

Original Research Article

HELMINTHOFAUNA OF THREE WILD *ACHATINA SPECIES* FROM A RURAL COMMUNITY OF SOUTH EASTERN NIGERIA: A POTENTIAL PUBLIC HEALTH PROBLEM

Abstract

A total of 405 land snails were collected by hand picking from the study area between November 2018 and August 2019. The snails were morphologically identified using standard procedures. Shannon Weiner index was used to determine the distribution of the snail species during sampling. Light and teasing methods were used to isolate some of the parasites from the snails. The snails were dissected and different parts of the viscera were examined for parasites, using standard parasitological techniques. The isolated parasites were identified using parasitological guides. *A. achatina* was the most abundant snail species while *A. marginata* was the least abundant. Greater numbers of snails were collected in the wet season than in the dry season, with no observed complete dominance of any of the species. In the dry season, *A. fulica* was infected by almost all the parasites, with an overall highest prevalence of 32.69%. More snails were infected in wet season in comparison with the dry season, with higher prevalence of (46.91% vs 32.69%). There was co-infection of the snails with at least two of the parasite species. In both wet and dry seasons, *A. marginata* had an overall least infection prevalence of 11.77% and 4.55% respectively while *A. fulica* was infected most with prevalence of (46.91% vs 32.69%). Proper cooking of snails, proper washing of vegetables, public health education and provision of good toilet facilities are recommended for control of snail-borne parasites especially the zoonotic ones.

Keywords: Helminthofauna, Achatina species, Rural Community, Problem, Nigeria

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INTRODUCTION

Achatina species are a group of snails that belong to the family Achatinidae. The genus *Achatina* has many species that include *Achatina fulica*, *Achatina achatina* and *Archachatina marginata*. These three are collectively known as giant African land snails (GALS). These snails have been reported to have originated from Africa and spread to other parts of the world. *A. achatina* originated from the West African rainforest belt from Guinea through Nigeria while *Archachatina marginata* has been reported to be a native of the humid African forest belt whose descent has been traced from southern Nigeria to Congo [1]. *A. fulica* has been reportedly distributed from Africa to other tropical and sub-tropical continents of the world and this wide spread distribution has been attributed to anthropologic activities [2] including transport and trade [3]. The spread of this species to new regions was propelled by its need as food, medicine, pets and for aesthetic purposes [4]. Fagbuaro *et al.* [5] has reported *A. achatina*, *A. fulica* and *A. marginata* as the common edible snails in Nigeria. Snails are phytophagous invertebrates that feed on variety of plant materials such as leaves, fruits and tubers. Some of them like *A. fulica* have been noted for being invasive and devastating crop pests wherever they are introduced.

Snail meat has become important delicacy for many Africans and its consumption has been on the increase, extending to new places of the world where it was not originally recognized as important kind of meat. Snail meat consumption is both nutritional and medicinal. The mineral composition of *Achatina species* has been reported to include zinc, iron, magnesium, calcium, phosphorus, potassium and sodium [5]. *Achatina* contains high levels of various minerals that can complement the minor and trace elements needed for proper human growth and development [1]. It has been reported that snail meat can provide the entire amino acid needs in humans [6] and contains high iron content with low fat [7]. Snail meat is commonly known as ‘Congo meat’ in Nigeria and has

become the most desirable source of meat. The shift in consumption behavior from some other types of meat to snail meat has been attributed to health challenges that arise from red meat consumption. Snails have been reported to constitute an important source of animal protein [5]. In addition to the nutritional values of edible land snails, they have been reported to play salient roles in folk medicine in some African countries and are known to contain certain aphrodisiac properties [1].

