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CO-OCCURRENCE OF *METAHAEMATOLOECHUS* AND *RHABDIAS* SPECIES IN THE LUNGS OF *HOPLOBATRACHUS OCCIPITALIS* SPECIMENS FROM PARTS OF RIVERS STATE, NIGERIA

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ABSTRACT: *Co-occurrence of Metahaematoloechus and Rhabdias species, lung parasites of amphibians, is a rare occurrence. It was supposed that both parasites excluded each other. This research investigated their co-occurrence in the anuran, Hoplobatrachus occipitalis, from two locations (Rumuesara and Agbada-2 flow station) in Rivers State, Nigeria. A total of forty-seven H. occipitalis specimens were collected from both locations. Nine hundred and thirteen (913) parasites (comprising of 574 Metahaematoloechus and 339 Rhabdias species) were recovered from their lungs. Among the twenty-three hosts captured at Rumuesara, nine were co-infected with Metahaematoloechus and Rhabdias species, accounting for a prevalence of 39.13%. Ten (43.5%) of the hosts were infected with only Metahaematoloechus sp., and four (17.4%) were not infected with either parasite. One of the host specimens from Agbada-2 was co-infected with both parasites, giving a prevalence of 4.2%. Eighteen (75%) of the hosts were infected with only Metahaematoloechus; five (20.8%) were not infected with either parasite. In both locations, no host was infected with Rhabdias alone. A preference for co-infection with both parasites was observed in the frogs from Rumuesara, in the rainy season. However, frogs from Agbada-2 preferentially harboured only Metahaematoloechus sp. It appeared that conditions around the flow station hindered the establishment of Rhabdias. Rhabdias sp. was completely absent from both locations during the dry season. Female hosts generally harboured more of both parasites. It is hereby established that Metahaematoloechus and Rhabdias can co-exist in the same host. There is however, need for further research into the effect gas flaring on the establishment of Rhabdias sp. in H. occipitalis.*

KEYWORDS: Co-occurrence, Metahaematoloechus, Rhabdias, Lung, Hoplobatrachus Occipitalis, Nigeria

INTRODUCTION

Trematodes, otherwise known as flukes or digeneans, may be the most widely recognized of the amphibian parasites (Densmore and Green, 2007). The trematodes *Metahaematoloechus* sp., are commonly known to infect the lungs of *Hoplobatrachus occipitalis* specimens all over the world. These digenean parasites of amphibians, are a species-rich genus with more than 50 species around the globe (Leon-Regagnon, 2011). *Rhabdias* species are a diverse group of nematodes infecting amphibians and reptiles. About forty species of *Rhabdias* have been described from amphibian and reptile hosts globally (Kuzmin *et al*., 2003). According to Shenandoah *et al*., (2010), the parasite exists as protandrous hermaphrodites in the lungs of infected hosts.

LITERATURE

Hoplobatracchus occipitalis specimens have been reported from many amphibian researches conducted in different parts of Nigeria (Anosike and Keke, 2002; Akani *et al*., 2011; Aisien *et al*., 2001; Ezemonye and Enuneku, 2011; Taiwo *et al*., 2014).

Cabrera-Guzmán *et al*. (2010) stated that the semiaquatic habits of frogs and the availability of particular feeding resources appear to determine the helminth composition and infection levels; however, co-speciation events also play an important role structuring these helminth communities. Details of co-speciation of *Metahaematoloechus* sp. and *Rhabdias* sp. may not be available, but research on the subject is important to establish the relationships between the two species.

Langford *et al*. (2013) reported on the co-occurrence of *Metahaematoloechus complexus* and *Rhabdias joaquinensis* in the Plains Leopard Frog from Nebraska. They found that *H. complexus* and *R. joaquinensis* do not competitively exclude each other from the lungs of *Lithobates blairi* in southeastern Nebraska. Their results showed that 256 *Rhabdias joaquinensis* and 225 *Metahaematoloechus complexus* were found in the lungs of *Lithobates (=Rana) blairi*. Thirty-six of the 44 (82%) frogs were co-infected with *H. complexus* and *R. joaquinensis* whereas 5 (11%) frogs were infected with only 1 species and 3 (7%) frogs were uninfected. Tests for association between *H. complexus* and *R. joaquinensis* found a significant positive relationship between the two parasites.

