

Prevalence and intensity of Parasites of Anurans in Selected Wetlands of Kogi State, North Central, Nigeria

ABSTRACT

Parasites infect nearly every species of animals, nevertheless the majority are inadequately studied except they have proven economic, medical or conservation significance. The role of parasites in the decline of anuran population is not clearly understood, therefore this study is aimed to assess the prevalence and intensity of parasites of Anurans in selected wetlands of Kogi State, North central, Nigeria. The study was conducted in Abu'ja wetland in Dekina Local Government area, and Egwubi seasonal wetland in Ejule, Ofu Local Government area of Kogi State. The Prevalence of parasites in Anurans was computed as percentage number infected divided by number examined, and compared between groups using chi-square statistic. Mean intensity of parasitic infection was computed as average number of parasites per infected group. Level of significance was $p < 0.05$. The data was analyzed using the Statistical Packages for Social Sciences (SPSS) version 20.0. Anuran species found in both wetlands included *Amietophrymus regularis*, *A. maculatus* and *Hoplobatrachus occipitalis*. Out of the 854 anurans collected, 25 were infected by ectoparasites, 37 by haemoparasites and 87 by gut parasites from Abu'ja station. Thirteen anurans were infected by ectoparasites, 14 by haemoparasites and 56 by gut parasites from Egwubi station. The ectoparasites found in anurans of both wetlands were Leech (*Hirudo medicinalis*) and ticks. Haemoparasites included *Folleyeloides* microfilaria, *Trypanosome* spp, and *Haemoigregarina* spp. The cestode, *Baerietta jaegerskioidi*, was found in the gut of *Hoplobatrachus occipitalis* from Egwubi wetland. Others are nematodes *Ampliceacum africanum*, *Cosmocercer onata*, and *Physaloptera* spp common to the three species of anurans from both wetlands. This study has shown the parasites of anurans from Kogi State, North Central, Nigeria. It also showed the biodiversity of anurans in the study area proving its relative abundance. Effort should be made to protect these anuran species from these parasites as they could be of public health concern if transmitted to humans more so they have very good ecological relevance.

Keywords: Prevalence, Intensity, Parasites, Anurans, Kogi State.

1. INTRODUCTION

Parasites infect almost all species of animals, however many are inadequately studied except they have proven economic, medical or conservation significance. This is mainly correct for parasites of wildlife where available data on parasite diversity and ecology is small [1]. One of the most endangered taxa of animals is the amphibians with nearly 40% of the roughly 5743 amphibian species globally reduced [1]. Infectious diseases like those caused by viruses, fungi and some parasites are key agents in the reduction of some animal species including anurans. Captive breeding and diseases prevention have been pin pointed as vital conservation apparatus that will be essential to guard amphibian populations worldwide. Without full knowledge of diseases causing agents in amphibians, measures to keep away from them cannot be victorious. Largely all the studies analysing community structure of helminths parasitizing anurans were done in temperate regions [2]. Researches on African tropical systems are even fewer, and in relation to the helminth fauna of anurans in Nigeria, there are just a few researches accessible [3]. In a research of South African Anuran, [4] reported a species of the Monogenean parasitic in the urinary bladder of *Schismaderma carens*. This was the third Eupolystoma species described from South Africa and the first *Polystoma* from *Schismaderma*, an anuran genus that is ancient with respect to the other African bufonids in which Eupolystoma has been seen. In their result, [4] reported that in a sample of 27 toads, 37% were infected with up to 130 parasites. In a parasite examination of the Anuran Leiuperidae from Corrientes, Argentina, [5] reported the first report of nematode parasites of *Physalaemus santafecinus*. In their study, 183 nematodes were obtained from 81 adults of *Leuperid Physalaemus santafecinus* investigated. In a different report a Lungworm nematode from amphibian hosts at Aswan Governorate in Egypt, [6] reported females of *Rhabdias species* from the lungs of the maculated toad *Bufo regularis*. [7] report on

