Prevalence of helminth partasites in rainbow lizard, *Agama agama* L. (Squamata: Agamidae) in Nsugbe, Anambra State, Nigeria

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Abstract

The study was under taken to determine the prevalence of helminthiasis in the rainbow lizard, Agama agama, in Nsugbe, Anambra State, Nigeria. Two hundred and fifty lizards comprised of 160 males and 90 females, 191 adult and 59 juvenile were caught by hand at night, in their roosting places, and were killed with chloroform in air tight killing jars. The oesophagus, stomach, small, intestine, rectum, visceral cavity, liver and lungs of the lizards were searched for helminths. Four species of helminths namely, Strongyloides brevicaudata, Parapharayngodon awokoyai, Foleyella candezei, (Nematoda) and, Oochoristica truncata (Cestoidea) were recovered from infected lizards. Two hundred and seventeen (217) (86.80 %) of the lizards were infected by helminthes. One hundred and thirty nine (139) (86.88 %) of male lizards and 78 (86.67 %) of females were infected, while 182 (95.29 %) and 35 (59.32 %) of adult and juvenile lizards respectively were infected. Strongyloides brevicaudata, had a prevalence of 85.60 %, followed by P. awokoyai (55.60 %), O. truncata (6.80 %) and F. candezei (2.00 %). Prevalence, abundance, mean abundance and mean intensity of infection did not differ significantly (p=0.05) with sex of lizard but all differed significantly with age of hosts (p=0.05). Two species concomitant infection occurred in 134 (92.41%) of the lizard sample while three species concomitant infection occurred in 11 (7.59%). Infection of the same lizard by S. brevicaudata and P. awokoyai accounted for 49.20% of multiple infections and was the most frequent of coinfections. Most of S. brevicaudata (98.95%) and P. Awokoyai (99.85%) inhabited the rectum, while 76.19 % of F. candezei inhabited the visceral cavity and 89.04% of O. trucanta occurred in the small intestine. The rectum was the most parasitized organ. Strongyloides brevicaudata, and P. awokoyai were recorded for the first time in A. agama in south-east Nigeria. It is suggested that S. brevicaudata is the most prevalent helminth parasite of A. agama in Nigeria, and that more studies on the helminth parasites of Nigerian reptiles should be under taken.

Keywords: Agama agama, Nsugbe, helminth parasite, concomitant infection.

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Introduction

The rainbow lizard, *Agama agama* (Linnaeus, 1758) (Sny *A. colonarum*) occurs throughout much of equatorial Africa from Senegal to Ethiopia and South to Angola and Tanzania, feeding primarily on insects and occasionally on plant materials. (Aden *et al*, 1995; Spawls *at al*, 2006; Harris, 1964), playing important role in the food-web of its terrestrial habitats. While feeding mainly on insects, it is in itself fed on by some carnivorous vertebrates, notably snakes and some birds of prey. Thus, the niche of *A. agama* in its habitat is multi-dimensional and its role in the balance of nature could be quite significant.

Over time A. agama has notched up a close ecological link with man. It roosts on walls of human dwellings and very frequently makes feeding runs into human houses, appearing undeterred by physical presence of man, quite unlike other wild vertebrates that would normally be scared by human presence. The close ecological association between humans and A. agama carries, a yet to be evaluated health, risk for the human

population. This is because the lizard has been reported to be hosts to a number of possible human parasites from different taxonomic divides, which include *Haemogregarina* species, *Eimeria* oocyst, *Plasmodium* (all protozoans) (Wekhe and Olayinka, 1999); *Lecudina* species (Protozoa) (Babero and Okpala, 1962; Wekhe and Olayinka, 1999), *Foleyella candezei* (Nematoda) (Babero and Okpala, 1962; *Capillaria* species (Nematoda) (Babero and Okpala, 1962; Cheng, 1999; Roberts and Janovy, 2006; Adeoye and Ogunbanwo, 2007), *Enterobius vermicularis*, *Hymenolepis nana*, *Taenia* sp. (all Cestoidea) (Biu *et al*, 2014) and pentastomes (Lindner, 1965, Adeoye and Ogunbanwo, 2007).

In spite of the medical and health implications of the presence of possible human parasites, some of which could cause fatal consequences to humans (Cheng, 1999), in *A. agama*, the study of parasite fauna of lizards in Nigeria appeared not to have attracted the attention of many parasitologists. Consequently, thesre is paucity of literature on the subject matter. Available information





seemed to be limited to those of Babero and Okpala (1962), Avery (1971), Obiamiwe and Iredu (1982), Wekhe and Olayinka, (1999), Biu *at el* (2014), Okafor (1988), Omonona *et al* (2011), and Adeoye and Ogunbanwo (2007). There is therefore an important need to undertake more studies on parasite fauna of *A. agama* in Nigeria.

