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[1]Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.
[2]Department of Chemistry, Shehu Shagari College of Education, Sokoto, Nigeria
[3]Department of Biological Sciences, Sokoto State University, Sokoto, Nigeria.

***Corresponding author's email:**

ibrahim.magami@udusok.edu.ng,

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Survey and Prevalence of Parasitic Species of Amphibians around River Rima and River Sokoto

Muhammad I. Magami,[1*] Tambuwal A. Arzika,[2] Moses O. Ode[1] and Tanko M. Muhammad[3]

Amphibian species in two major rivers in Sokoto (Rima and Sokoto) were investigated for prevalence of parasitic species. Adult amphibians (120) from both rivers were captured within the months of April to August 2017 and studied for presence and types of parasitic species. The results showed that a total of seven species of amphibians (*Amietophrynus africanum*, *Amietophrynus maculatus*, *Bufo bufo, Ptychadenama scareniensis*, *Ptychadena longirostris, Rana catesbeiana* and *Rana clamitans*) were encountered. Out of the hundred and twenty (120) specimens examined for parasites, 100(83.33%) were infected, while 20 (16.67%) were uninfected. Also, in the 959 parasites recorded, 621 were found in amphibians from River Rima while 338 existed in amphibians from River Sokoto. These comprises; two (2) Apicomplexa: *Isoporasp* and *Crytosporidium spp*, one (1) Opalina (*Opalina ranarum*), one (1) Sarcodine; *Entamoeba ranarum*, one (1) acanthocephala; *Acanthocephalan cysthacanth* and four (4) nematodes; *Strongyloides spp*, *Ascaridoid sp* eggs, *Physaloptera sp* and *Cosmocerca ornata*. Nematodes had the highest occurrence, infecting amphibians from both rivers whereas River Rima had the highest mean intensity (86.20). Generally, the study concludes that even though amphibians around both rivers were infected with parasitic species, amphibians from River Rima had the higher infection rate.

Keywords: Amphibians, *Bufo bufo***,** Parasitic**,** Rima, Sokoto

1. Introduction

Research on amphibians is on the increase in Nigeria and this is largely due to the global knowledge of amphibian declines (Blaustein and Wake, 1995). Some researchers have reported on the causes of amphibian declines in the country (Akani and Luiselli, 2001; 2002; Amuzie, 2017). Most other reports have concentrated on the parasitic fauna of different amphibian species in Nigeria (Aisien *et al*., 2001; 2003; 2004a; Ayodele and Akinpelu, 2004; Nworah and Olorunfemi, 2011; Iyaji *et al*., 2015) and on the influence of various environmental factors on the community structure of the parasite species (Aisien *et al*., 2004b; 2009; 2011; 2015). Many other findings on the ecotoxicological effects of heavy metals in amphibians has been carried out (Ezemonye and Enuneku, 2006; 2011; Tyokumbor and Okorie, 2011; Idowu *et al*., 2014). Amphibians can be infected by different types of parasites. These parasites among others include the intestinal parasites. The intestinal parasites of amphibians are usually; protozoa, flagellates, ciliates and worms (such as helminthes), which are the most common intestinal parasites. Since amphibians are usually found in association with other living organisms, and man; some species of amphibians are edible and provides sources of protein in some species and other nutrients for man. They may also cause human infections by these intestinal parasites. Therefore, these parasites could pose a great threat to the amphibians themselves and other animals they interact with. The research aim was to evaluate and compare parasitic species of amphibians around the rivers Rima and Sokoto and to identify the organs of predilection of these parasites.

2. Methodology

2.1 Study area

This study was carried out in Sokoto Metropolis cutting across two rivers (Rima and Sokoto) of Sokoto State. Sokoto is located on latitude 13°05N 05°15E and longitude 13.083°N 5.250°E. Sokoto metropolis has a population of 4,998,100 (NBS, 2017). Rainfall in Sokoto is between May/June to early November, when the natural

water bodies are often flooded (Umar and Ipinjolu, 2010). Annual rainfall in Sokoto ranges between 500 and 1300 mm with the highest peak in August, while the dry season sets in first with the cold harmattan from November to February, and a hot period comes in from March to the end of May when temperatures reach 40ºC (which is the extreme) during the day with humidity less than 20% and the rain begins in June to early November (Umar and Ipinjolu, 2010).

