AJHSE Vol: 2 (2): 166-182, 2021
DOI: 10.52417/ajhse.v2i2.176
Accepted Date: Nov. 30, 2021
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www.ajhse.org

African Journal of Health, Safety and Environment
An official publication of the
Applied Environmental Bioscience and Public Health Research Group
University of Benin, Benin City, Nigeria
Open Access | Bi-annual | Peer-reviewed | International
ISSN (Online): 2695-1819 | ISSN (Print): 2695-2386

RESEARCH ARTICLE **AJHSE-OWSD-16**

PARASITIC NEMATODE INFECTIONS OF ANURANS FROM A DERIVED SAVANNA BIOTOPE IN EDO STATE, NIGERIA

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ABSTRACT

As part of a broader study to increase the information of parasite infections of anurans from the savanna biotope, an investigation of the nematode parasites of anurans in Agbede, a location in the derived savanna of Edo State, Nigeria was undertaken from August 2007 to July 2008. Anurans were collected at night using the visual acoustic encounter survey (VAES) sampling method, dissected and examined for nematode infections. A total of 329 anurans belonging to sixteen taxa, were examined, of which 235 were infected with an overall prevalence of 71.43%. Seasonal prevalences were 85.63% for wet season and 52.48% for the dry season ($p<0.05$). Ten nematode species, namely: *Amplicaecum africanum*, *Cosmocerca ornata*, *Oswaldocruzia hoeplii*, *Physaloptera* sp., *Rhabdias africanus*, *Rhabdias* sp., *Camallanus dimitrovi*, an unidentified oxyurid nematode, *Ophidascaris* larva, and an unidentified nematode were recovered from the hosts examined. The most common nematode species was *Amplicaecum africanum* (31.31%), whereas the least common was an unidentified oxyurid nematode (0.30%).*Physaloptera* sp. is a new record for the anurans of the derived savanna.

Keywords: *Amphibians, anurans, nematodes, savanna, parasitic, Nigeria*.

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INTRODUCTION

The various bioclimatic zones in Nigeria range from mangrove and fresh water swamps of the Niger Delta, to the rainforest, derived savannah, guinea savannah, Montane Forest and the Sahel savannah of the extreme North (Aisien *et al*., 2017). Investigations of the parasites of amphibians have extensively been carried out in the rainforest biotope (characterized by high levels of precipitation and atmospheric humidity which thus supports high canopy trees and thick undergrowth. These prevailing environmental and atmospheric conditions in the rainforest thus supports the study of the wide diversity of amphibians and their parasites in the country by various authors (Aisien *et al*., 2001, 2003, 2004a, b, 2009, 2011a, 2017; Imasuen and Aisien 2012, 2015, Amuzie *et al*.2018; Oniya and Adeyekun, 2019; Edo-Taiwo and Aisien 2020).

The savannah biotope is one characterized by shorter duration of rainfall (six months) when compared to the rainforest. The vegetation consists of grass and deciduous trees which offers very limited canopy cover. These conditions pose great threat to the survival of amphibians in this terrain and impair the life cycle and transmission of nematode parasites. Unlike anurans from the rainforest which have been studied in great detail for their helminth parasitic fauna, not so much attention has been devoted to anuran species from the savanna biotope. Information available in the literature about studies in the savannas of Nigeria include the studies by Avery (1971); Oladimeji (1990); Aisien *et al*. (2004); Iyaji *et al*. (2015); Anele *et al*. (2020).

The only report on the helminth parasites of anurans from the savanna mosaic zone of Edo State is that undertaken by Aisien *et al*., (2003) from five different locations, excluding Agbede and over a short duration. The latter limitation necessitated the present study to enable a detailed study, carried out over a longer duration. An earlier report dealt with the digenetic trematodes infecting anurans from this study area (Ozemoka and Aisien, 2020). In this paper we report the nematodes parasitizing anurans investigated at Agbede in the derived savannah of Edo State. The study yielded ten nematode parasites with *Physaloptera* sp. as a new record for the anurans of the derived savanna.