helminths community of the tree frogs at the Okomu National park, Edo State Nigeria. The tree frogs of the Okomu National Park were examined for helminths parasitic infections. The 24 species of tree frogs discovered belong to the families' Arthroleptidae, Ranidae and Rhacophoridae. The 24 species of tree frogs in the study included. Cestoda: *Baerietta* species (7.4%) Monogenea: *Polystoma chiromantis* (4.7%), *Polystoma dorsalis* (3.7%) *Polystoma gracie* (6.7%). The trematoda seen included *Mesocoelium monodi*, *Mesocoelium monas* and *Osteoloides rappiae* which were seen to be multi host parasites. The nematodes seen are *Cosmocerca ornata* and an unidentified Oxyurid nematode which was observed to infect a wide range of host. [8] studied the endoparasite of amphibians from localities in the rainforest and mangrove forest zones of south western Nigeria. Amphibian examined included *Bufo regularis*, *Dicroglossus occipitalis*, *Ptychadena oxyrhynchus*, *Xenopus muelleri* and *Hemisus mamoratus*. The investigation yielded the following parasites. A pentastomid of the *Raillietiella* species Cestoda: *Cephalochlamys namaquensis*, *Cephalochlamys* sp and *Cylindrotaenia jaegerskioeldi*, Trematode: *Diplodiscus fischthalicus*, *Progonimidiscuss doyeri*, *Ganeo africana*, *Mesocoelium monodi* *Haematoloechus exoterochis* and *Prosotocus exovitellous* nematode: *Rhabdias bufonis*, *Cosmocerca onata*, *Paracosmecerca* species, *Chabaudus leberec*, *Amplicercum africanum*, *Cammallanus dimitroi*, *Camallanus* sp *Batrachamallanus xenopodis*, *Batrachamallus* sp *Oswadocruzia hoeplii* and some immature filarids. Nearly every parasites found in this study were reported for the first time in Nigeria but a number of them have circulation in other African countries. Amphibians obtained from the rainforest zone contain more parasites species than those from the mangrove forest zone. *Dicroglossus occipitalis* housed the utmost number of parasite species followed by *B. regularis*, *X. muelleri* and *P. oxyrhynchus*. [9] studied the helminths parasites of anurans from the savannah-mosaic zone of south-western Nigeria.

Parasites of helminths from 5 areas in the Savana-mosaic and 1 in the transitional vegetation Zone of Edo state Nigeria were studied. Amphibian host examined included *Bufo regularis*, *Dicrodiscus occipitalis*, *Bufo maculatus*, *Ptychdena oxyrhynchus*, *P. mascereniansis*, *P. pumilio*, *P. schubtzi*, *Xenopus mueller*, *Silurana tropicalis*, *Rana galamensis* and *Leptopelis viridis*. Some parasites infecting amphibians in the rainforest and mangrove, such as *Cephalochlanys mamaquensis*, *Mesocoelium monodi*, *Haematoloechus exoterorchis*, *Diplodiscus fischthalicus*, *Prosotocus exovitellousus*, *Ganeo africana*, *Rhabdias bufonis*, *Cosmocerca ornata*, *Camallanus dimitrovi*, *Amplicacum africanum* and *Bartrachocamallanus xenopodis* also infected anurans of the Savannah mosaic. In a different study on helminths parasites of anurans at new Bussa in the guinea Savannah zone of Nigeria. [10] reported that, anuran hosts investigated included *Burn regularis*, *Burn maculatus*, *Dicroglossus occipitalis* and *Rana galamensis*. The cestodes *Cylindrotaenia species*, was obtained from *Burn regularis* and *Burn maculatus*. Monogeneans were represented by *Eupolystoma alluaudi* and *Polystoma galamensis*; trematodes by *Haematoloechus exoterorchis*, *Haematoloechus micrurus*, *Mesocoelium monondi* and *Diplodiscus fischthalicus*. Nematode parasites included *Rhabdias bufonis*, *Amplicercum africanum*, *Camallanus dimitrovi*, *Cosmocerca ornata* and larval ascaridoid nematode. From every indication in literature, there is paucity of information on parasites of anurans in Kogi State, Nigeria. This work was designed to determine the prevalence and intensity of parasites of anuran in Kogi State, North Central, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Abu'ja wetland in Dekina Local Government area, and Egwubi seasonal wetland in Ejule, Ofu Local Government area of Kogi state. Kogi experiences tropical

hinterland type of climate, with high temperatures of 27 °C – 30 °C. The highest temperature is obtained between the period of March and April, while the lowest temperature is obtained between the period of December and January. The annual rainfall is 100cm -150cm, with duration of six to eight months. The relative humidity is high during the rainy season; this is about 80% during this period; whereas the relative humidity is low during the dry season, as low as about 5%. The dry season lasts for about five to six months. The atmosphere is usually cloudy during rainy season, as opposed to dryness and dust in dry season. The climatic conditions of the study area and the duration of rain described favours the persistence of anuran species and breeding periods.