Anthropogenic activities such as urbanization have effects on amphibian populations and the parasite communities infecting them (Sparling *et al*. 2001; Aisien *et al*., 2008). There is paucity of data in Nigeria on the co-occurrence of both parasites in the lungs of their hosts. We hereby present results of investigation into the co-occurrence of *Metahaematoloechus* and *Rhabdias* in the lungs of *H. occipitalis* caught from Rumuesara and Agbada-2 flow station, Rivers State, Nigeria.

METHODOLOGY

Description of Sampled Locations: Rumuesara area was a developing residential area, with several houses under construction, and the vegetation was mostly secondary growth. The access road was not tarred, though it was still motorable. There was a block moulding industry, an aluminium window manufacturing shop, and other activities associated with housing construction. Agbada-2 flow station, on the other hand, was mostly forest vegetation. It was characterized by a vertical, constant gas flaring column. There were a few residential houses, fewer than at Rumuesara, about 100m away from the flow station.

Forty-seven specimens of *Hoplobatrachus occipitalis* were caught from two locations in Rivers State, Nigeria, for the isolation and numeration of *Metahaematoloechus* sp. and *Rhabdias* sp. between the months of June, 2015 and March, 2016. The first location was Rumuesara in Esara kingdom with the co-ordinates E4^{o} 54$^{'}$ 26.37$^{''}$, N7^{o} 1$^{'}$ 58.128$^{''}$, while the second area surveyed was Agbada-2 Flow Station (E 4^{o} 55$^{'}$ 57.006$^{''}$, N 7^{o} 1$^{'}$ 13.692$^{''}$), close to Igwuruta, Rivers State, Nigeria.

Both areas were visited once monthly during the period of investigation, and as many *Hoplobatrachus occipitalis* specimens as could be captured between the hours of 7.00pm to 10.00pm were collected. The visual encounter and acoustic survey method (VEAS) Method (Crump and Scott, 1994) was employed for the sampling, combined with auditory surveys for calling anurans at night, on each sampling day in the selected site with the aid of flashlights. Visual searches and refuge examinations, such as under fallen logs, stones and in leaf litter, were also done.

The anurans were transported to the Department of Animal and Environmental Biology, Rivers State University, Port Harcourt, for further investigation. The anurans were identified using appropriate keys (Roedel, 2000), and euthanized by exposure to Chloroform vapour in a tightly closed jar. Measurements of snout vent length (SVL) and total body weight (TBW) were taken for each specimen.

The specimens were dissected and the lungs were placed in a Petri dish containing 0.72% saline solution for examination and collection of parasites. The *Metahaematoloechus* sp. were flattened between two microscope slides and fixed in 5% formol saline. The fixed specimens were preserved in the same medium (fixative).

Permanent mounts were made in Canada Balsam after the worms were washed to remove the fixative, stained in acetocarmine for 6 hours, dehydrated in alcohol series (50%, 70%, 90% and 100%) and cleared in Xylene (50% and 100%). Species of *Rhabdias* were fixed in hot 70% alcohol and preserved in fresh 70% alcohol. They were cleared in lactophenol before examination. Parasites were identified to the possible taxonomic level with the aid of keys from Prudhoe and Bray (1982).

Prevalence and mean intensity of helminth parasites were calculated according to Bush *et al*., 1997.

RESULTS AND DISCUSSION

Forty-seven hosts were captured and investigated for the co-ocurrence of *Metahaematoloechus* sp. and *Rhabdias* sp. in their lungs. A total of 913 individuals of both parasites were recovered from all hosts examined. This was made up of 574 *Metahaematoloechus* species and 339 *Rhabdias* sp.

