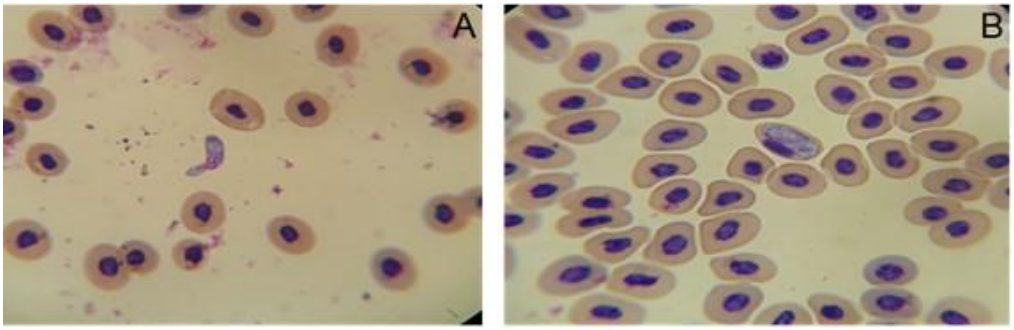

Plate 1. *Haemogregina sp* infecting *Amietophrynus regularis:* **A**) Merozoite, and **B)** Trophozoite.

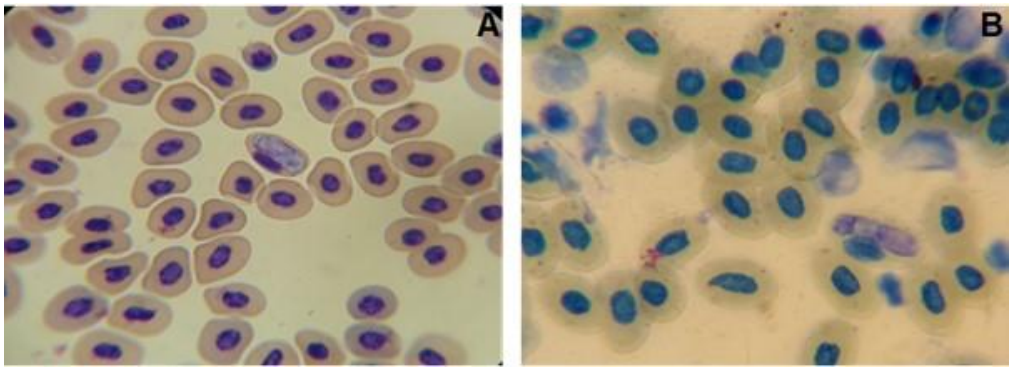

Plate 2. *Haemogregina sp* infecting *Amietophrynus regularis*: **A)**Young broad form, and **B)** Young thin form.

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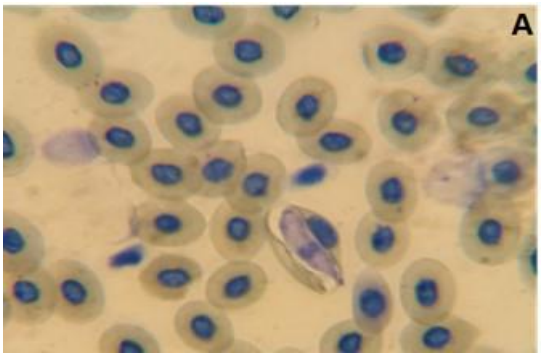

Plate 3. *Haemogregina sp* infecting *Amietophrynus regularis*: **A**) Gamonts

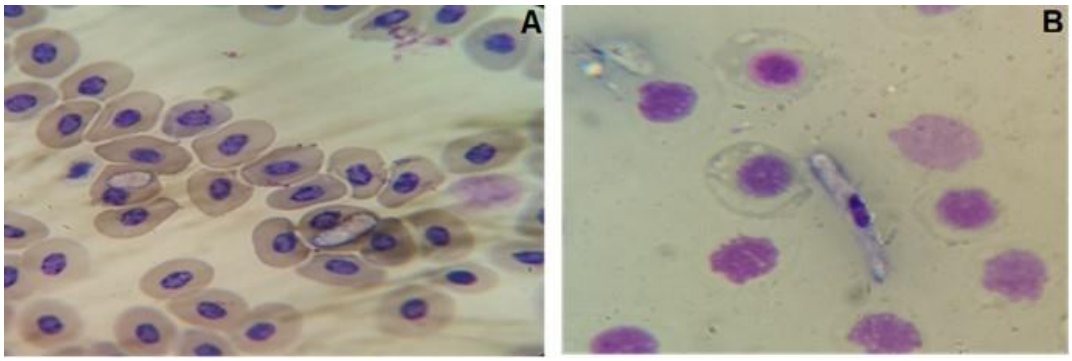

Plate 4. A) Trophozoite, and **B**) Merozoite of *Hepatozoon sp* infecting *Amietophrynus regularis.*