The paper reports on investigation of helminth parasites of *A. agama* in Nsugbe, Anambra State, Nigeria.

Materials and methods

The study-area

The study was carried out in Nsugbe community in Anambra East Local Government Area of Anambra State, Nigeria, with the consent of the local government authority and the leadership of Nsugbe Community. Nsugbe is one of the communities located within the River Anambra plains which lies between Longitudes 6030' and 7015'E and Latitudes 6010' and 708'N (Ezenwaji and Ilozumba, 1992). The climate is tropical rainforest type with double rainfall maxima which occur in July and September. Annual temperature ranges from 24°C to 31°C (Odo, 2004), while mean annual rainfall is between 1500 mm and 2000 mm (Ilozumba, 1980). The vegetation is equatorial rainforest type, although pressured to meet the varied needs of the ever growing human population, it has decimated the original vegetation, thus creating a derived savannah in most places. Nsugbe is a rural community and most of her adult population are engaged in agricultural activities, notably crop production and fishing. Lizards and other reptiles are important members of the fauna, and are active all year round.

Collection and examination of lizards for infection by helminths

Two hundred and fifty (250) *A. agama* comprising 160 male and 90 female were captured by hand at night in their roosting places. Forty seven (47) of the 160 males and 12 of the 90 females were juveniles.

The lizards were killed with chloroform in air tight killing jar. The snout-vent length and the weight of each lizard were thereafter measured, using a measuring board and Adams electronic balance (Model AQP 600) respectively. The sex of adult lizards was determined using the colour differences between adult male *A. agama* and the female, as well as the presence of preanal pads in the males (Harris, 1964). Juvenile lizards were identified as male through the presence of preanal pads, and a pair of ovoid testes within the visceral cavity. Both features are absent in females.

The contents of the oesophagus, stomach, small intestine and rectum were washed into separate Petri dishes which contained normal saline and thoroughly searched for helminths, using a hand-lens and a dissecting microscope. The visceral cavity of each lizard was carefully searched for helminths using a hand lens, while the liver and lungs were teased out in Petri dishes and examined with a dissecting microscope. The linings of

the different sections of the alimentary canal were scrapped with scalpel blade, and the scrapings were examined as wet mount, using low and medium power objectives. Note was taken of the contents of the stomach which served as clue to the food of the lizards. Recovered helminths were washed in cold normal saline, killed, counted and preserved for identification. Live nematodes were killed in hot 70% alcohol and then preserved in cold 70% alkanol to which a few drops of glycerine were added to reduce extreme dehydration by the alcohol. Cestodes were allowed to die in warm normal saline and then preserved in 4% formaldehyde solution. For identification, nematodes were cleared in undiluted glycerol or lactophenol while castodes were stained with Delafields hematoxylin. Cleared and stained parasites were mounted in Canada balsam and examined with Leica ATC 2000 compound microscope using low and medium power objective lenses. The helminths were identified using the keys of Babero and Okpala (1962), Yorke and Maplestone (1969) and Wardle et al (1974). The identities of the helminths were confirmed by Dr. Charles Bursey of Pennstate-Shenango, Sharon PA 16146, USA. Infection parameters were defined in accordance with the terminologies of Bush et al (1997) as follows:

Prevalence =
$$\frac{\text{Number of infected host}}{\text{Number of host examined}} \frac{100}{1} \%$$

Abundance = Total number of parasites collected from all infected hosts

Number of parasites of a species collected from Infected hosts

Mean Abundance = Number of host examined

Mean intensity of Infection = $\frac{\text{Number of parasites of a species}}{\text{Number of infected Host}}$

The infection parameters were subjected to statistical test using *chi*-squared (x^2) test.

Results

Two hundred and seventeen (217) (86.80%) of the 250 A. agama examined were infected by helminth parasites. One hundred and thirty nine (139) (86.88%) of 160 male lizards were infected while 78 (86.67%) of the 90 females were infected. One hundred and eighty two (182) (95.29%) adult lizards and 35 (59.32%) juveniles were infected. Differences in prevalence, of helminth parasites in male and female lizards was not significant (p=0.05).