In the host specimens from Rumuesara, a total of 301 *Metahaematoloechus* sp. and 338 *Rhabdias* sp. were recovered, while in those from the Agbada-2 flow station area, a total of 273 *Metahaematoloechus* and only one *Rhabdias* species were recovered (Table 1). Both parasites were more abundant in Rumuesara than in the gas flaring community of Agbada-2 flow station. In both sites, however, it was observed that the occurrence of *Rhabdias* sp. was only in the presence of *Metahaematoloechus* and only during the rainy season. *Metahaematoloechus* sp., on the other hand, occurred alone in the lungs of ten host specimens from Rumuesara and in eighteen from Agbada-2 flow station.

Of the twenty-three (23) hosts, mean SVL 8.8±1.12 and mean TBW 56.0±19.82, collected from Rumuesara, 15 were males and there were 8 females, and a total of 639 parasites were recovered from their lungs. Twenty-four hosts, 14 males and 10 females, were collected from the Agbada-2 flow station. They had a mean SVL of 9.1±1.55 and mean TBW of 57.4±21.13; a total of 274 parasites were recovered from their lungs.

Prevalence and mean intensity of infection were variously computed for both sites. In the hosts from Rumuesara, 9 (39.1%) out of the 23 hosts were co-infected with *Rhabdias* and *Metahaematoloechus*; none was infected with only *Rhabdias*; 10 (43.5%) were infected with only *Metahaematoloechus* sp., and 4 (17.4%) were not infected with either of the parasites. In the hosts collected from Agbada-2 flow station, only 1 (4.2%) host was infected with both *Metahaematoloechus* and *Rhabdias* in their lungs; none was parasitized with only *Rhabdias* sp., while 18 (75%) hosts were infected with only *Metahaematoloechus*. Five hosts (20.8%) were neither infected with *Metahaematoloechus* nor *Rhabdias* species (Figure 1).

The total prevalence of infection with *Metahaematoloechus* was 82.6% at Rumuesara while mean intensity was 16. The total prevalence of infection with *Rhabdias* was 39.1%, while mean intensity was 38. At the Agbada-2 flow station, the total prevalence of infection with *Metahaematoloechus* was 79.2%, while mean intensity was 14. Total prevalence of infection with *Rhabdias* sp. was 4.2%, and the mean intensity was 1.

The prevalence of infection with both *Metahaematoloechus* and *Rhabdias* occurring together was found to be 39.1%, and the mean intensity was 49 in the hosts from Rumuesara. Prevalence and mean intensity were also calculated for infection with only *Metahaematoloechus*, 43.5% and 19, respectively. In the host specimens from the flow station area, prevalence of infection with both *Metahaematoloechus* and *Rhabdias* was calculated to be 4.2%, while mean intensity was 5. Prevalence of infection with only *Metahaematoloechus* was 75%, while mean intensity of infection with only *Metahaematoloechus* was 15.

As such, it was found that the prevalence and mean intensity of infection with both *Metahaematoloechus* and *Rhabdias* co-occurring in the lungs of *Hoplobatrachus occipitalis* specimens was higher in the host specimens collected from Rumuesara than in those collected from the area around the flow station. It appeared that habitat alteration associated with construction of houses, such as cutting down of forest cover, and the presence of secondary and isolated stands of grass bushes favoured the co-establishment of both *Metahaematoloechus* and *Rhabdias*. *Rhabdias* sp., in particular, flourished in the frogs collected from Rumueasara. It was recorded only once at the flow station area, raising questions as to the effect of gas flaring on the ability of *Rhabdias* sp. to get established in the frogs collected from areas around a flow station. The prevalence and mean intensity of infection with *Rhabdias* were both higher at Rumuesara than at Agbada-2 flow station.