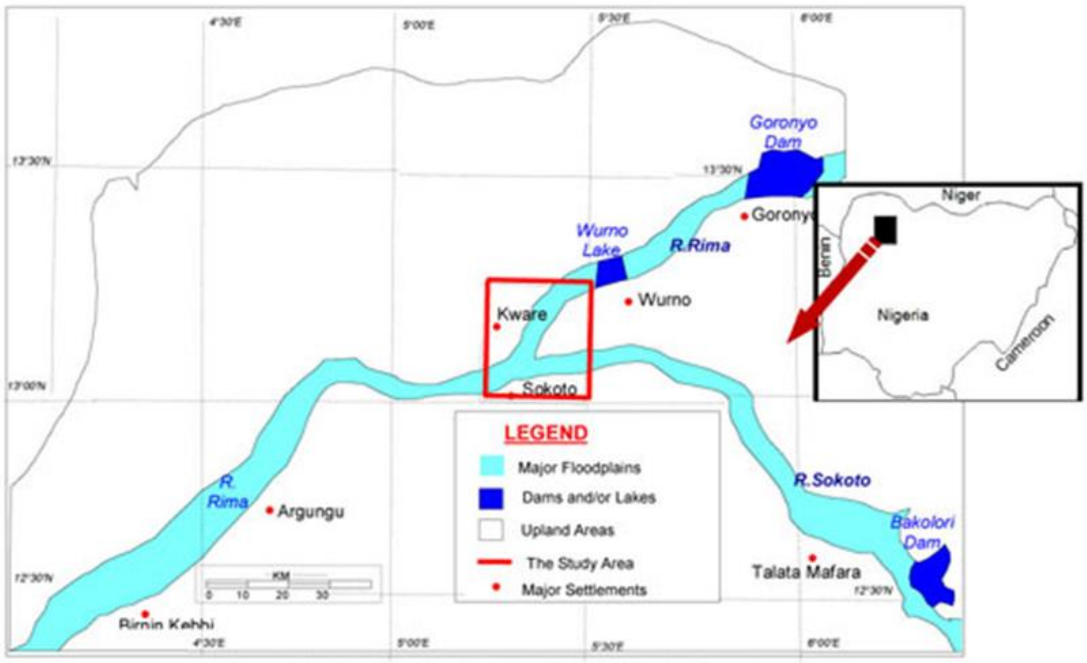

Figure 1. *Map showing River Rima and River Sokoto in Sokoto State, Nigeria*

2.2 Survey of Animals

Adult amphibians (120) were captured within the months of April to August, 2017. The amphibians were caught from two rivers namely; River Rima, and River Sokoto all within Sokoto metropolis. The amphibians were categorized based on sampling locations; they were labeled appropriately; indicating sample number, collection day, and centers respectively. The amphibians were taken to parasitology Laboratory of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto immediately after collection for Laboratory analysis.

2.3 Sample Collection and Preparation

Collection of amphibians was carried out very early in the morning by fishermen and farmers living around the locality between 7am to 9am using nets. Species of amphibians were identified according to the descriptions of Schiotz (1999); Roedel (2000) and Inger and Stuebing (2005).

2.4 Dissection and Examination of Amphibians for Parasites

The frogs and toads were dissected ventrally from the cloacae region to the anterior end on the dissecting board. The Oesophagus, stomachs, small and large intestine and rectums were removed from the frogs. In addition, the lungs and urinary bladder were also removed and placed separately into different petri dishes. Later, the organs were opened longitudinally using a forceps and scissors and the contents were kept in the petri-dishes. 10 mL of normal saline solution was poured into the petri dishes. The contents were observed under the dissecting microscope. Helminthes were picked and kept in 70% ethanol glass bottles and labelled (Rahman and Shakinah, 2015).

2.5 Collection and Identification of Helminthes

All nematodes and acanthocephalans were cleared in lacto phenol. They were then killed in hot 70% ethanol. Nematodes were preserved in 70% glycerine alcohol, whereas acanthocephalans were preserved in 70% ethanol. All helminthes were identified according to the descriptions of Yamaguti, (1961).

2.6 Preparation of Specimen for Microscopic Examination

Different organs were cut open with the aids of blunt forceps and the contents were poured into different petri dishes containing sufficient amount of normal saline solution. Three methods were used in the preparation of the sample for microscopic examination; Direct Wet Mount Fecal Exam, Modified Wisconsin Sugar

Floatation Method, and Ethyl-acetate Sedimentation Method. The specimen was identified using identification charts and the parasites were identified using appropriate protocols (Yamaguti, 1961; Prudhoe and Bray, 1982; Khalil *et al.,* 1994).