Three species of nematodes namely, Strongyloides brevicaudata, Mueller 1894, Parapharyngodon awokoyai, Babero and Okpala 1962, Foleyella candezei, Fraipont (1882), Securat (1917), and one species of cestode, Oochoristica truncata, Krabbe (1976), Zschoke (1905), were recovered from infected lizards. S. brevicaudata had a prevalence of 85.60%, P. awokoyai 55.60%, F. candezei 2.00%, and O. truncata 6.80% (Table 1).

Table 1. Overall prevalence of helminth parasites in *A. agama* in Nsugbe, Anambra State, Nigeria.

Helminth species	No. of Lizards infected	Prevalence (%)		
S. brevicaudata	214	5.60		
P. awokoyai	139	55.60		
F. candezei	5	2.00		
O. truncata	17	6.80		

Table 2. Prevalence of helminth parasites in *A. agama* in Nsugbe, Anambra State, by sex of host.

Helminth species	Number Infected	Prevalence (%)	Abundance	Mean abun- dance	Mean intensity
Male (<i>n</i> =160)					
S.					
brevicaudata	138	86.25	3169	19.80	22.96
P. awokoyai	34	52.50	381	2.38	4.56
F. candezei	3	1.88	11	0.07	3.67
O. truncata	10	6.25	41	0.26	4.1
Female (<i>n</i> =90)	ı				
S.					
brevicaudata	76	84.44	1099	12.21	14.46
P. awokoyai	55	61.11	291	3.23	5.29
F. candezei	2	2.22	10	0.11	5.00
O. truncata	7	7.78	32	0.36	4.57

Infection parameters for the recovered helminths by sex of lizard is shown on Table 2. It is seen from the Table, that for *S. brevicaudata* all infection parameters had higher values for male lizards, than for females. For *P.awokoyai*, abundance was higher for males, but other parameters-prevalence, mean abundance, and mean intensity of infection, had higher values for female lizards. *Foleyella candezei* had higher values of prevalence, mean abundance and mean intensity of infection for females, but higher abundance for males. The trend in infection parameters for *O. truncata* was similar to those for *F. candezei*. The differences in infection parameters between male and female lizards for the four helminth species were not significant (*p*=0.05).

Infection parameters for the helminth parasites for adult and juvenile lizards are shown on Table 3. The table shows that all infection parameters had higher values for adults lizard than for juveniles. The differences were significant (*p*=0.05). *Foleyella. candezei* and *O. truncata* were not recovered from juvenile lizards.

Concomitant (mixed) infection occurred in 145 (58.00%) of the lizard sample. One hundred and thirty four (134) (92.41%) of infected lizards harboured two species of helminths while 11 (eleven) (7.59%), harboured three species of helminths (Table 4). Strongyloides brevicaudata occurred in all recorded cases of infection, and its combination with *P. awokoyai* was the most frequent concomitant infection, accounting for 49.20% of such events. Infection involving the pair of

S. brevicaudata and O. truncata was the second most frequent, accounting for 3.20 % of infection, while the pair of S. brevicaudata and F. candezei accounted for 1.20%. Three species concomitant infection involving the triads of S. brevicaudata, P. awokoyai and O. truncata on the one hand, and S. brevicaudata, P. awokoyai and F. candezei on the other hand, were recorded. No case of F. candezei and O. truncata infecting the same lizard was encountered in the study. Also, and no case of all four helminth parasites infecting the same lizard was recorded.

Table 3. Prevalence of heminth parasites in *A. agama* in Nsugbe, Anambra State, by age of host.

Helmith species	Number infected	Preva- lence (%)	Abun- dance	Mean abundance	Mean intensity
Adult (<i>n</i> =191)					
S.					
brevicaudata	181	94.76	4078	21.35	22.53
P. awokoyai	118	61.78	619	3.24	5.25
F. candezei	5	2.62	21	0.11	4.20
O. truncata	17	8.90	73	0.38	4.29
Juvenile (n=90))				
S.					
brevicaudata	33	55.93	190	3.22	5.75
P. awokoyai	21	35.95	53	0.89	2.52
F. candezei	0	0.00	0.00	0.00	0.00
O. truncata	0	0.00	0.00	0.00	0.00

Table 4. Concomitant infections in *A. agama* in Nsugbe, Anambra State, Nigeria.