The lung fluke, *Metahaematoloechus* sp., did not appear to be hindered at both sites. In the host specimens from Rumuesara, prevalence and mean intensity of infection with only *Metahaematoloechus* were 43.5% and 20 respectively, while at Agbada-2 flow station, they were 75% and 15, respectively. More frog hosts at Agbada-2 flow station were infected with only *Metahaematoloechus* than at Rumueasara. Aisien *et al*., 2001, stated that the overall prevalence (%) of *Metahaematoloechus exoterorchis* and *Rhabdias bufonis* recovered from *Dicroglossus* (now *Hoplobatrachus*) *occipitalis* from south-western Nigeria were 24.0 and 9.8 respectively, but did not state whether both parasites co-existed in the same host.

The preference of host sex to co-infection with *Metahaematolochus* and *Rhabdias* was examined only in the hosts from Rumuesara. This is because only one host collected from the flow station was infected with both lung parasites, and it was a male host. So, in the Rumuesara frogs, out of the nine hosts co-infected with *Metahaematoloechus* and *Rhabdias*, four were males and 5 were females. The four male host specimens were altogether infected with 39 *Metahaematoloechus* sp. and 73 *Rhabdias* sp., making a total of 112 parasites. The five female host specimens were found to be infected with more of both parasites: 65 *Metahaematoloechus* sp., and 265 *Rhabdias* sp., giving a total of 330 parasites. As such, the female *H. occipitalis* specimens were more parasitized with both parasites hereby investigated than the males. Other researchers, however, report a higher infection of helminth parasites in male than in female hosts (Begum and Banu, 2012; Oladimeji *et al*., 1990). In fact, Kennedy and Lie (1974) stated that the female sex estrogen might inhibit the rate or ease of infection with parasites.

The effect of season was also evaluated among the hosts from Rumuesara. The rainy season covered the period from June to November, 2015. The total number of hosts was 17, while the number of hosts co-infected with both lung parasites was 9, and 7 were infected with only *Metahaematoloechus*, while one host was not infected. Prevalence and mean intensity of infection with both parasites were 52.9%, and 49, respectively. On the other hand, prevalence and mean intensity with only *Metahaematolochus* were found to be 41.2%, and 19 respectively. During the rainy season, the lungs of the frogs were preferentially co-infected with both parasites.

In the dry season, which spanned from December, 2015 to March, 2016, only six hosts were captured at Rumuesara; none of them was co-infected with both parasites. Three were infected with only *Metahaematoloechus* and another three were free from both parasites. So, prevalence and mean intensity of infection with only *Metahaematoloechus* were 50% and 22, respectively. Infection with *Rhabdias*, either alone or in association with *Metahaematoloechus* was not observed in the dry season. Actually, earlier workers commonly reported higher rate of infection during the rainy season (Chandra and Gupta, 2007; Begum and Banu, 2012). The complete absence of *Rhabdias* in the dry season could be due to the absence of standing pools of water which promoted contact between the larval infective stage and the anuran host (Shenandoah *et al*., 2010).

CONCLUSION

It is concluded that *Metahaematoloechus* and *Rhabdias*, both helminth parasites infecting the lungs of frogs, can occur together in the same lungs of the same host specimen. They do not necessarily exclude each other even in hosts where they occur independently. In such cases, some other factors may be responsible for the non-coexistence. It is also noted that *Rhabdias* and its co-occurrence with *Metahaematoloechus* was recorded only during the rainy season. The species appeared to be adversely affected by dry season conditions. It was also observed that the presence of a flow station seemed to hinder the establishment of *Rhabdias* infection in *H. occipitalis* specimens, a trend which was not observed in infection with *Metahaematoloechus* species. It is also of note that in both locations surveyed, *Rhabdias* sp. was only found in co-infection with *Metahaematoloechus*; it never infected any host unaccompanied. However, *Metahaematoloechus*, co-occurred with *Rhabdias*, but preferentially occurred alone in a larger number of hosts.

Future Research

The impact of gas flaring activities on *Rhabdias* species need further investigation.