MATERIALS AND METHODS

The anurans examined in this study were collected from Agbede, a location in the derived savanna biotope in Edo State, from August 2007 to July 2008, using the Visual Acoustic Encounter Survey (VAES) techniques (Crump and Scott, 1994). Sampling was carried out at night on a monthly basis (once a month) for twelve months. Agbede is located in Etsako West Local Government Area of Edo State, Nigeria and lies roughly within latitude 06º50´N and 06º60´N and longitude 06º1 0´E and 06º20´E (Fig. 1). The anurans collected were identified according to Rödel, (2000). In the laboratory, the anurans were euthanized in Benzocaine solution, dissected and examined. The gastro-intestinal tract (oesophagus, stomach, small intestine, large intestine and rectum) was examined for parasites. Other parts examined included the lungs, body cavity and the urinary bladder. The contents were mixed with 0.72% of NaCl solution and examined under a dissecting microscope. The nematode parasites recovered were fixed in hot 70% alcohol and preserved in the same medium. The worms were cleared in lactophenol and examined as temporary mounts. Photomicrographs of the parasites were taken (using a digital camera attached to a binocular microscope) from which we made line drawings. Prevalence rate of parasites was calculated as a percentage of the number of a particular host species infected with a specific helminth parasite divided by the total number of hosts examined. The

mean intensity of infection refers to the number of parasites per host (calculated only for the infected hosts examined). Differences in the level of significance of infection according to season of infection, were determined using the one-way ANOVA at a 5% level of significance. All statistical analysis were done using SPSS (Statistical Package for the Social Sciences) version 20 for windows.

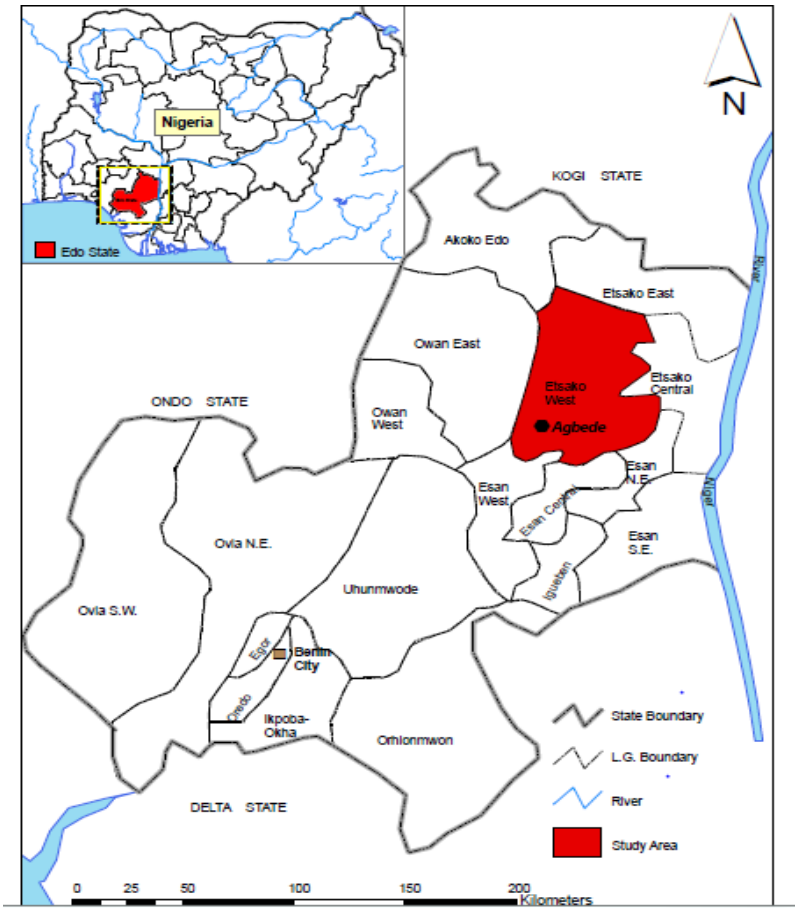

Figure 1. Map of Nigeria showing the study area**.**

RESULTS

In this study, 329 anurans which spread across four families, eight genera and sixteen species were examined. The species of anurans examined included *Sclerophrys regularis*, *S. maculata*, *Sclerophrys* sp. *Arthroleptis poecilonotus*, *Arthroleptis* sp. *Leptopelis viridis*, *Hylarana galamensis*, *Hyperolius occidentalis*, *Ptychadena pumilo*, *P. bibroni*, *P.oxyrhynchus*, *P. schubotzi*, *P. mascareniensis*, *Ptychadena* sp., *Hoplobatrachus occipitalis* and *Phrynobatrachus natalensis*. The study yielded ten nematode parasites as shown in Table 1. Overall prevalence of nematode parasites was 71.43% while infection intensity ranged from 3.00 to 17.33 parasites per infected host. The parasites encountered included: *Amplicaecum africanum*, *Cosmocerca ornata*, *Oswaldocruzia hoeplii*, *Physaloptera* sp., *Rhabdias africanus*, *Rhabdias* sp., *Camallanus dimitrovi*, unidentified oxyurid nematode, *Ophidascaris* larva and an unidentified nematode species (Table 1). Nematode parasites with appreciable prevalence included *A. africanum* (31.3%), *Oswaldocruzia hoeplii* (21.58), *C. ornata* (27.54) and *R. africanus* (15.81%). Prevalence for the remaining

parasites ranged from 0.30 –2.73%. Mixed infections of *A. africanum* and *O. hoeplii* and *A. africanum* and *Physaloptera* sp., respectively were also encountered.