It is unfortunate and paradoxical that snails that have both nutritional and medicinal values, harbour wide array of different helminthic parasites of public health concern. Wild snails are known to be phytophagous and coprophagous and feed on various decomposing matter. As they forage, they are exposed to infections by soil-transmitted helminthes, which have been reported to survive and develop in certain climatic conditions of optimum temperature and humidity [8], similar environmental requirements for the snails to survive and thrive. In addition to exposure to infections by geohelminths, wild land snails are hosts to other public health zoonotic parasites, including the dangerous rat lungworm nematodes known as *Angiostrongylus cantonensis* and *Angiostrongylus costariensis* [9]. *A. cantonensis* is commonly known as the rat lungworm because its adult stage is always found in the pulmonary arteries of the definite hosts, which are rats that belong to two species called *Rattus rattus* and *Rattus norvegicus*. The intermediate hosts of *A. cantonensis* are snail species from as many as 51 families, including the Achatina species. The paratenic hosts include frogs, crabs, shrimps, toads, planarians and lizards whereas humans are accidental hosts who can be infected by consumption of raw or poorly cooked snail meat or any of the paratenic hosts or vegetable contaminated with the third stage larvae (L₃). In humans, the larvae migrate to the brain where their development is terminated and it eventually dies. The niche of the adult of *A. cantonensis* is in the pulmonary arteries of the definitive hosts (*R. rattus* or *R.*

norvegicus). The female worms lay eggs in the arteries and the eggs develop into the third stage larvae (L₃) that migrate to the inside part of the alveoli, the pharynx and are swallowed. They pass down the gastrointestinal tract and are passed out in faeces. The snails become infected by ingestion of rat dung that contains the first stage (L₁) larvae of the parasite. Inside the snail intermediate hosts, the larvae moult two times within 21 days post infection to become infective third stage (L₃) larvae. The rats become infected by ingesting the L₃ larvae-infected snail intermediate hosts. The larvae penetrate the intestinal wall and migrate to the blood stream and reach the pulmonary circulation and are dispersed to various organs through arterial circulation. Some of them enter the brain and moult to become L₄ larvae and again finally moult to become the 5th larvae (L₅) and finally migrate to the pulmonary arteries within 25 days of infection. The worms mature approximately 35 days post infection and L₁ larvae of the parasite can be found in the rats' faeces after 42 days of exposure to the previous generation of L₁ larvae. The life cycle is completed when any of the land or aquatic snails ingest infected rat faeces or through larval penetration.

Human angiostrongyliasis can be characterised by abdominal symptoms or eosinophilic meningoencephalitis, depending on the causative parasite species. *A. costariensis* has been reported to be the aetiologic agent that causes a pathological abdominal syndrome that is characterised by eosinophilic infiltration, vascular abnormality and granulomatous reactions [10]. *A. cantonensis* causes eosinophilic meningoencephalitis with characteristic presumptive clinical signs that include eosinophilic meningitis, eosinophilic encephalitis and ocular angiostrongyliasis [11]. It has been reported that human angiostrongyliasis is usually mild and self-limiting and that recovery occurs within one week, though heavy infections that are characterised by paraesthesiae and muscular weakness may persist for years [12]. Massive exposure to infective L₃ larvae has

been reported to be fatal in some cases [13]. Clinical symptoms characterized by severe headache, stiffness of neck, nausea, vomiting, confusion, blurred vision and hyperaesthesia have also been reported [14]. Less commonly reported observed symptoms include muscle weakness, retro-orbital pain, ataxia, abdominal pain, body and extremity aches, convulsion, facial paralysis, somnolence and incontinence [15,16].

The parasitic infections of edible land snails are enormous and cannot be overemphasized. Therefore, this study appraises the helminth parasite status of some edible land snails in the study area and reports the helminthofauna of three wild *Achatina species* from rural settlements of an eastern state in Nigeria: a potential public health problem.

Materials and Methods

The study Area

The study was carried out in Oshiri Community in Onicha Local Government Area of Ebonyi State, Nigeria. Ebonyi State is characterized by tropical rainforest vegetation, with two distinct seasons of dry and wet. The former commences from November and ends in March while the later starts from April and stops in October. Oshiri community is located on latitude $6^{\circ}8'5^{11}N$ and Longitude $7^{\circ}53'22^{11}E$. Farming is the main occupation of the people in the state, an occupation that is probably encouraged and favoured by the availability of expanse fertile arable lands and surrounding Ebonyi River and its tributaries. Rice, yams, maize, sweet potatoes and cassava are the main crops cultivated in the state. Apart from the metropolitan city of Abakaliki, other parts of the state are rural with limited facilities such as good toilet systems, tap water supply, and electricity. In communities of the LGA, including Oshiri (the study area), open defecation on farmlands and bushes is still being practised.