2.2 Study Design and Sampling

Anuran samples were randomly collected from the water and surrounding wet grass of the wetlands used to avoid removing excessive number of anuran individuals from same population for conservation reasons. Overall, 854 specimens of anurans were collected within a period of eighteen months in order to minimize seasonal variation on the parasite infra-community structure occurring in the hosts [11, 2]. A total of 3 sample sites was marked out in each wetland; (site 1), water merging with vegetation (site2) open water body, and (site 3) vegetation around water, was selected for proper coverage. Each of the areas were visited twice each month during the study period between 8 am-11am to collect anurans. Methods implored during the collection process included; Hooks baited with body parts of the anurans, this served as a lure trap for them, Direct chase and capture; this involved the active chase of any spotted frog or toad and Direct light flash: this was implored for night catches. The specimens were transported alive to the laboratory where they were sacrificed with MS 222, commercially available as Tricaine Methane Sulfonate (TMS). Tricaine is a benzoic acid derivative, in water of low alkalinity, the

solution was buffered using sodium bicarbonate. A 10g/L-1 stock solution was made, and sodium bicarbonate was added to saturation, resulting in a pH between 7.0 and 7.5 for the solution [12]. External parasites were isolated by close examination of the anuran using magnifying lens and bright, cool light. The body cavity was opened by longitudinal incision from throat to vent, the gut was detached by cutting across the oesophagus and rectum [13]. Thereafter, it was shared into four parts: oesophagus, stomach, intestine and colon. Each segment was opened longitudinally and placed separately in a watch glass containing normal saline to extract the helminths, preserved in vials containing 70% ethanol until examination. Nematodes was identified after clearing in a drop of undiluted glycerol on a glass slide; cestodes and trematodes were stained in haematoxylin, dehydrated in graded ethanol and mounted in balsam for examination [13]. Blood sample was collected from the heart ventricle of the animal using 5ml syringe and needle. The blood samples were preserved in EDTA bottles. Haematological examination was conducted by preparation of thin smear of the blood sample, air dried and fixed in absolute alcohol for 3 minutes and stained with Giemsa stain and then observed using photomicrograph with camera under the microscope to detect the presence of blood parasites. Microscopic examination of faecal sample was done according to [14].

2.3 Anuran Species Identification and Preservation

Frogs collected were identified using available keys and taxonomic standards. Frogs examined for helminths and ectoparasites were transported alive to the laboratory and screened respectively, and later preserved in 10% formalin. All frogs investigated for helminths were sacrificed and the gut contents examined. The parasites obtained were identified using available keys and taxonomic standards.

2.4 Data Analysis

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The data was analysed using the Statistical Packages for Social Sciences (SPSS) version 20.0, PAST (Paleontological Statistics) version 3.14 and Microsoft Office Excel. Prevalence of parasites in Anurans was computed as percentage number infected divided by number examined, and compared between groups using chi-square statistic. Mean intensity of parasite infection was computed as average number of parasites per infected group. Level of significance was $p < 0.05$.

3. RESULTS

A total 584 anurans were examined for parasitic infection at the Abu'ja station. They were of three species, *Amietophrymus regularis*, *Amietophrymus maculata* and *Hoplobatrachus occipitalis*. Out of the 584 examined, 25 (4.3%) were infected by ectoparasites, 37 (6.3%) by haemoparasites, and 87 (14.9%) by gut parasites. The prevalence of ectoparasites were similar among the three anuran species ($\chi^2 = 1.813$, $p = 0.404$). The haemoparasite prevalence was also similar among the three anuran species (Table 1). The prevalence of gut parasites were relatively higher, and was significantly different among the anuran species ($p < 0.05$). *H. occipitalis* and *A. maculata* had 20.8% and 14.0% prevalence compared to 11.5% prevalence in *A. regularis*. The intensity of gut parasites were relatively higher than that of other parasites types (Table 1). Mean intensity of infection at the Abu'ja station was usually higher in the genus *Amietophrymus*.

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Table 1: Prevalence and intensity of parasites in Anuran species at Abu'ja station

Anuran species	No. examined	No. infected (%)	Intensity*
Ectoparasites			
<i>Amietophrymus regularis</i>	252	10 (4.0)	1.80 ± 0.92
<i>Amietopheymus maculate</i>	164	5 (3.0)	2.60 ± 1.14
<i>Hoplobatrachus occipitalis</i>	168	10 (6.0)	2.10 ± 0.99
		25 (4.3)	
	$\chi^2 = 1.813, p = 0.404$		
Haemoparasites			
<i>Amietophrymus regularis</i>	252	12 (4.8)	6.08 ± 3.94
<i>Amietopheymus maculate</i>	164	10 (6.1)	5.70 ± 3.43
<i>Hoplobatrachus occipitalis</i>	168	15 (8.9)	4.40 ± 3.46