With respect to season, 188 anurans were examined in the wet season and 141 anurans in the dry season. Infection prevalence was higher in the wet season when 161 of the 188 anurans examined were infected with a prevalence of 85. 63%. Prevalence in the dry season was 52.48% (74/141) and these differences was statistically significant ($p<0.05$).

Some nematodes including *A. africanum*, *O. hoeplii C. ornata*, *R. africanus*, C. *dimitrovi*, *Physaloptera* sp. and *Ophidascaris* larva, occurred in both seasons in some hosts while others (e.g. the unidentified Oxyurid nematode) were recorded in the wet season while *Rhabdias* sp., the unidentified nematode species and mixed infection of *A. africanum* and *Physaloptera* sp. were recorded in the dry season (Table 2.)

Among the nematodes recovered, *A. africanum*, *O. hoeplii* and *C. ornata* were generalists as they infected multiple hosts. For example, *Cosmocerca ornata* infected thirteen of the sixteen anuran species examined. It had a prevalence of 27.40%. One of the *S. regularis* examined harboured as many as 70 worms in the rectum. *Oswaldocruzia hoeplii* infected eight of the anuran species examined. The prevalence of this parasite ranged from 0.30% in *P. pumilo, P. mascareniensis, H. galamensis* and *H. occipitalis*, respectively, to 24.62% in *S. regularis*. One of the *S. maculata* examined harboured as many as 24 worms. *Amplicaecum africanum* parasitized six species out of the 16 species examined. The prevalence values for the parasite in most hosts were very low except for *S. regularis* (36.78%). As many as 55 worms were recovered from the stomach of one of the *S. regularis* examined.

Table 1: Overall prevalence and mean intensity of nematode parasites in anurans from Agbede.

Parasite	**Prevalence (%)**	**Mean intensity (±SEM)**	**Infection site**
Amplicaecum africanum	31.31	7.10 ± 0.80	Stomach, s/intestine
Oswaldocruzia hoeplii	21.58	7.52 ± 0.90	Stomach, s/intestine
Cosmocerca ornata	27.40	17.33 ± 1.52	L/intestine/ Rectum
Rhabdias africanus	15.81	7.15 ± 2.26	Lungs
Rhabdias sp.	0.61	3.5 ± 0.50	Lungs
Physaloptera sp.	2.73	5.00 ±1.82	Small intestine
Camallanus dimitrovi	0.61	13.00 ± 9.00	Small intestine
Unidentified oxyurid nematode	0.30	8.00	Stomach
Ophidascaris larva	0.61	3.00	Body cavity
Unidentified nematode	0.61	3.00	Stomach, s/intestine
A. africanum & *O.hoeplii* (mixed infection)	3.64	6.16 ± 0.94	Stomach, s/intestine
A. africanum & *Physaloptera* sp. (mixed infection)	0.30	15.00	Small intestine

Table 2: Prevalence of nematode parasites in the anuran species from Agbede according to season.

Parasite	Wet season		Dry season	
	Prevalence (%)	Mean intensity (±SEM)	Prevalence (%)	Mean intensity (±SEM)
Amplicaecum africanum	22.34	6.52 ± 1.10	43.97	7.44± 1.13
Oswaldocruzia hoeplii	29.26	7.32 ± 1.04	11.34	8.18± 1.50
Cosmocerca ornata	33.51	18. 52± 1.85	19.14	14.60 ± 2.60
Rhabdias africanus	14.90	10.80 ±4.00	17.02	2.91 ± 1.04
Rhabdias sp.	-	-	1.42	3.5 ± 0.50
Physaloptera sp.	4.26	3.62 ± 0.60	1.42	10.50 ± 8.50
Camallanus dimitrovi	0.53	4.00	0.71	22.00
Oxyurid nematode	0.53	8.00	-	-
Ophidascaris larva	0.53	3.00	0.71	3.00
Unidentified nematode	-	-	1.42	3.00
A. africanum & *O. hoeplii* (mixed infection)	3.19	6.83 ± 1.53	4.25	5.50± 1.18
A. africanum & *Physaloptera* sp. (mixed infection)	-	-	0.71	15.00

Rhabdias africanus was recovered from the lungs of *S. regularis* and S. *maculata* with higher prevalence (13.87%) in *S. regularis* than in *S. maculata* (1.82%). Two of the *S. regularis* examined harboured as many as 80 worms each in their lungs. Unidentified *Rhabdias* spp. were recovered *P. bibroni* and *H. galamensis* with prevalence values of 0.30% and 1.22%, respectively. Four *Ptychadena* spp. harboured larval *Physaloptera* sp. with low prevalence values ranging from 0.30% to 1.52%.