Snail collection and Identification

The snail species used in the study were collected two times in a month (fortnightly) from Nov 2018 to August 2019. The snails were collected by hand picking mainly in the evening hours (between 7:00pm and 9:00pm) from farm lands, gardens, bushes and on forest floors. Also, some of them were collected during daytime in holes and in tree trunks. Snails were identified by comparison of shell morphology, adhering to instructions accordingly [3,17,18,19,20].

Isolation and Identification of Parasites

Isolation of parasites followed the methods according to Cheesbrough [21] and Onyishi *et al.* [22]. The method according to Onyishi *et al.* [22] describes light and teasing procedure which involves dipping the snail in a water container and exposing it under electric light for 2 hours and thereafter transferring the content to a petri dish and examining it for parasites. The shell of each snail was broken to expose the body of the snail. The snail species were further dissected to expose the entire viscera including the stomach and intestine which were separately dissected and teased in petri dishes containing normal saline and examined, using wet preparation techniques. The contents of the alimentary canal were further processed with formal ether concentration technique. The tissues of the snails were further digested within 1hr at 37⁰C, as modified from Wallace and Rosen [23]. The isolated parasites were identified according to guides and illustrations of [17,21,24,25,26]. The identification of recovered larvae of *Angiostrongylus* was limited to genus level.

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Did they not have a standardized sampling methodology? There are numerous articles published in Brazil in which Fischer determined a sampling protocol. I highlight some:

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https://www.scielo.br/scielo.php?pid=S1676-06032005000100014&script=sci_arttext

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https://www.researchgate.net/profile/Marta_Fischer/publication/216828840_Estimativa_e_inferencias_do_metodo_de_controle_do_molusco_exotico_Achatina_fulica_BOWDICH_1822_STYLOMMATOPHORA_ACHATINIDAE_em_Pontal_do_Parana_Litoral_do_Estado_do_Parana/links/056895ac42a146c66313ec0c.pdf

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Statistical Analysis

Shannon Wiener index was used to determine the distribution of the snail species during sampling. Data obtained were presented using descriptive statistics.

Results and discussion

A. achatina was the most abundant snail species (N = 182) and the highest number (N= 90) of snails was collected in May 2019. However, there was no complete dominance of any of the snail species across the months on the course of snail collection ($D = \sum p_i^2 = 0.1447$). Similarly, the Shannon Wiener index value ($H = -\sum p_i \ln(p_i) = 2.0065$) indicated that there was almost equal abundance of snails from the three species across the months of sampling (Table 1).

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Table 1: Species-specific distribution of monthly collected snails in rural villages of Oshiri Community

| Month/year | <i>A. fulica</i> | <i>A. achatina</i> | <i>A. marginata</i> | Total | Ratio (Pi) | D (Pi ²) | H (Pi ln Pi) |
|--------------|------------------|--------------------|---------------------|-------|------------|----------------------|--------------|
| Nov, 2018 | 18 | 25 | 10 | 53 | 0.1309 | 0.0171 | -0.2662 |
| Dec, 2018 | 11 | 18 | 07 | 36 | 0.0889 | 0.0079 | -0.2152 |
| Jan, 2019 | 10 | 17 | 05 | 32 | 0.0790 | 0.0062 | -0.2005 |
| Feb, 2019 | 13 | 15 | 00 | 28 | 0.0691 | 0.0048 | -0.1847 |
| May, 2019 | 20 | 31 | 39 | 90 | 0.2222 | 0.0498 | -0.3342 |
| June, 2019 | 24 | 35 | 13 | 72 | 0.1778 | 0.0316 | -0.3071 |
| July, 2019 | 17 | 29 | 06 | 52 | 0.1284 | 0.0165 | -0.2636 |
| August, 2019 | 20 | 12 | 10 | 42 | 0.1037 | 0.0108 | -0.2350 |
| Total | 133 | 182 | 90 | 405 | 1.0000 | 0.1447 | 2.0065 |