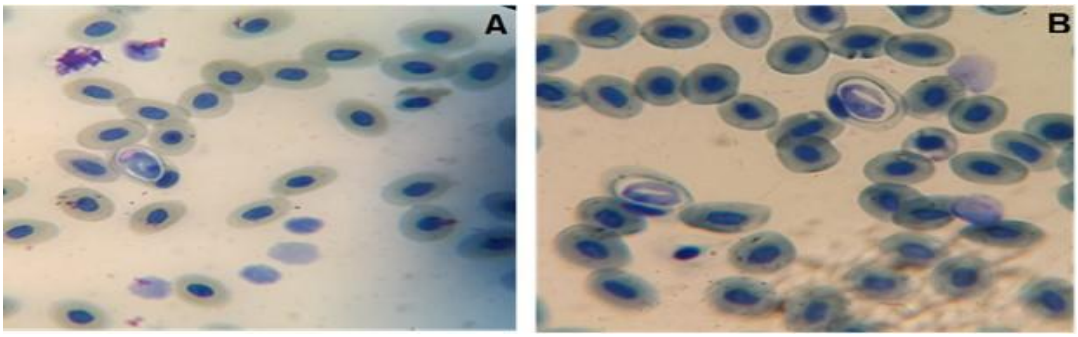

Plate 5. A) Mature gamont well-developed capsular cap, and **B**) Mature gamont displaying a recurved tail gamonts of *Hepatozoon sp* infecting *Amietophrynus regularis.*

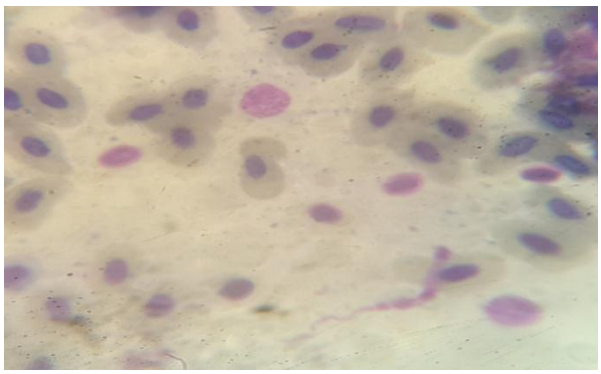

Plate 6. Trypanosoma species infecting *Amietophrynus regularis.*

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Infection of *A. regularis* in relation to sex showed that females were more infected with the *Haemogregarina* sp. Although *Hepatozoon* sp. has the lowest prevalence in both sexes, but the males were more infected with *Hepatozoon* sp. than the females. There was a significant difference (χ^2 =12.520, p=0.05) in the infection rate between males and females.

The relation of *A. regularis* parasites infection with age group showed that mature toads were more infected than the immature toads with *Haemogregarina* sp. and *Hepatozoon* sp. had a low prevalence in both age group and immature group was more infected with *Hepatozoon* sp. than the mature group. There was a significant difference in the infection of mature group compared with immature (χ^2 =19.471, p=0 .05).

The prevalence of infection increased with the low weight of the host. A negative correlation (r_s = –0.022, p = 0.814) was found between the host weight and parasitic infection. Recover parasite species in relation to the weight ranges showed that the *Haemogregina* sp. had the highest prevalence of 56.07% in 10 to 30.50 g weight range. Meanwhile, the *Hepatozoon* sp. had lowest prevalence in 10 to 30.50 g weight range with 6.54% prevalence.

Parasitic infection in relation to length ranges of the hosts examined shows that the length ranges of 8.0 to 8.9 cm had the highest prevalence of infection (82.35%). There was a negative correlation (r_s = –0.004, p = 0.966) in the infection with length.