DISCUSSION

The number of species of anurans examined in this study, was sixteen which was more than the number that have been reported in other savannah locations (Avery, 1971; Aisien *et al*., 2003, 2004; Iyaji *et al*., 2015; Anele *et al*., 2020). *Xenopus muelleri* was the only anuran reported by Avery, (1971). Eleven species were recorded in the previous study undertaken by Aisien *et al*. (2003) in derived savannah of Edo State. In the Guinea savanna at new Bussa, Aisien *et al*. (2004) recorded and examined only four species. The numbers recorded were understandably so because of the short durations of the respective studies. Our record is however, still higher than the nine species reported by Anele *et al*. (2020) from Zaria in an investigation that spanned over two seasons.

The finding of 10 nematodes species from only one location (Agbede) as reported in this study brings two important points to the fore. There is the need to undertake studies of this nature over longer durations to ensure a proper capture of the parasite diversity present in the anurans of any biotope while also avoiding an over harvest of the anuran hosts. It also gives an insight into the influence of atmospheric humidity and vegetation cover in the transmission of nematode parasites. Although the derived savannah in Edo State has only six months of rainfall, the canopy cover is denser than other savanna biotopes further north. Therefore, anurans from areas with comparable rainfall data with lower canopy cover recorded lower nematode diversity: two in Katsina (Oladimeji *et al*., 1988-1990); four at New Bussa (Aisien *et al*., 2004); three from Ayingba (Iyaji *et al*., 2015) and four from Zaria (Anele *et al*., 2020)

One finding of interest in this study was the occurrence of *Physaloptera* sp. in five anuran species (*P. pumilo*, *P. bibroni*, *P. oxyrhynchus*, and *P. schubotzi* and *H. occipitalis*). This nematode was not recorded in the

anurans investigated earlier by Aisien *et al*., (2003) in the savanna mosaic of Edo State. This nematode was first reported in the anurans from the Gelegele Forest Reserve in Edo State by Aisien *et al*., (2009) in *P. bibroni*, *P. oxyrhynchus* and *P. longirostris*, Thereafter, it has been reported in other anurans from different habitats, including, the brackish water environment (Uwasadhiuka, 2010; Aisien *et al.,* 2015), freshwater creek biotope (Aisien *et al*., 2017), Guinea savanna (Iyaji *et al*., (2015) and cocoa plantations (Edo-Taiwo and Aisien, 2020). In this study, the parasite was recovered from *Pty. schubotzi* which thus becomes a new host record for the parasite. With regards to site of infection, *Physaloptera* sp. was recovered from the stomach of the infected hosts. This is in agreement with the reports of Iyaji *et al.,* (2015), Gonzalez and Hamann (2006) and Edo-Taiwo and Aisien, (2020) who all reported the parasite from the stomach. This is in contrast to the report of Aisien *et al*., (2009), who reported the parasite from the small intestine. Iyaji *et al*., (2015) attributed the occurrence of the parasite in the small intestine to less nutrition in the stomach of captive host, which may have caused the movement of the parasite down to the small intestine.

Amplicaecum africanum infected more hosts (five) than was previously reported (four) by Aisien *et al*., (2003) in other locations of the savanna-mosaic in Edo State and three in the Guinea savanna (Aisien *et al*. (2004). The prevalence of (31. 31%) in *S. regularis* was lower than the value recorded for this host in Auchi (43.5%) but much lower when compared with the report for Igarra, Edo State, where 64.9% prevalence was recorded for the same host (Aisien *et al*., 2003). A much higher prevalence (91.67%) was reported by Iyaji *et al*. (2015) at Ayingba in the same host (formerly *Amietophrynus regularis*). Infections with this parasite have been reported in amphibians and reptiles of countries of East, West and Central Africa (Baker, 1987).