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		37 (6.3)	
		$\chi^2 = 2.971, p = 0.226$	
		Gut parasites	
<i>Amietophrymus regularis</i>	252	29 (11.5)	12.55 ± 10.22
<i>Amietophrymus maculate</i>	164	23 (14.0)	16.48 ± 11.02
<i>Hoplobatrachus occipitalis</i>	168	35 (20.8)	9.26 ± 6.50
Total	584	87 (14.9)	
		$\chi^2 = 7.051, p = 0.029$	

*intensity as mean ± standard deviation. Total in bold font

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A total of 270 anurans were examined at Egwubi station, they were also of same species as found at Abu'ja station. In total, 13 (4.3%) were infected with ectoparasites, 14 (5.2%) with haemoparasites and 56 (20.7%) with gut parasites. The prevalence of ectoparasites and haemoparasites were not different among the three anuran species ($p < 0.05$). However, infection with gut parasites were higher in *H. occipitalis* (37.7%) and *A. maculate* (20.5%) compared to *A. regularis* (14.4%); the difference was significant ($p < 0.05$).

Table 2: Prevalence and intensity of parasites in Anuran species at Egwubi station

Anuran species	No. examined	No. infected (%)	Intensity*
<hr/>			
Ectoparasites			
<i>Amietophrymus regularis</i>	139	6 (4.3)	2.00 ± 0.89
<i>Amietopheymus maculate</i>	78	4 (5.1)	2.25 ± 0.96
<i>Hoplobatrachus occipitalis</i>	53	3 (5.7)	3.00 ± 2.00
13 (4.3)			
$\chi^2 = 0.175, p = 0.916$			
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Haemoparasites			
<i>Amietophrymu sregularis</i>	139	6 (4.3)	1.50 ± 0.55
<i>Amietopheymus maculate</i>	78	5 (6.4)	1.20 ± 0.45
<i>Hoplobatrachus occipitalis</i>	53	3 (5.7)	1.33 ± 0.58

14 (5.2)

$$\chi^2 = 0.467, p = 0.788$$

		Gut parasites	
<i>Amietophrymus regularis</i>	139	20 (14.4)	17.25 ± 12.30
<i>Amietophrymu smaculata</i>	78	16 (20.5)	27.19 ± 12.00
<i>Hoplobatrachchu socciptalis</i>	53	20 (37.7)	18.25 ± 12.58
Total	270	56 (20.7)	

$$\chi^2 = 12.727, p = 0.002$$

*intensity as mean ± standard deviation

4. DISCUSSION

The prevalence of ectoparasites was similar among the three genera of anurans, the ectoparasites fauna includes leech (aquatic blood sucking annelid of class, Hirudinea *Hirudo medicinalis*), and ticks which was diagnosed through gross observation. The prevalence of the haemoparasite was also similar among the three genera of anurans studied and included *Folleyelides* microfilaria, *Trypanosoma* Spp. and *Haemogregarina* spp. Earlier study has reported Flagellates in poison dart frogs [15]. The intensity of gut parasites among the three genera of anurans were relatively higher than that of other parasites. Parasites recovered are nematode parasites which were common among the three genera of anurans in the two wetlands. While the cestode *Baerietta jaegerskioidi* was found only in *Hoplobatrachchus occipitalis* from Egwubi wetland, this is

opposed to the report of [16] that reported the presence of this parasite in *Amietophrymus regularis* nevertheless it could be an indication that the cestodes are not host specific. The nematodes *Ampliceacum africanum*, *Cosmocerca onata* and *Physaloptera* Spp were common in the three genera obtained from the two wetlands, but *A. africanum* had the highest prevalence. This may be due to the fact that savanna zone experiences less rainfall and its vegetation consists mainly of grassland, deciduous trees and differences in their abiotic factors. It is expected that the prevailing ecological conditions may affect the amphibian fauna composition of this area as well as influence the parasites infecting the anuran resident there [17]. Parasites may play a role in promoting biodiversity and functions as indicators of ecosystem productivity and resilience. Among the parasites recovered, nematodes had the highest infection rate. This high rate of nematode infection in the anurans conforms to the finding in Ile-Ife and Awka [18] reported that anurans serve as intermediate hosts of vertebrate parasites especially those inhabiting the same habitat. The cestode parasite recovered occurred in very low prevalence which is similar to the report of [19] but is opposed to the report of [18] where no cestode was recovered from the same kinds of anurans species.

5. CONCLUSION

This study has shown the parasites of anurans from Kogi State, North Central, Nigeria. It also showed the biodiversity of anurans in the study area proving its relative abundance. Effort should be made to protect these anuran species from these parasites as they could be of public health concern if transmitted to humans more so they are of good ecological relevance.