(a) Direct Wet Mount Fecal Exam

A small amount of feces was placed on a microscope slide. A drop of liquid (normal saline) was added to the feces and mix thoroughly on a microscopic slide, the slide was covered with a cover slip. Then the entire cover slid was examined under the microscope (Amite *et al.,* 2004).

(b) Modified Wisconsin Sugar Floatation Method

A 15 mL test tube was filled with 10 mL of Sheather's solution. Three (3) grams of feces was weighed and placed into a beaker. Sheather's solution from the test tube was poured into the beaker and mixed well. Funnel was placed into the test tube and a strainer was placed into the funnel and the fecal-sugar solution mixture through the strainer was poured into the test tube. Tongue depressor was used to squeeze the liquid out of the feces that was left in the strainer. The tube was then centrifuge (1500 to 2500 rpm) for 5 minutes. The tube was filled to the brim with more Sheather's solution and cover slide was placed onto the meniscus. The tube was left for about 5 minutes and the cover slide was removed and placed on a slide. The entire cover slid was examined under the microscope (Khalil *et al.,* 1994; Amite *et al.,* 2004).

2.7 Statistical Analysis

Prevalence (%), mean intensity and mean abundance were calculated according to Margolis *et al.* (1982) and Bush *et al.* (1997). T-test analysis at 95% significance level was used to compare the mean intensity and mean abundance of infection from both rivers.

3. Results

A total of seven species of amphibians were encountered (*Amietophrynus regularis, Amietophrynus maculatus, Bufo bufo, Ptychadenama scareniensis, ptychadena longirostris, Rana catesbeiana and Rana clamitans*). The amphibian species and their distribution in each location are presented in Table 1. Six species of amphibians were encountered at River Rima while seven species were recorded from River Sokoto. At River Rima, *Amietophrynus regularis* was the dominant species with a total of fifteen individuals (12.50%) while in River Sokoto *Ptychadenama scareniensis* was the dominant species with a total of fifteen individuals (12.50%).

At River Rima, the prevalence of parasites ranged from 20% to 100% while the mean intensity of infection ranged from1.0 to 10.0 and mean abundance of infection ranged from 0.4 to 10.0 with *Isospora sp*, *Physaloptera sp, Strongyloides sp,* and *Ascaridoid sp* each having the highest prevalence of 100% but the mean intensity and mean abundance of infection differed. The overall mean intensity and mean abundance of infection was 86.20 ± 2.79 and 58.17 ± 2.76 respectively (Table 5). The overall distributions of parasite species of amphibians from River Rima are shown in Table 2.

Out of a total of 120 amphibian hosts examined for parasitic infections, 100 (83.33%) were found to be infected with a prevalence of 83.33%, with mean intensity of 13.30 and mean abundance of 11.08 while 20(16.67%) were not infected. Total of (621) parasites were identified from amphibian host examined from River Rima and (338) parasites were recovered from amphibian host examined from River Sokoto.

The overall prevalence of parasitic infection in relation to the site of infection shows that *Opalina ranarum* has the highest prevalence of 100% in the rectum; *Entamoeba ranarum* has the highest prevalence of 100% in the large intestine while *Physaptera sp* has the highest prevalence of 100% in the esophagus and stomach. *Ascaridoid sp was observed to have* the highest mean intensity of 10.0 in the urinary bladder, stomach*, A. cysthacanth* had the highest mean intensity of 10.0 in the small intestine while *Physaloptera sp* has the highest mean abundance of 6.67 in the esophagus and stomach (Table 4).

The overall mean intensity and mean abundance of infection from both rivers was 137.24 ± 24.86 and 99.27 ± 12.07 respectively, with amphibians from River Rima possessing a mean intensity and a mean abundance of 86.20 ± 2.79 and 58.17 ± 2.76 respectively, while amphibians from River Sokoto have the overall mean intensity and mean abundance of 51.04 ± 2.12 and 41.10 ± 2.22 respectively. The result showed that there was no significant different between the mean intensity and mean abundance of infections from both rivers at $P<0.05$(Table 5).