A. fulica was infected by all the parasites except hookworms. It had the highest prevalence of all the parasites and an overall highest prevalence value of 32.69%. *Angiostrongylus* infected *A. fulica* most when compared with infection prevalence across other snail species. *A. marginata* was only susceptible to *Ascaris lumbricoides*, with a prevalence of 4.55% (Table 2).

Table 2: Dry season prevalence of parasites among *Achatina species* from rural villages of Oshiri Community

| Parasite Taxa | Snail species | | | | | |
|------------------------|---------------------------|----------------|---------------------------|----------------|------------------------------|----------------|
| | <i>A. fulica</i> (N = 52) | | <i>A. achatina</i> (N=75) | | <i>A. marginata</i> (N = 22) | |
| | Number Infected | Prevalence (%) | Number Infected | Prevalence (%) | Number Infected | Prevalence (%) |
| <i>Angiostrongylus</i> | 8 | 15.39 | 2 | 2.67 | 0 | 0.00 |
| <i>Hookworms</i> | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| <i>A. lumbricoides</i> | 6 | 11.54 | 3 | 4.00 | 1 | 4.55 |
| <i>S. stercoralis</i> | 3 | 5.77 | 0 | 0.00 | 0 | 0.00 |
| Total | 17 | 32.69 | 5 | 6.67 | 1 | 4.55 |

Table 3 shows wet season prevalence of parasites among *Achatina species* from rural villages of Oshiri Community. All snail species were infected with different species of parasites (i.e. co-infections) with the exception of *A. marginata* that was not susceptible to *Strongyloides stercoralis*. *A. fulica* was infected most with an overall prevalence of 46.91% and was also infected most by *Angiostrongylus*. However, *A. marginata* had an overall infection prevalence of 11.77%, with highest infection prevalence observed in *A. lumbricoides*.

Table 3: Wet season prevalence of parasites among *Achatina species* from rural villages of Oshiri Community

| Parasites | Snail species | | | | | |
|------------------------|---------------------------|----------------|----------------------------|----------------|------------------------------|----------------|
| | <i>A. fulica</i> (N = 81) | | <i>A. achatina</i> (N=107) | | <i>A. marginata</i> (N = 68) | |
| | Number Infected | Prevalence (%) | Number Infected | Prevalence (%) | Number Infected | Prevalence (%) |
| <i>A. cantonensis</i> | 21 | 25.93 | 5 | 4.67 | 1 | 1.47 |
| <i>Hookworms</i> | 2 | 2.47 | 10 | 9.35 | 2 | 2.94 |
| <i>A. lumbricoides</i> | 13 | 16.05 | 12 | 11.22 | 5 | 7.35 |
| <i>S. stercoralis</i> | 4 | 4.94 | 2 | 1.87 | 0 | 0.00 |
| Total | 38 | 46.91 | 29 | 27.10 | 8 | 11.77 |

A total of 405 *Achatina species* were collected from November 2018 to August 2019. More snails were collected in the wet season (May 2019 to August 2019) than in the dry season months (November 2018 to February 2019). *A. achatina* was the most abundant species while *A. marginata* was the least abundant, though no complete dominance was observed. The highest number of snails was collected in May. That more snails were collected in the rainy season than in the dry season is in line with the normal biology of snails. Snails hibernate under prolonged dry conditions and aestivate under prolonged hot conditions. The period of the year with extreme weather conditions are spent in dormancy, a behavioural survival strategy that sustains the different species. During hibernation and aestivation, only a few of the snails are seen foraging on the ground surfaces. Hence, the fewer number collected in the dry season and more in the wet season. It has been reported that Nigerian snails aestivate in the dry weather and when the phenomenon of aestivation is in progress, the aperture is usually temporarily closed by epiphragm and when rains begin to fall, the epiphragm opens and releases the snail to forage [27,28]. The

highest number of snails collected in May is in line with the report of Hodasi [29], who observed that *Achatina* breeds in the main rainy season from April to July.