4. Discussion

Amietophyrnus regularis from two localities in Khartoum State were found infected with a large number of parasites comprising one parasite taxa and two parasites' species. On the whole, 64.16% (77/120) of the toads in this study were infected with at least one haemoparasite group, some infected with two. This was similar to previous studies such as that of Bell *et al*. (1986) in White Nile near (Sonot) Acacia forest at Khartoum State documenting 60% (12/20) prevalence and also with 53.33% recorded in 15 samples examined in Jebel Aulia by Hassan *et al*. (2015). According to Hudson *et al*. (2006), parasites may play a role in promoting biodiversity and function as indicators of ecosystem productivity and resilience. Among the parasites taxa recovered, Apicomplexa had the highest infection rate. The high infection rate of Apicomplexa in *A. regularis* is in conformity with other findings in Khartoum State (Hassan *et al*., 2015).

In this study, *Haemogregarina* sp. show a highest prevalence rate of 54.16% which corresponds with the findings of Bell *et al*. (1986) who reported 50% prevalence rate in *Bufo regularis* in Sudan. The mosquitoes, mites and ticks were founded in Jebel Aulia and Al-Sorojia area, these arthropod hosts most likely to transmit haemogregarines (Gardiner *et al*., 1988; and Rose, 2005).

Other Apicomplexa species reported was *Hepatozoan* sp. which occurred in a very low prevalence rate of 7.5%. This is contrary to the survey done in Uganda by Readel and Goldberg (2010) who recorded 39.0% prevalence rate. Also, Ball (1967) found a considerably higher prevalence rate of 29% in a study conducted in Tanzania and Kenya. In another

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research done by Hassan *et al*. (2015), no *Hepatozoan* sp. was recovered. The higher prevalence rate in some of these studies were attributed to availability of insect vectors (Readel and Goldberg, 2010).

Trypanosome species (*Trypanosoma* spp.) occurred in low prevalence of 0.83% which is similar to the report of Bell *et al*. (1986), Aisien *et al*. (2015), and Hassan *et al*. (2015) who examined the same species. The results of the present and the previous studies conducted in Africa are in contrast to what has been recorded in other similar studies but on different continents (Barta and Desser, 1984; Barta *et al*., 1989; and Readel & Goldberg, 2010), in which the *Trypanosoma* demonstrated a higher prevalence to that of *Hepatozoon* or any other haemoparasite groups (Werner, 1993). Distinctive species within the genus *Trypanosma* can infect different animals, including mammals, aves, reptiles, amphibians, and fish. Numerous researchers reported the detection of *Trypanosma* spp. in amphibian early (Werner *et al*., 1988) and recently, the natural infection of sandfly species with amphibian *Trypanosma* was accounted for in *Phlebotomus kazeruni* (Leal *et al*., 2009; and Kato *et al*., 2010).

Mixed infections of an intracellular blood parasite, were recorded in *Amietophrynus regularis* from Al-Soroja. Studies by other investigators have also revealed the occurrence of mixed infections of blood parasites in anurans from elsewhere (Desser, 2001; Leal *et al*., 2008; Stenberg & Bowerman, 2008, 2010; Readel & Goldberg, 2010; and Aisien *et al*., 2015).

In relation to sex, this study has shown that female anurans had higher prevalence rate of 80.95% among the 17 of 21 female specimens examined, as compared to only 59.59% (60 of 99) of male specimens examined. The reason for these differences in infection prevalence is not clear. However, more samples need to be examined to validate these differences. The parasites infection rate was higher in mature toads than in immature ones. This may be due to older hosts having longer time to accumulate parasites than younger hosts and they provide more internal and external space for parasite establishment. But there were differences which could be explained by ontogenetic changes in the host by behavior and ecological factors; both characteristics have important roles in parasite induction and may be correlated with the host's body size (Poulin, 2000).

Parasite infections in relation to the size of the hosts showed higher prevalence, mean intensity and abundance rates in toads of small size than those with large. This result was in contradiction with the finding in the previous studies by Brickle *et al*. (2003) and Nworah and Olorunfemi (2011). They affirmed a positive correlation between the host size and parasitic infection which contradicts this study as a negative correlation was observed between host size and parasitism. This may be related to the kinds of investigated parasites; all the previous studies were on helminthes parasites which are more different on their way of establishment in host.

The comparing among the areas of study, host sex and host age showed no significant interactions between these factors. This may be due to the time for collected sample where it was at the end of the summer season and the beginning of the short period fall season. The

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convergence of latitude and longitude of the two areas and the combination of samples environments may also be a factor. This is not sufficient to show the impact of climate change on parasites.

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