REFERENCES

1. Thompson RCA, Lymbery A, Smith A. Parasites, emerging disease and wildlife conservation. *International Journal for Parasitology*. 2010; 40(10): 1163 -1170

2. Bolek MG, Coggins JR. Seasonal Occurrence and Community Structure of Helminths Parasites in Green Frogs, *Rana clamitans melanata*, from South western Wisconsin, U. S. A. *Comparative Parasitology*. 2001, **68**:164-172.
3. Aisien S, Francisca BA, Kareem B. Helminths parasite of anurans from the savana mosaic zone of Southwestern Nigeria. *Acta Parasitologica*. 2003; **48**(1): 47-54.
4. Louis HD, Richard CT, Rafael D. Polystomatidae (Monogenea) of South African Anura: *Eupolystoma vanasi* n sp. Parasitic in *Schismaderma carens* (smith). *Systematic Parasitology*, 2003; **54**: 34 - 36.
5. Cynthia EG, Monika IH. First Report of Nematode Parasites of *Physalaemus santafecinus* (Anura: Leiuperidae) from Corrientes, Argentina. *Revision of Mexican Biodiversity*, 2010; **81**:677-687.
6. Atef IS, Rufuat K, Mustafa N. Studies on the life cycle and identity of *Paracosmocerca macronata* (Nematode: Cosmocercidae) in amphibians under experimental conditions. *World Journal of Zoology*, 2009; **4**(1): 29 -36.
7. Imasuen AA, Ozemoka HJ, Aisien MSO. Anurans as intermediate and paratenic hosts of helminths infections in the rainforest and derived savanna biotopes of Southern Nigeria. *International Journal of Zoology*. 2012; 1 - 7.
8. Aisien SO, Ugbo AD, Ilavbare N, Ogunbor, O. Endoparasites of amphibians from South-Western Nigeria. *Acta Parasitologica*, 2001; 46(4): 299 - 305.
9. Aisien SO., Ajakaiye FB., Salami K. Helminth parasites of anurans from the savannah-mosaic zone of south-western Nigeria. *Acta Parasitologica*. 2003; 48(1): 47 - 54.
10. Aisien SO, Ayeni F, Ilechie I. Helminth fauna of anurans from the Guinea savanna at New Bussa, Nigeria. *African Zoology*. 2004; 39(1), 133 –136.
11. Verma AK, Singh GA. A Quantitative Analysis of Gastrointestinal Helminths (Trematode: Digenea) Infection in Ranid Frogs in Jammu. *Zoos Print. Journal*, 2000; **15**:233-238.

12. Anderson RC. *Nematode Parasites of Vertebrates: Their Development and Transmission*. 2nd Edition, CAB International, Wallingford Oxon Uk. 650. Anonymous Guidelines for Euthanasia of Nondomestic Animals. American Association of Zoologists and Veterinarians, 2006, New York.
13. Goldberg SR, Bursey CR. Helminthes of Two Frogs, *Lithobates viallanti* (Ranidae) from Costa Rica. *Caribbean Journal of Science*. 2007; **43**: 65 -72.
14. Cheesbrough M. District Laboratory in Tropical Countries, Part 1, 2nd Edition, Cambridge University Press, 2005, Cambridge.
15. Cooper JE. *Host-Parasite relations in reptiles and Amphibians*. Proc. 2001 ARAV Conference, Orlando, Fl. 2001, p. 249-250.
16. Iyaji FO, Medayedupin IT, Echi PC, Falola OO, Omowaye OS. Helminths Parasites of *Amietophrymus regularis* (African Common Toad) in Anyigba community, Kogi State, Nigeria. *Animal Research International*, 2015, **12**(2) xxxx-xxxx
17. Aisien MSO, DuPreez LH, Imasuen, AA. *Polystoma okomuensis* n sp. (Monogenea: Polystomatidae) from Bolengers Striped Frog, *Phlyctimatis boulengeri* (Perret 1986) in Nigeria. *Journal of helminthology*. 2010; **85**:153-159.
18. Nworah DC, Olorunfemi OJ. The Helminth Parasitofauna of *Bufo Regularis* (REUS) in Awka. Anambra State Nigeria. *International Journal of Parasitology Research*, 2011; **3**(2) 26-30.
19. Ayodele HA, Akinpelu AI. The Helminths parasitofauna of *Bufo regularis* (Reuss) in Ile-Ife. *Ife Journal of Science*, 2004; **6**(2): 101-104.

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