In the present study, *A. achatina* was the most abundant. This finding does not agree with Okeke *et al.* [30], who reported *A. achatina* to be second in abundance in their study at Awka, Anambra State, Nigeria. However, the finding in the present study that *A. marginata* was the least abundant snail species, is in line with Naggs and Raheem [31] and Okeke *et al.* [30]. In this study, *A. fulica* was found to have been infected by all the parasites with the exception of hookworms. This observation is in agreement with previous reports that some snail species are more susceptible to certain parasites than others with multiple infections [8]. It was observed that *Angiostrongylus* infected *A. fulica* more than the other snail hosts. It has been reported that *A. fulica* is the major snail host of *Angiostrongylus species* all over the world [32] and that observation has been attributed to its ubiquity and high level of susceptibility to the parasite [33].

The prevalence of parasites among the snails was higher in the wet season when compared with that of the dry season and the parasites were observed to be more dispersed among the snail species in the wet season.

Hookworms that infected no host in the dry season and *S. stercoralis* that infected only *A. fulica* in the dry season infected all the snail hosts with high prevalence in the wet season. Igbinosa *et al.* [8] made and reported a similar observation. This observation could be attributed to the fact that the snail species had the freedom of mingling together and transmitting the parasites as they come out to feed. The larvae (L_1) of *Angiostrongylus species* under normal conditions should survive better in the rainy season and should be able to penetrate the snail intermediate hosts better in the rainy season than in the dry season. In the dry season, the phenomena of aestivation and hibernation reduce snail exposure to the parasites because only very few snails forage during that period. In

the wet season, the soil-transmitted helminthes (Hookworms, *A. lumbricoides* and *S. stercoralis*) infected almost all the snail species with higher prevalence than in the dry season. Most of the farmlands and bushes that the snails inhabited were contaminated with human faeces as a result of lack of good toilet facilities that encourage open land defaecation by the people of the study area. As the wild snails forage, they become more exposed to the geohelminths that have been reported to survive and develop in climate conditions of optimum temperature and humidity [8] as observed in the wet season months.

Angiostrongylus infected all the snail host species, an indication of wide host range and non-host-specificity. This observation has been previously reported and has been known to also occur among the freshwater snail intermediate hosts [34]. *A. lumbricoides* infected all the snail intermediate hosts in both seasons, with less prevalence in the dry season. The versatility in infectivity of *A. lumbricoides* with respect to seasons and hosts as observed in the present study has been reported and attributed to the resilience of its ova which has been reportedly observed to withstand extreme environmental conditions [35]. In addition, the mucopolysaccharides that coat the ova have been reported to be responsible for rendering them adhesive to environmental surfaces [36].

