Ecology of snail intermediate host of *Schistosoma haematobium* in adim and abini communities of Biase Local Government Area, Cross River State, Nigeria

Essien, Ibok Eyo[a]; Akpan, Samuel Sunday[b]; Afahanam, Lucy Emmanuel[c]; Inyang-Etoh, Paul[b]& Agana, Ibibong Okpashi[d]

[a]*Department of Microbiology, Faculty of Biological Sciences, University of Calabar, Cross River State, Nigeria.*

[b]*Department of Medical Parasitology and Entomology, Faculty of Medical Laboratory Science, College of Medical Sciences, University of Calabar, Cross River State, Nigeria.*

[c]*Department of Genetics and Biotechnology, Faculty of Biological Sciences, University of Calabar, Cross River State, Nigeria.*

[d]*Department of Medical Bacteriology, Virology and Mycology, Faculty of Medical Laboratory Science, College of Medical Sciences, University of Calabar, Cross River State, Nigeria.*

Corresponding author: ibokessien001@gmail.com

Abstract

Freshwater snails are widespread in both tropical and temperate regions of the world where they serve as intermediate hosts for larval stages of parasitic trematodes which cause schistosomiasis. This disease is endemic in areas where the snail intermediate host breeds in water contaminated by urine and faeces of infected persons. The study was conducted to investigate the distribution of snail intermediate host of Schistosoma haematobium in Adim and Abini communities of Biase Local Government Area, Cross River State, Nigeria. The snails were collected and grouped according to villages, after which they were induced to shed cercaria. The snails were further processed for DNA extraction and quantification to determine their species. Four hundred and twenty (420) snails were collected from streams in Adim and Abini, out of which 105(25%) were Bulinus africanus in Adim and 105(25%) were Bulinus africanus in Abini. Out of the 105 snails in Adim, which were identified as B. africanus, 10(4.8%) were positive for cercaria of S. haematobiumi while in Abini 17(8.1%) were positive for cercaria of S. haematobium out of the105 snails collected there. A prevalence of 7(17.5%) was recorded for urogenital schistosomiasis among residents of Adim and Abini . Temperature readings were taken on site using mercury in glass thermometer; pH and conductance readings of the samples were quickly determined. Analyses revealed that temperature, pH, conductivity and TDS range of 26.0-29^{0}C, pH was 4.9-6.5, conductivity 32.0-67.0 scm^{-1}, TDS 21.44-44.9mgL^{-1}, DO 2.7-4.2. The study shows that the prevalence of cercariae of S. haematobium in snails in studied community is still high. Continuous health intervention should be carried out as well as biological and chemical control measures to eradicate infected snails in these villages.

Keywords: Cross River State, Ecology, Molecular Characterization, Schistosomiasis, Snails.

Écologie de l'hôte intermédiaire de *Schistosoma haematobium* dans les communautés d'Adim et d'Abini de la zone de gouvernement local de Biase, État de Cross River, Nigéria

Résumé

Les mollusques d'eau douce sont répandus dans les régions tropicales et tempérées du monde où ils servent d'hôtes intermédiaires aux stades larvaires de trématodes parasites responsables de la schistosomiase. Cette maladie est endémique dans les zones où l'hôte intermédiaire se reproduit dans de l'eau contaminée par l'urine et les selles de personnes infectées. L'étude a été menée pour étudier la distribution de l'hôte intermédiaire de Schistosoma haematobium dans les communautés d'Adim et d'Abini de la zone de gouvernement local de Biase, État de Cross River, Nigeria. Les mollusques ont été collectés et regroupés par village, puis induits à libérer des cercaires. Les mollusques ont ensuite été traités pour l'extraction et la quantification de l'ADN afin de déterminer leur espèce. Quatre cent vingt (420) mollusques ont été collectés dans les ruisseaux d'Adim et d'Abini, dont 105 (25 %) étaient des Bulinus africanus à Adim et 105 (25 %) des Bulinus africanus à

Abini. Sur les 105 mollusques identifiés comme B. africanus à Adim, 10 (4,8 %) étaient positifs pour les cercaires de S. haematobium, tandis qu'à Abini, 17 (8,1 %) sur les 105 mollusques collectés étaient positifs. Une prévalence de 7 (17,5 %) a été enregistrée pour la schistosomiase urogénitale parmi les résidents d'Adim et d'Abini. Les températures ont été relevées sur site à l'aide d'un thermomètre à mercure ; le pH et la conductivité des échantillons ont été rapidement déterminés. Les analyses ont révélé des plages de température de 26,0-29,0°C, un pH de 4,9-6,5, une conductivité de 32,0-67,0 µ scm^{-1}, des TDS (Solides Dissous Totaux) de 21,44-44,9 mg L^{-1} et de l'OD (Oxygène Dissous) de 2,7-4,2. L'étude montre que la prévalence des cercaires de S. haematobium chez les mollusques dans la communauté étudiée reste élevée. Des interventions sanitaires continues ainsi que des mesures de contrôle biologique et chimique devraient être mises en œuvre pour éradiquer les mollusques infectés dans ces villages.