Table 1. *Amphibian Species examined from River Rima and River Sokoto*

Amphibian Species	Locations			
	River Rima	River Sokoto	Total	Prevalence (%)
Amietophrynus regularis	15	10	25	20.83
Amietophrynus maculates	5	10	15	12.50
Bufo bufo	10	5	15	12.50
Ptychadenama scareniensis	10	15	25	20.83
Ptychadena longirostris	12	10	22	18.33
Rana catesbeiana	8	5	13	10.83
Rana clamitans	-	5	5	4.17
Total	**60**	**60**	**120**	**100**

Table 2. *Occurrence of Parasite Species of Amphibians for River Rima*

Parasite	Site	Prev. (%)	Mean intensity	Mean abundance.
Isospora spp	2	100.00	2.0	2.00
	2	20.00	5.0	1.00
E. ranarum	3	62.50	6.0	3.75
A. cysthacanth	2	40.00	1.0	0.40
	2	80.00	10.0	8.00
	2	66.70	9.5	6.33
Physaloptera sp	1,4	83.33	4.0	3.33
	1,4	100.00	6.7	6.70
C. ornata	3,7	33.33	6.0	2.00
	7	60.00	7.0	4.20
	7	41.70	6.0	2.50
Strongyloides sp	6	100.00	3.5	3.50
	6	62.50	4.0	2.50
	6	53.33	2.5	1.33
	6	20.00	3.0	0.63
Ascaridoid sp	4,5	100.00	10.0	10.00

Footnote:1 = esophagus, 2= small intestine, 3= large intestine, 4 = stomach, 5 = urinary bladder, 6 =lungs, 7= rectum.

Table 3. *Prevalence of parasite species of amphibians from River Sokoto, 2017*

Parasite	Site	Prev. (%)	Mean Intensity	Mean Abundance
Opalina ranarum	7	100.00	6.0	6.00
Entamoeba ranarum	3	100.00	6.0	6.00
Strongyloides sp	6	100.00	5.0	5.00
	6	80.00	4.75	3.80
	6	20.00	1.0	0.20
	6	60.00	3.3	2.00
Crytosporidium sp	2	80.00	6.0	4.80
	2	40.00	1.0	0.40
Cosmocerca ornate	3,7	70.00	4.29	3.00
	3,7	50.00	6.0	3.00
	3,7	90.00	7.70	6.90

Footnote*:1 = esophagus, 2= small intestine, 3= large intestine, 4 = stomach, 5 = urinary bladder, 6 =lungs, 7= rectum.*

Table 4: *Overall parasite infection of amphibian in relation to organ of infection, 2017*

Parasite	Host	Site	Pre. (%)	Mean Intensity	Mean abundance
Opalina ranarum	*R. clamitans*	7	100.0	6.0	6.0
E. ranarum	*R. catesbeiana*	3	38.46	6.0	2.31
	R. clamitans	3	100.0	6.0	6.00
A. cysthacanth	*A. regularis*	2	40.00	9.5	3.80
	p. mascareniensi	2	82.00	10.0	3.20
Physaloptera sp	*P. longirostris*	1,4	45.50	4.0	1.81
	A. regularis	1,4	100.0	6.70	6.70
Crytosporidium sp	*P. longirostris*	1,4	36.40	6.0	2.18
	Bufobufo	4	13.33	1.0	0.13
Isospora spp	*R. clamitans*	2	33.33	2.0	0.67
	A. maculatus	2	66.67	5.0	0.33
Ascaridoid sp (lavae)	*P. mascareniensis*	4	40.00	10.0	4.00
C. ornate	*A. regularis*	7	48.00	5.0	2.40
	A. maculatus	7	53.33	6.4	3.40
	P. longirostris	7	63.64	6.4	3.40
Strongyloides sp	*P. mascareniensis*	6	40.00	3.5	1.40
	R. catesbeiana	6	38.50	4.0	1.54
	A. maculatus	6	53.33	5.0	2.67
	Bufo bufo	6	20.00	3.33	0.66

Footnote: 1=*Oesophagus,* ***2= S****mall intestine****, 3=*** *Large intestine****, 4= S****tomach,* ***5=*** *Urinary bladder,* ***6****= Lungs,* ***7****= Rectum*

Table 5. *Overall mean intensity and mean abundance of infection from both rivers, 2017.*

Locations	Mean Intensity	Mean Abundance
River Rima	86.20 ± 2.79	58.17 ± 2.76
River Sokoto	51.04 ± 2.12	41.01 ± 2.22
Overall	137.24 ± 24.86	99.27 ± 12.07

4. Discussion

The number of species encountered was rather few, perhaps due to the short duration of the study which was during raining season. Nevertheless, this study has given an insight to some of the parasitic species infecting amphibians around River Rima and River Sokoto. Nine (9) parasites species encountered in this study have been reported in previous studies undertaken in other locations in Nigeria and elsewhere (Dobell 1918; Aisien *et al*., 2001; Aisien *et al*., 2003; Aisien *et al.,* 2004; Aisien *et al*., 2009; Avery1971; Oddo 2008; Iyaji *et al.,* 2015). Among these parasites only three (3) species (*Entamoeba ranarum, Cosmocerca ornata and Strongyloides spp*) were common to host examined from the two locations indicating their abilities to complete their life cycles and establish infections in their respective hosts irrespective of the prevailing environmental conditions in these habitats.