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REFERENCES

1. JR Cobbinah, A. Vink and B. Onwuka. Snail Farming in West Africa: Production, Processing and Marketing. A Practical Guide. Technical Centre for Agriculture and Rural Cooperation. 2008.
2. E Dharmaraju. Transport and spread of crop pests in Tropical Polynesia. Pages 257-272. In: Commerce and the spread of Pests and Disease Vectors, Laird, M. (Editors). Praeger, New York, USA. 1984
3. JC Bequaert. (1950). Studies in *Achatinidae*, a group of land snails. *Bulletin of Museum of Comparative Zoology*, 1950; **105**(1), 1-216.
4. RH Cowie and DG Robinson. Pathways of introduction of non-indigenous land and freshwater snails and slugs: Pages 93-122. In: *Invasive Species Vectors and Management Strategies*. Ruitz, J.M and Carlton, J.T (Editors). Island Press, Washington, USA. 2003
5. O. Fagbuaoro, JA Oso, JB Edward and RF Ogunleye. Nutritional status of four species of giant land snails in Nigeria. *Journal of Zhejiang University Science B*, 2006; **7**(9), 686-689. doi:10.1631/jzus.2006.B0686.
6. EI Adeyeye. Waste yield, proximate and mineral composition of three different types of land snails found in Nigeria. *International Journal of Food Science and Nutrition*, 1996; **42**(2), 111-116.
7. OM Agbogidi, BC Okonta and EI Ezeani. Effects of two edible fruits on the growth performance of African giant land snail (*Archachatina marginata*. Swainson). *ARPJN Journal of Agricultural and Biological Sciences*, 2008; **3**(3), 26-29.
8. IB Igbinoso, C Isaac, HO Adamu and GC Adeleke. Parasites of edible land snails in Edo State, Nigeria. *Helminthologia*, 2016; **53**(4), 331-335. DOI10.1515/helmin-2016-0031.
9. DM Spratt. (2015). Species of *Angiostrongylus* (Nematoda: Metastrongyloidea) in wildlife: a review. *International Journal of Parasitology: Parasites and Wildlife*, 2015; **4**, 178-189.
<http://dx.doi.org/10.1016/j.ijppaw.2015.02.006>.
10. C Graeff-Teixeira, L Camillo-Coura and H Lenzi. Abdominal angiostrongyliasis and undiagnosed disease. *Memorias do Instituto Oswaldo Cruz*, 1987; **82**(4), 353-354.
11. K Sawanyawisuth and K Sawanyawisuth. (2008). Treatment of Angiostrongyliasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2008; **102**(10), 990-996.
12. CE Archer, CC Appleton, S Mukaratirwa and KJ Hope (2011). The rat lungworm, *Angiostrongylus cantonensis*: a first report in South Africa. *South African Medical Journal*, 2011; **101**(3), 174-175.
13. ARMC Lima, SD Mesquita, SS Santos, ERP Aquino, AO Teixeira, ZRS Carta and MLB Ferreira. Neuroinfestation by *Angiostrongylus cantonensis* in Recife, Pernambuco Brazil. *Arq. Neuro-Psiquiat*, 2009; **67**(4), 1093-1096.
14. AL Morassutti, SC Thiengo, M Fernandez, K Sawanyawisuth and CG Teixeira. Eosinophilic Meningitis caused by *Angiostrongylus cantonensis*: an emergent disease in Brazil. *Memorias do Instituto Oswaldo Cruz*. 2014; **109**(4), 399-407. doi:10.1590/0074-0276140023.
15. Q Wang, D Lai, X Zhu, X Chen and Z Lun. Human angiostrongyliasis. *The Lancet Infectious Diseases*. 2008; **8**(10), 621-630.