Mots-clés : État de Cross River, Écologie, Caractérisation Moléculaire, Schistosomiase, Mollusques.

تنتشر القواقع المائية العذبة على نطاق واسع في المناطق الاستوائية والمعتدلة من العالم، حيث تعمل كمضيفات وسيطة للمراحل اليرقية للمثقوبات الطفيلية التي تُسبّب داء البلهارسيا. ويكون هذا المرض متوطّنًا في المناطق التي تتكاثر فيها القواقع المضيفة الوسيطة في مياه ملوّثة ببول وبراز الأشخاص المصابين. أُجريت هذه الدراسة للتحقّق من توزيع القواقع المضيفة الوسيطة لطفيلي Schistosoma haematobium في مجتمعي أديم (Adim) وأبيني (Abini) التابعين لمنطقة الحكم المحلي بياسي (Biase) بولاية كروس ريفر، نيجيريا. جُمعت القواقع وصُنّفت حسب القرى، ثم أُحفزت لإطلاق السركاريا. وبعد ذلك خضعت القواقع لمزيد من المعالجة لاستخلاص الحمض النووي (DNA) وقياس كميته لتحديد أنواعها. تم جمع أربعمائةٍ وعشرين (420) قوقعة من الجداول المائية في أديم وأبيني، كان منها 105 (25%) من نوع Bulinus africanus في أديم و105 (25%) من النوع نفسه في أبيني. ومن بين 105 قواقع في أديم التي حُدِّدت على أنها B. africanus، كانت 10 (4.8%) إيجابية لوجود سركاريا S. haematobium، بينما كانت 17 (8.1%) إيجابية في أبيني من أصل 105 قواقع جُمعت هناك. وسُجِّل معدل انتشار قدره 7 (17.5%) لداء البلهارسيا البولية التناسلية بين سكان أديم وأبيني. أُخذت قراءات درجة الحرارة ميدانيًا باستخدام ميزان حرارة زئبقي زجاجي، كما تم تحديد قراءات الأسّ الهيدروجيني (pH) والتوصيلية الكهربائية للعينات بسرعة. وأظهرت التحاليل أن مدى درجة الحرارة، وpH، والتوصيلية، والمواد الصلبة الذائبة الكلية (TDS) كان على التوالي: 26.0–29.0°م، و4.9–6.5، و32.0–67.0 ميكروسيمنز/سم، و21.44–44.9 ملغم/لتر، في حين تراوح الأكسجين الذائب (DO) بين 2.7 و4.2. وتبيّن من الدراسة أن معدل انتشار سركاريا S. haematobium في القواقع بالمجتمعات المدروسة ما يزال مرتفعًا. لذا يُوصى بتنفيذ تدخلات صحية مستمرة، إلى جانب إجراءات المكافحة البيولوجية والكيميائية، للقضاء على القواقع المصابة في هذه القرى.

الكلمات المفتاحية : ولاية كروس ريفر، علم البيئة، التوصيف الجزيئي، داء البلهارسيا، القواقع.

Introduction

Schistosomiasis is prevalent in areas where the snail intermediate hosts breed in waters which are contaminated by urine or faeces of infected persons. It is acquired through penetration of the unbroken skin, due to repeated contacts with freshwater during fishing, farming, swimming, washing, bathing and recreational activities (Akinwale *et al*., 2010).
2012).