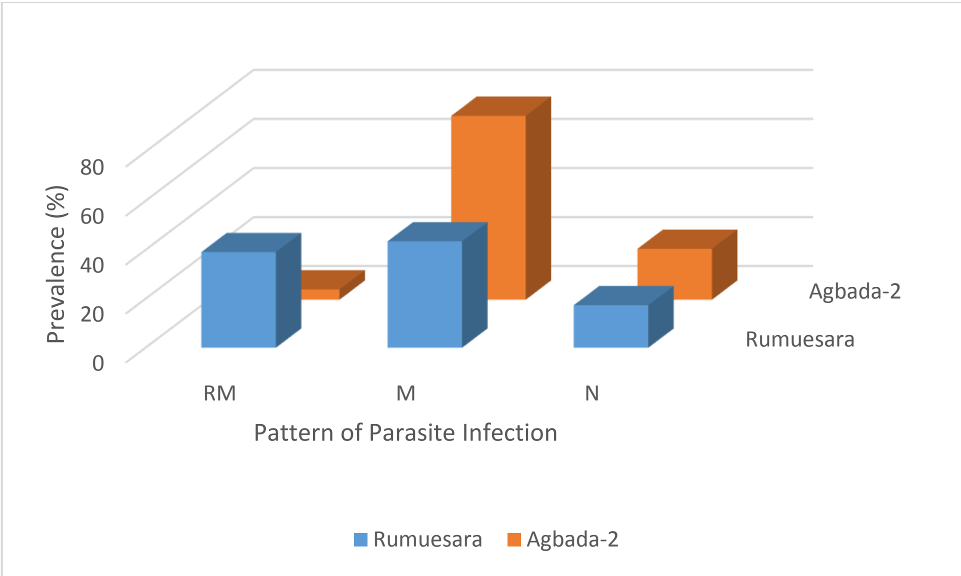

Fig. 1: Prevalence of Lung Infections in *H. Occipitalis* from the Study Locations

(Key: RM=co-occurrence of Rhabdias and Metahaematoloechus; M=infection with Metahaematoloechus alone; N=non-infection with either parasite)

Table 1: Summary of Host and Parasite Characteristics from Study Locations

			Rumuesara	Agbada-2
Host	Sex	No. of Male Specimens	15	14
		No. of Female Specimens	8	10
	Size	SVL Range (cm)	6.9-10.4	6.3-12.1
		TBW Range (cm)	24.6-95.5	22.0-98.1
Parasites		Total No. of *Metahaematoloechus* Recovered (Range)	301 (0-51)	273 (0-71)
		Total No. of *Rhabdias* Recovered (Range)	338 (0-160)	(0-1)
		No. of Hosts Co-infected	9	1
		No. of Hosts Infected with only *Metahaematoloechus*	10	18
		No. of Hosts Infected with only *Rhabdias*	0	0
		No. of Hosts not Infected with Either Parasite	4	5

REFERENCES

Aisien, S.O., Ogoannah, S.O., and Imasuen, A.A. (2008). Helminth parasites of amphibians from a rainforest reserve in south-western Nigeria. African Zoology, 44, 1-7.

Aisien, S.O., Ugbo, A.D., Ilavbare, A.N. and Ogunbor, O. (2001). Endoparasites of amphibians from south-western Nigeria. Acta Parasitologica, 46, 299-305.

Akani, G.C., Luiselli, L., Amuzie, C.C. and Wokem, G.N. (2011). Helminth community structure and diet of three Afro tropical anuran species: a test of the interactive-versus-isolationist parasite communities hypothesis. Web Ecology, 11, 11-19.

Anosike, J.C. and Keke, I.R. (2002). A survey of gastrointestinal helminths of frog (*Dicroglossus occipitalis*) in south eastern Nigeria. African Journal of Applied Zoology and Environmental Biology, 4, 47-49.

Begum, A. and Banu, N. (2012). Sex, organal and seasonal differences of helminthofauna of toad, *Bufo melanostictus* (SCHNEIDER, 1799). Bangladesh Journal of Zoology, 40: 155-164.