16. K Sawanyawisuth, K Takahashi, T Hoshuyama, V Senthong, P Limpawattana, PM Intapan, D Wilson, S Tiamkao, S Jitpimolmard and V Chotmongkol. Clinical factors predictive of encephalitis caused by *Angiostrongylus cantonensis*. *American Journal of Tropical Medicine and Hygiene*, 2009; **81**, 698-701.
17. LR Ash. Diagnostic morphology of the third stage larvae of *Angiostrongylus cantonensis*, *Angiostrongylus vasorum*, *Aeluro strongylus rostratus* (Nematoda: Metastrongyloidea. *Journal of Parasitology*, 1970; **56**, 249-253.
18. CYL Schotman. Data sheet on the giant African land snail *Achatina fulica* Bowdich (Mollusca: Achatinidae). Food and Agriculture Organization Regional Office of Latin America and the Caribbean. Plant Quarantine Action Programme. 1989; 16-21.
19. D Herbert and D Kilburn. Field Guide to the Land Snails and Slugs of Eastern South Africa. Pietermaritzburg: Natal Museum, South Africa. 2004
20. KC Emberton. (2010). Diversities and distribution of 80 land snail species in Southeastern most Madagascan rainforest with a report: lowlands are richer than high lands in endemic and rare species. *Biodiversity and conservation*, 2010; **6**, 1137-1154.
21. M Cheesbrough. *District Laboratory Practice in Tropical Countries, Part 1*. Cambridge University Press, United Kingdom. 2010.
22. GC Onyishi, IO Aguzie, JO Okoro, CD Nwani, N Ezenwaji, NS Oluah and FC Okafor. Terrestrial snail fauna and associated helminth parasites in tropical semi-urban zone, Enugu State, Nigeria. *Pakistan Journal of Zoology*, 2018; **50**(3), 1079-1085. DOI: <http://dx.doi.org/10.17582/journal.pjz/2018.50.3.1079.1085>.
23. GD Wallace, and L Rosen. (1969). Techniques for recovering and identifying larvae of *Angiostrongylus cantonensis* from mollusks. *Malacologia*, 1969; **7**, 427-438.
24. IR Ash and TC Orihel A Guide to Laboratory Procedure and Identification of Parasites: ASCP Press, Chicago. 1991.
25. PL Chiodini, AH Moody and DW Manser. Atlas of Medical Helminthology and Protozoology. Fourth Edition. Harcourt Publishers Limited. 2003
26. RH Cowie. Biology: Taxonomy, Identification, and Life Cycle of *Angiostrongylus cantonensis*. Rat lungworm Disease Scientific Workshop Held between 16-18 August, 2011 in Honolulu, Hawai. 2011
27. RH Nisbet. The life of Archachatiniidae in London. Proceedings of Malacological Society of London. 1974; **41**, 171-183.
28. AB Odaibo. Snail and Snail Farming: Nigerian Edible Land Snails. Stirling Hordon Publishes, Ibadan, Nigeria. 1997
29. JKM Hodasi. (1979). Life history studies of *Achatina (Achatina) Achatina* (Linne). *Journal of Molluscan Studies*, 1979; **45**(3), 328-339.
30. JJ Okeke, CE Samuel, TC Mogbo, DC Ezeonyejiaku, CA Otikpa, CO Okoye and CH Asolo. (2016). A study on the diversity of snail species within Nnamdi Azikiwe University, Awka. *Research Journal of Agriculture and Environmental Management*, 2016; **5**(7), 207-212. <http://www.apexjournal.org>.
31. F Naggs and D Raheem. Sri Lankan snail diversity: faunal origins and future prospects. Records of the Western Australian Museum Supplement. 2005; **68**, 11-29.
32. J Alicata. Biology and distribution of the rat lungworm, *Angiostrongylus cantonensis* and its relationship to eosinophilic meningoencephalitis and other neurological disorders of man and animals. *Advances in Parasitology*, 1965; **3**, 223-248.

33. A Vitta, R Polseela, S Nateeworanart and M Tattiyapong (2011). Survey of *Angiostrongylus cantonensis* in rats and giant African land snails in Phitsanulok Province, Thailand. *Asian Pacific Journal of Tropical Medicine*. 2011; **4**, 597-599.
34. SC Thiengo, A Maldonado, EM Mota, EJM Torres, R Caldeira, OS Carvalho, APM Oliveira, RO Simoes, M Fernandez and RM Lanfredi. The giant African snail *Achatina fulica* as natural intermediate host of *Angiostrongylus cantonensis* in Pernambuco, Northeast Brazil, *Acta Tropic.*, 2010; **115**, 194-199. doi:10.1016/j.actatropica.2010.01.005.
35. PJ Hotez, N DeSilva, S Brooker and J Bethony. Soil-transmitted helminth infections: the nature, causes and burden of the condition. Working Paper No 3: Disease Control Priority Project. Bethesda, M.D. Fogarty International Centre, National Institute of Health. 2003.
36. DWT Crompton. Biology of *Ascaris lumbricoides*. Pages 9-44 in: *Ascariasis and its Prevention and Control*. D.W.T.Crompton., M.C.Nesheim and Z.S. Pawlowski (Editors). Taylor and Francis, London, NewYork and Philadelphia. 1989.

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