Helminth parasites combinations	No. Infected (<i>n</i> =250)	Frequency (%)	
S. brevicaudata and P. awokoyai	123	49.20	
S. brevicaudata and O. truncata	8	3.20	
S. brevicaudata and F. candezei	3	1.20	
S. brevicaudata, P. awokoyai and O. truncata	9	3.60	
S. brevicaudata, P. awokoyai and F. candezei	2	0.80	
Total	145	58	

The distribution of helminth parasites in the organs of *A. agama* in Nsugbe is shown on Table 5. Most of *S. brevicaudata* (98.95%) and *P. awokoyai* (99.85%) were recovered from the rectum. Most of *F. candezei* (76.19%) were recovered from the visceral cavity, while 23.81% were recovered from the stomach. Most of *O.*

truncata (89.04%) were taken from the intestine, while 10.96% were recovered from the stomach. Items found in the stomach of the lizards were a variety of insects –

beetles, grasshoppers, termites, ants and occasionally grass.

Table 5. Distribution of helminth parasites in the organs of A. agama in Nsugbe, Anambra State, Nigeria.

Parasite	Abundance						
		Oes.	Sto.	Int.	Rec.	Vca	Lun
S. brevicaudata	4268	2 (0.05%)	0 (0.00%)	43 (1.01%)	4223 (98.95%)	0 (0.00%)	0 (0.00%)
P. awokoyai	672	1 (0.15%)	0 (0.00%)	0 (0.00%)	671 (99.85%)	0 (0.00%)	0 (0.00%)
F. candezei	21	0 (0.00%)	5 (23.81%)	0 (0.00%)	0 (0.00%)	16 (76.19%)	0 (0.00%)
O. truncata	73	0 (0.00%)	8 (10.96%)	65 (89.04%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Total	5034 (100.00%)	3 (0.06%)	13 (0.26%)	108 (1.15%)	4894 (97.22%)	16 (0.32%)	0 (0.00%)

Key: Oes. = Oesophagus; Sto. = Stomach; Int. = Small intestine;

Rec. = Rectum; Vca. = Visceral cavity; Lun. = Lung: (%) = percentage of helminth population.

Discussion

A prevalence of 86.80% for helminthiasis in *A. agama* in Nsugbe recored in this study is high, although it is lower than the figures of 100%, and 97.6% reported for *A. agama* in Lagos, Nigeria, by Babero and Okpala (1962) and Adeoye and Ogunbanwo (2007) respectively. It is also lower than a prevalence of 92% reported by Biu *at al* (2014) for the same species in Maiduguri, Nigeria, while Omonona *et al* (2011) recorded 87.50% prevalence of helminthiasis for *A. agama* in Ibadan, Nigeria. These results coming from different regions of the country suggest that high prevalence of helminthiasis could be natural to *A. agama* in Nigeria.

Prevalence and other infection parameters were found to be independent of sex, but they varied significantly with age. Babero and Okpala (1962), and Adeoye and Ogunbanwo (2007) obtained similar results in their studies of helminthiasis in A. agama in Lagos, Nigeria. Similarly, Amo et al (2005) and Fadiel et al (2005) reported that sex did not appear to influence prevalence of helminthiasis in the Mediterranean lizard, Lacerta lepida and the Libyan lizard Chalcides ocellatus, respectively. It would therefore seem that sex does not play significant roles in helminthiasis in lizards generally. On the other hand, this study recorded significant differences in all infection parameters investigated between adult and juvenile lizards, as did Adeoye and Ogunbanwo (2007) and Amo et al (2005). Since adult and juvenile A. agama feed on similar items, as discerned from examination of stomach contents, and there is no age-related immunity in the lizard (Adeoye and Ogunbanwo (2007), differences in prevalence of helminthiasis between adult and juvenile lizards could be attributed partly to differences in length of exposure to infective agents, and partly to differences in physical size. Naturally, adult lizards by the fact of their haven lived for a longer period of time in the habitat than juveniles, would have had greater numbers of encounter with infective agents, and, therefore would have accumulated more parasites in comparison to the juveniles. Also, the larger physical size of the adult lizards would naturally translate to larger spatial compartments and, therefore, larger potential habitats for helminths, which would translate to higher abundance, since more helminths would be accommodated by the larger adults as deposed by Adeoye and Ogunbanwo (2007).