The Prevalence of Schistosomiasis in Nigeria has serious effect on many people in different communities in Nigeria (Njepuome *et al*. 2009). The disease constitutes a major public health problem in Nigeria, affecting mainly rural and sub-urban populations. Ecological areas with high prevalence of schistosomiasis are represented by five pilot projects of the National Schistosomiasis Control Programme. The disease is essentially an infection of agricultural communities where the way of life of people promotes the contamination of inland water with human excreta (Akinwale *et al.* 2010). This highlights the need for proper control of the disease in order to improve the

socioeconomic status of these people. Although schistosomiasis is endemic in Nigeria, it is usually a neglected common parasitic disease of childhood (Bello and Edungbola, 1992). Nigeria is one of the countries known to be highly endemic for urinary schistosomiasis with estimated 101.28 million persons at risk and 25.83 million people infected (Chitsulo *et al*. 2000). Morbidity estimates are high and school-age children usually presented with the highest prevalence and intensity of *Schistosoma haematobium* infection (WHO, 2002b). Reports on the knowledge, attitudes and practices on schistosomiasis have also been documented. Ukwandu and Nmorsi (2004) reported that 42.0% of the inhabitants admitted knowledge of schistosomiasis while 0.4% knew about the aetiologic agent. A total 5.0% of the respondents admitted procuring treatment, while 5.0% declined to seek treatment of any sort. Headache (43.0%) and fever (31.0%) were the major clinical signs recognized by the respondents, while sexual pains (22.0%) were The use of parasitological techniques to study the prevalence of schistosomiasis in Nigeria has been reported earlier. Adeoye and Akagbogu (1996) reported from a study among residents of Ado Odo/Ota of Ogun State, that 40% tested positive for urinary schistosomiasis. The highest prevalence of 48.6% was recorded in the age group 11–20 years, followed by 43.2% in 1–10 years old and the females had higher prevalence compared to the males. The intensity of infection in males almost doubled that of females while 95% of the infected people had light infections. This calls for interventions to halt the transmission of the disease in these areas. Abolarinwa (1999) reported moderate (30.6%) prevalence of urinary schistosomiasis from randomly selected school pupils in Esie, Kwara State. The infection rate was significantly higher among males (36.5%) than females (21.7%), reaching the peak in the 11–14 years age group in both sexes. Intensity of infection was higher among males than females in all age groups with disproportionately high egg output among few infected children. The prevalence and intensity of infection was higher the least. Ekpo *et al*. (2010) reported from Ilewo-Orile in Ogun State that members of the community are aware of the symptom of urinary schistosomiasis which is passing blood in the urine called "Atosiaja" in the local language. However, they did not consider their river as the source of infection. They believed that the disease is only a sign of virility and coming to adulthood, which is also common in other communities near them (Ekpo *et al*. 2010). They ignorantly submitted that any infection that is contacted through water and penetration of the skin must show on the skin surface and not in their urine. They submitted that urinary schistosomiasis has existed in their community for many years and that they do not consider it as their major health problem when compared to malaria fever (Ekpo *et al*. 2010). There is urgent need to provide health education to the people on the disease to improve their knowledge and equip them to accept and plan control interventions.

among pupils of the public schools than those of private schools. Dunah and Basiri (2000) studied the prevalence of *Schistosoma haematobium* among primary school pupils in Mayo-Belwa Local Government Area of Adamawa State and reported that the overall prevalence was 27.2%. The highest prevalence was recorded among pupils in Mayo Lamja (14.4%) followed by Mayo Sanagnere (8.3%) and Kudaku (4.4%). The infection rate varied with age, reaching peak among children 8–13 years old and dropping sharply thereafter. Males were more infected than females. The infection was found to be more in children whose parents were civil servants and all infected children had previous history of contact with the infested streams. Bassey and Umar (2004) reported 50.3% prevalence of schistosomiasis among children aged 5–17 years in Garun–Babba, Kadawa and Kura in Kano state. The report further stated that in Kura, the recorded prevalence of 20.3% far exceeded previous records while there was significant difference from that of Garun-Babba (17.1%) and Kadawa (12.9%). There were variations in prevalence

between the different communities. Therefore, planning of control should take into consideration these variations to maximize interventions in a cost-effective manner based on available resources. A good understanding of the factors that contribute to these variations is therefore imperative (Bassey & Umar, 2004).