Cosmocerca ornata was the most dominant nematode infecting the anurans from Agbede, infecting 13 of the 16 host species examined. The generalist status of this parasite has similarly been reported in all the bioclimatic zones investigated in Nigeria (Aisien *et al*., 2001, 2003, 2004a, b, 2009, 2011a, 2017; Iyaji *et al*., 2015; Amuzie *et al*., 2018).

The two bufonid anurans in this study (*S. regularis* and *S. maculata*) were both infected with *R. africanus* with an overall prevalence of 15.81% and a mean intensity of 7.15 parasites/ infected host. This prevalence value was however lower than the values recorded in other locations in savanna-mosaic: 36.20% in Igarra, 28.20% in Agenebode in *S. regularis*. The prevalence value recorded in this study was however higher than the 1.4% recorded at Ihievbe 4.8% in Ozalla and 3.5% in Ogbonna (Aisien *et al*., 2003). The *Rhabdias* spp. in *Pty. bibroni* and *H. occipitalis* need to be identified. Kuzim (2001) stressed the need for thorough examination of various *Rhabdias* specimens occurring in the different anurans investigated in Nigeria. *Rhabdias* spp.are lungworms which are problematic and can cause pulmonary damage and eosinophilic pneumonia in captive anurans (Christine and David 2007). *Hoplobatrachus occipitalis* is a new host record for *Rhabdias*.

Camallanus specimens were isolated from three specimens of *H. occipitalis* and one specimen of S. *regularis* with a low prevalence value of 0.61%. Higher prevalence values were reported in *H. occipitalis* (*D. occipitalis*) at Ihievbe (76.5%) and Ogbonna (23.3%) (Aisien *et al*., 2003). The authors also recovered a *Camallanus* sp. from *B. regularis* with a prevalence value of 0.2%. From the findings of this study, there is need for appropriate

description and identification of the parasite from *S. regularis* to determine if it is *Camallanus dimitrovi* or a different species.

Ophidascaris larva occurring in *Pty. mascareniensis* and *Pty. pumilo* has been reported from various anuran species in many locations (Aisien *et al*., 2001, 2003, 2004a, b, 2009, 2011a, 2015, 2017a, b; Imasuen and Aisien, 2015) and *Agama colonarum* (Odigwe, 1985). A recent study by Edo-Taiwo *et al*., (2020) reported high prevalence of this parasite in the body cavity of *S. regularis* (50%), *S. maculata* (31.8) and *Ptychadena oxyrhynchus* (50%) in Benin City. These parasites are believed to use anurans as transport or paratenic hosts (Imasuen *et al*., 2012). Adults of these nematodes have been recovered from snakes in Benin City, Nigeria by Awharitoma *et al*. (2017).

The mixed infection of *A. africanum* and *Physaloptera* sp. in *P. schubotzi* and of *A. africanum* and *Oswaldocruzia hoeplii* in *S. regularis* and in *P. bibroni* is a new phenomenon that have not been previously reported in the anurans from the savanna mosaic.

The wet season recorded a higher prevalence of nematode parasites which further buttresses our earlier assertion that rainfall and canopy cover are important factors in the transmission of nematode parasites. Some nematodes (*A. africanum*, *Oswaldocruzia*, *hoeplii Cosmocerca ornata*, *Physaloptera* sp. *Ophidascaris* larva, *Rhabdias africanus* and Camallanus *dimitrovi*) occurred in both seasons in some hosts, while some others were either recorded in the wet season (unidentified Oxyurid nematode) or in the dry season (*Rhabdias* sp., unidentified nematode and mixed infection of *A. africanum* and *Physaloptera* sp.).

CONCLUSION

In conclusion, all the parasites recorded in this study have previously been reported by other authors in various biotopes. This is the first report of *Physaloptera* sp. from the savanna mosaic zone. *Ptychadena schubotzi* is a new host record for this parasite. *Hoplobatrachus occipitalis* is similarly a new host record for the *Rhabdias* sp. There is need for further research to properly describe and identify *Rhabdias* sp. from anurans in Nigeria. There is also the need for appropriate description and identification of the *Camallanus* specimens from *S. regularis*. The co-occurrence of *A. africanum* and *Physaloptera* sp. in *Pty. schubotzi* as well as *A. africanum* and *Oswaldocruzia hoeplii* in *S. regularis* and in *Pty.bibroni* is a new record.

ACKNOWLEDGEMENT

We acknowledge the cooperation of the Traditional Head of Agbede town, the Agbede Vigilante Group and the Agbede Police Division for providing security during the period of this investigation. Ibrahim Musa and Dauda Musa are also appreciated for their technical assistance during the field collections.

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