The amphibians from River Rima harbored more numbers of parasites species which is an indication of the habitat suitability which was perhaps supporting the successful transmission of these parasites to their susceptible hosts. According to Pietrock and Marcogliese, 2003, unsuitable environmental conditions results in impaired survival and/or reduce infectivity of free-living parasitic stages. All the parasites identified with the exception of *Entamoeba ranarum, Cosmocerca ornate* and *Stongyloides spp* which were common to the host examined from the two locations, infect amphibians host from different locations.

The acanthocephalan cysthacanth identified in the amphibians examined from River Rima and River Sokoto had been previously recorded in amphibian studied in other locations of Nigeria (Aisien *et al.,* 2015), but their prevalence rate, mean intensity and mean abundance of infection differed significantly. According to Imasuen *et al.,* (2012), Acanthocephala cysthacanth recovered from anurans used these frogs as transport hosts.

The protozoan parasites (*Opalina ranarum* and *Entamoeba ranarum*) found in this study was host specific, only in Rana species such as *Rana catesbeiana* and *Rana clamitans*. This result was in conformity with the findings of Dobell (1918), who found the same parasite in the rectum of *Rana clamitans*. On the contrary though, finding

Entamoeba ranarum in the large intestine of *Rana clamitan* and *Rana catesbeiana* did not agree with his findings. From River Rima, *Isospora sp, Physaloptera sp* and *Strongyloides sp* had the highest prevalence rate of 100% respectively while the mean intensity and mean abundance of infection varied respectively.

All the Nematodes found in this present study had been previously recorded in amphibian host studied in other locations in Nigeria (Aisien *et al.,* 2001, 2003, 2004a). *Cosmocerca ornata* occurred only in the large intestine and rectum of the host examined in the study. This is in line with Aisein *et al.* (2009), Dusen (2011) and Imasuen *et al.* (2012) in which the parasites species were all recovered from the large intestine and rectum of the different kind of anurans examined. *Physaloptera sp* was recovered from the esophagus and stomach of the host examined in this study which is the same with the result of Anderson (2000), who found *Physaloptera sp* which persisted in the gastric mucosa of toads for varying periods of time without reaching the adult stage or by larvae of *Mesocestoides sp*. which remained encapsulated in the mesenteries. This was in contrast to the report of Gonzale and Hamann (2010) where the parasite was only recovered from the stomach of the anurans host examined. It can therefore be deduced that *Physaloptera spp* has the ability to attach itself to the stomach wall of its host using its mouth, and so it is not easy for this parasite to be pushed by peristaltic movement into the intestine.

In the present study, the main group of helminthes found parasitizing amphibians were nematode species. This result is consistent with previous findings on the toads, *Bufo ictericus* (Luque *et al*., 2005), *Amietophrynus regularis* (Ibrahim, 2008) and *Rhinella icterica* (Santos *et al*., 2013). This is because, on land, anurans are more exposed to nematodes with monoxenous life cycles such as *Strongyloides spp*., *Rhabdias spp*., and cosmocercids, because the majority of nematodes infect anurans through cutaneous penetration or the ingestion of eggs. Similarly, previous studies have shown that the bufonid amphibians tend to harbor a higher number of nematode species than trematodes, as was seen in this study (Bolek and Coggins, 2003). This is likely to be because the terrestrial toads predominantly feed on ants, beetles and other terrestrial invertebrates (Hirai and Matsui, 2002) and are therefore less prone to infection from the wide range of species of trematodes which commonly infect aquatic amphibians. Additionally, it has been reported that there is a greater incidence of infection of anurans with nematodes than with cestodes infections (Mohammad *et al.*, 2010).

5. Conclusion

Significant findings of amphibian survey and parasites, the level of multiple infections and the number of worms recovered in the study were an indication of the rich parasitic fauna of amphibians in Sokoto State. This study has given an insight into some of the parasitic infections of amphibians from the two rivers in Sokoto State of Nigeria and these findings have opened the door for further research on other aquatic fauna parasitism, its impact to human health as they may infect frogs and fishes in water, since they play vital roles in the balancing of food chains and webs in the ecosystem. The study has shown that the amphibians found around River Rima and River Sokoto in Sokoto State, Nigeria were rich in parasitic fauna.

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Conflict of interest

The authors declare no conflict of interest.

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