Urogenital schistosomiasis constitutes a major public health challenge in Africa. Numerous researches have been carried out to authenticate the prevalence of schistosomiasis in Cross River but none on the snail intermediate host. Hence there is need to study snail which is the intermediate host of urogenital schistosomiasis. Aquatic snail host of *Schistosoma* occurs in shallow water and their density vary significantly with the season. Despite the provision of effective chemotherapy for mass treatment of the disease by different research group, re-infection seems to occur rapidly because people living in affected areas depend on contaminated water, and lack of residual. In Cross River state, most of the population is involved in agriculture. The environment is also conducive for the thriving of the snail intermediate host. During recreational activities people who live in these endemic areas come in contact with the infected snail; these factors have contributed to the prevalence of urogenital *Schistosomiasis* (Ejezie*et al.,* 1991). To better have a clear view of the study, the following research questions were computed; which species of snails are prevalent as intermediate host for schistosomiasis in Abini and Adim community of Biase LGA and at what percentage of these snails are carriers of the cercaria stage of *S. haematobium*. The hypotheses were Null hypothesis which stated that the species of snails in Abini and Adim community of Biase LGA, of Cross River state are not intermediate host for *S. haematobium and the* Alternate hypothesis whichstated that the species of snails in Abini and Adim community of Biase LGA, of Cross River state are intermediate host for *S. haematobium.* The aim of this work was to study the ecology of snail intermediate host of urogenital schistosomiasis in Abini and Adim communities of Biase LGA of Cross River state. The objectives of this work include; to identify and determine the species of the freshwater snail intermediate host of *Schistosomiasis* abundant in Abini and Adim community of Biase LGA, to establish their distribution and determine the presence of cercaria in the snail host, to recommend ways to better control the disease and intermediate host in the community based on the outcome of this research.

Fig. 1: *Bulinus globosus* shell (Source: Kane 2008)

Fig. 2: *Bulinus forskalii* shell (Source: Kane 2008)

Fig. 3: *Bulinus tropicus* shell (Source: Kane 2008)

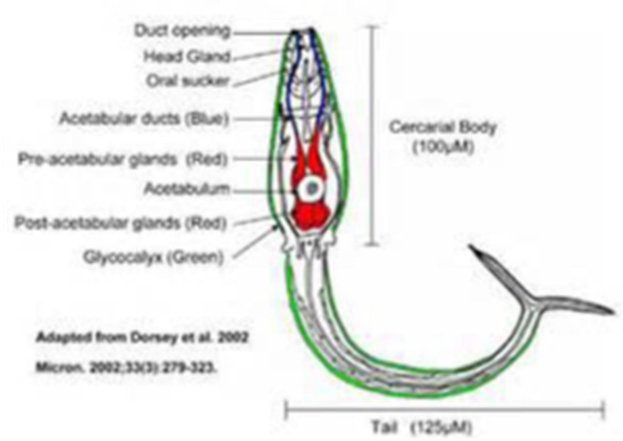

Fig. 4: *Schistosoma Haematobium cercaria* (Source: Ubaka, 2019)

Methodology

The research adopted an experimental descriptive study design on ecology of snail intermediate host of *Schistosoma haematobium* in Adim and Abini communities of Biase Local Government Area of Cross River State. These communities have streams which makes the snail environment to thrive and survive very well. Members of these communities do visit the streams for purposes like swimming, bathing, washing, fetches the water for home chores etc. The names of the streams in Adim were Ette-Oke,/Anijak while in Abini are Afifia/Emmorrow. These communities have a mean annual rainfall of 401-600 mm, a temperature of 25-30^{o}c. with a 97km distance from Calabar on Latitude: 5°44'0"N Longitude: 8°2'0"E. The two communities has a combine population of about 44,940, with their occupation mostly farming and fishing. Ethical consideration was sought for and obtained from the Cross River State Ministry of Health. The sample size was determined by the formula to produce a total sample size of 210.

$$N = \frac{Z^2p}{d^2}$$

Where N = minimum sample size
Z = the level of the coefficient interval at 95% (1.96)
p = proportion of occurrence
q = (1-p) proportion of non-occurrence
d= precision.

Using the formula; $N = \frac{Z^2\ P(1-P)}{d^2}$

At a prevalence rate of 16% (Adie *et al.,* 2005)

$$N = \frac{1.96^2 \times 0.16 \times 0.84}{0.05^2}$$

$$N = 206$$

Snails were collected from different streams in Adim and Abini communities using a scoop net and transported in a polythene bag to the laboratory. Water samples were collected from streams, rice field, irrigational canal and pounds in Adim and Abini communities for water analysis into sterile containers put inside cooler that contains ice packs and transported to the laboratory. Urine samples were collected randomly at noon from 20 residents from Adim and Abini, respectively into a sterile universal container and taken to the laboratory for analysis.