Three out of the four species of helminths recorded from *A. agama* in this study were nematodes namely, *S. brevicaudata, P. awkokoyai* and *F. candezei*. Nematodes were also more prevalent in lizards from Lagos (Babero and Okpala, 1962; Adeoye and Ogunbanwo, 2007), in Ibadan (Omonona *et al*, 2011), and, in Maiduguri (Biu *et al*, 2014). It would therefore seem that nematodes are the most prevalent helminth parasites of *A. agama* in Nigeria. Borkovcova and Kopriva (2005) reported that nematodes were the most frequent helminths of Czech reptiles. The findings of this and the other studies in Nigeria indications that such may be the case with reptiles in Nigeria. Studies of helminth fauna of reptiles in Nigeria will help to elucidate the situation, and enable a more unequivocal conclusion to be drawn about the matter.

Stronayloides brevicaudata had been reported from different geographical locations in Nigeria (Babero and Okpala, 1962; Avery, 1971; Adeoye and Ogunbanwo. 2007; Omonona at al, 2011), and would therefore appear to be the commonest nematode parasite of A. agama in

Nigeria. The same species had also been reported to infect other African agamids in Kenya (Schmidt and Canaris, 1968) and Tanzania (Baylis, 1919), and could therefore pass for a cosmopolitan helminth of African lizards. To the best of our knowledge, the finding of *S. brevicandata* and *P. awokoyai* in *A. agama* in this study is the first report of the helminths in *A. agama* from south-east Nigeria and therefore constitutes a new geographical record for the two species.

Capillaria, Oxyuris, and Thelandros species (all nematodes) which were found in A. agama in other locations in Nigeria (Babero and Okpala, 1962; Adeoye and Ogunbanwo, 2007; Omonona at al, 2007) were not recorded in the present study. Other helminth species reported from A. agama in Nigeria but which were not recorded in the present study include Monocoelum species (Trematoda) (Babero and Okpala, 1962; Adeoye and Ogunbanwo, 2007), Enterobius vermicularis, Heterakis spumosa (nematodes) (Biu et al, 2014) Adeoye and Ogunbanwo (2007) also found Raillietiella (Pentastomida) in A. agama in Lagos, but no pentastomid was recovered in the lizard sample used in the present study. Examination of larger samples of A. agama in Nsugbe will help to ascertain the correct situation as concerns the above listed species in A. agama in Nsugbe. However, there is need to revisit the identity of the parasites listed as Oxyuris, Enterobius vermicularis, Heterakis spumosa, Taenia species and Hymenolepis nana by Biu et al (2014), because those species have so far been regarded as parasites of mammals (Yorke and Maplestone, 1969; Cheng, 1999; Roberts and Janovy, 2006).

The distribution of helminth parasites in the organs of A. agama in the present study could be attributed to host environment factors and parasite physiology. Oochoristica truncata (cestode) was found in the small intestine mainly. Cestodes lack alimentary canal and as such depend on host digestive physiology for nutrients. They are limited to the parts of the host where soluble nutrients which they can absorb through the tegument are available, and this happens to be the intestine (Kennedy, 1975). Nematodes generally possess functional digestive system and by virtue of that fact can inhabit more diverse locations which could differ in nutrient availability. This aspect of their biology could explain the finding of nematodes in the stomach (F. candezei), intestine (S. brevicaudata), rectum (S. brevicaudata and P. awokoyai) and visceral cavity (F. candezei). Adeoye and Ogunbamwo (2007) attributed the large nematode community of the rectum of A. agama to abundance of undigested food in that location. In the present study, 97.22% of the helminths recovered inhabited the rectum, and only nematodes were found to infect that organ. This result gives credence to the opinion of Adeoye and Ogbunbanwo (2007). That F. candezei and O. truncata were not found in the same host individual in the present study is worthy of note.

Analysis of distribution of helminth species in infected lizards reveal that both species could inhabit the small intestine, and both occured in different combinations of concomitant infection with the other species. More studies, involving more samples from more locations in the study area need to be undertaken in order to elucidate the correct situation as concerns concomitant infection of *A. agama* by *F. candezei* and *O. truncata*.

The result of this and similar studies conducted in Nigeria show high prevalence of helminthiasis generally but variations in the helminth fauna of A. agama. There is therefore need for more studies to be undertaken in different parts of the country so as to establish species diversity and richness of the helminth fauna of A. agama more accurately. Foleyella candezei, one of the helminths recovered in the present study, which is transmitted by haematophagous anthropod vector (Anderson, 2000), could be of clinical significance to other animal populations in Nigeria (Babero and Okpala, 1962). In the face of such possibilities, and the report of human infection by Railletiella species in Ibadan (Lindner, 1965). There is need for Nigeria parasitologists to enkindle interest in the study of parasites of A. agama and other lizards, so that the health-risk to man of A. agama contact can be properly evaluated.

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