Bush, A.O., Lafferty, K.D., Lotz, J.M. and Shostak, A.W. (1997). Parasitology meets ecology on its own terms: Margolis *et al*., revisited. Journal of Parasitology, 83, 575-583.

Cabrera-Guzmán, E., Garrido-Olvera, L. and León-Règagnon, V. (2010). Helminth parasites of the leopard frog *Lithobates* sp. Colima (amphibia: ranidae) from Colima, Mexico. Journal of Parasitology, 96, 736-739.

Chandra, P. and Gupta, N. (2007). Habitat preference and seasonal fluctuations in the helminthofauna of amphibian hosts of Rohilkhand Zone. Indian and Asian Journal of Experimental Science, 21, 69-78.

Crump, M. and Scott Jr, N. (1994). Visual encounters surveys. In: Measuring and monitoring biological diversity - standard methods for amphibians, W. Heyer, M. Donnelly, R. McDiarmid, L. Hayek and M. Foster (Ed.), 84-91, Smithsonian Institution Press, ISBN-10 1560982845, Washington, United State of America.

Densmore, C.L. and Green, D.E. (2007). Diseases of amphibians. *ILAR J (2007)* 48, 235-254. *doi:* 10.1093/ilar.48.3.235..

Ezemonye, L.I. and Enuneku, A.A. (2011). Histopathological alterations in the liver and lungs of *Hoplobatrachus occipitalis* exposed to sub lethal concentrations of cadmium. Australian Journal of Basic and Applied Sciences, 5, 1062-1068.

Kennedy, C.R. and Lie, S.F. (1974). The distribution and pathogenecity of strongyllids (nematode) in brown trout *Salmo trutta* (L) in Feraworthy Reservoir. Devonian Journal of Fishery of Scotland, 8, 293-302.

Kuzmin,Y., Tkach,V.V. and Snyder, S.D. (2003). The Nematode Genus *Rhabdias* (Nematoda: Rhabdiasidae) from Amphibians and Reptiles of the Nearctic. Comparative Parasitolology, 70(2), 101–114.

Langford, G.J., Vhora, M. S., Bolek, M.G. and Janovy Jr., J. (2013). Co-Occurrence of *MetaMetahaematoloechus complexus* and *Rhabdias joaquinensis* in the plains leopard frog from Nebraska. Journal of Parasitology, 99, 558-560.

Leon-Regagnon, V. (2011). Evidence of new species of *Haematoloechus* (Platyhelminthes: Digenea) using partial *coxl* sequences. Mitochondrial DNA, 21, 12-17.

Oladimeji, A.A., Abuh, S.J. and Sadiku, S.O.E. (1990). Parasitic helminths of the frog *Dicroglossus occipitalis.* Nigerian Journal of Parasitology, 9-11, 145-148.

Prudhoe, S. and Bray, R.A. (eds.) (1982). Platyhelminth parasites of the amphibian. British museum (Natural History). Oxford University Press, London.

Rodel, M.O. (2000). Herpetofauna of West Africa, Vol. 1: Amphibians of the West African savanna. *Edition Chimaira*, Frankfurt/M.

Shenandoah, R.M., Johnson, S.A., Haraa, A.H. and McGarrity, M.E. (2010). Preliminary evaluation of the potential of the helminth parasite *Rhabdias elegans* as a biological control agent for invasive Puerto Rican coquís (*Eleutherodactylus coqui*) in Hawaii. Biological Control, 54, 69–74.

Sparling, D.W., Fellers, G.M. and McConnell, L.L. (2001). Pesticides and amphibian population declines in California, USA. Environmental Toxicology and Chemistry, 20, 1591–1595.

Taiwo, I.E., Amaeze, N.H., Adie, P.I., and Otubanjo, O.A. (2014). Heavy metal bioaccumulation and biomarkers of oxidative stress in the wild African tiger frog, *Hoplobatrachus occipitalis.* African Journal of Environmental Science and Technology, 8, 6-15.