Sample analysis

Snails samples collected from the different streams in Adim and Abini communities were sorted out and grouped accordingly; the snail's samples were rinsed in cool tap water for few seconds to remove debris, counted and identified according to shell morphology by a snail specialist. Each snail was tested by placing in a glass tube containing 10mls of filtered water and was exposed to bright electric light for 30-40 minute to include cercarial shedding. The Schistosome cercarial issued by infested snails were examined as wet mount under the microscope to ascertain for *S.haematobium.* Morphometric parameters of the snails were recorded. The sample snails were soaked in70% ethanol for molecular analysis.

Molecular analysis

i. DNA extraction from snail

To 1g of the snail foot part, 20 µl of proteinase k was added and mixed thoroughly by vortexing for 10-15 seconds and then incubated at room temperature for 20 minutes. One millilitre of Genomic Binding Buffer was added to the digested sample and mixed thoroughly. The mixture was transferred to a Zymo-spin TMIIC-XLR column in a collection tube, centrifuged at ≥ 12000 x g for 1minute.the collection tube was discarded with the flow through 4000 µl DNA Pre-Wash Buffer was added to the spin column in a new collection tube and centrifuged at ≥ 12000 xg for 1 minutes. The collection tube was emptied. Seven hundred microlitres (700 µl) of g- DNA wash Buffer was added to spin column, centrifuged at ≥12.000g for 1 minute and the collection tube was emptied. Two hundred microliter (200µl) g-DNA Wash Buffer was added to the Spin Column, centrifuged at ≥ 12,000 g for 1 minute and the collection tube with the flow through was discarded. The Spin column was transferred to a clean micro centrifuge tube,50µl of DNA Elution Buffer was added directly on the matrix, incubated for 5minutes at room temperature then centrifuged at maximum speed for 1minute to elute the DNA. The eluted DNA was stored ≤ -20^{0}C for future use.

ii. DNA quantification

The extracted genomic DNA was quantified using Nanadrop 1000 Spectrophotometer. The software of equipment was lunched by double clicking on the Nanodrop icon. The equipment was initialized with 2µl of sterile distilled water and blanked using normal saline. Two microliter of the extracted DNA was loaded onto the lower pedestal, the upper pedestral was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the measured button.

iii. Amplification of the Cox gene of the snails

The amplification of the Cox gene of the snail was amplified using ETTS1 and ETTS10 primer on ABI 9700.Applied Biosystems thermal cycle at a final volume of 30 microlitres for 35 cycles.The PCR mix included; the X5 Dream Taq Multiplex Master Mix supplied by Inquaba, South Africa (Taq polymerase, DNTPs,MgCL),the primers at a concentration of 0.4M and 50ng of the extracted DNA as template. The PCR conditions were as follows; Initial denaturation,95^{0}c for 5 minutes, denaturation 95^{0}c for 30seconds; annealing, 56^{0}c for 40 seconds; extension 72^{0}c for 50 seconds for 35 cycles and final extension, 72^{0}c for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator for product sizes of 680bp

Water Analysis

i. Temperature reading, Water pH and Water Conductivity

Mercury in glass thermometer calibrated to 100^{0}c / 110^{0}c was immersed in water by inserting the bulb of the thermometer in to the

stream water about 1000ml. Direct reading as indicated in the thermometer was recorded. A standard pH meter with two point calibration and two standard buffer solutions of 4.00 PH and 9.18 pH in each buffer was used. Enter button was pressed to finish calibration, then it was inserted into water sample and the reading was recorded. Conductivity meter was switch on and preheat for 30 minutes, then calibrated by turning the range selection adjuster was check position. The constant adjuster was turn to "I", the temperature component adjuster was turn to 25^{o}c and the calibration adjusted until it displayed 100µs/cm. Then calibration finished. It was inserted into the sample and the reading recorder.

ii. Dissolve Oxygen Reading and Total Dissolved Solids

The dissolve oxygen analyzer was warm up for 5 minutes, electrodes was set into fresh 5% sodium sulphate solution for 5 minutes the reading which was stabilized at 0.00. The electrode was taken from the solution cleaned with fresh water and the water drop was removed carefully from the membrane surface using absorbing paper, left in the air for few minutes to make the read out stabilized. The span calibrating knob was adjusted to make the readout value of the dissolved oxygen of the water samples. A Total Dissolve Solid meter which measures electrical conductivity (µs|cm) was used to estimate Total Dissolve solid in the water samples.

Statistical Analysis

Data were analyzed using a cross sectional descriptive statistical tool of measures of central tendencies, using the statistical package for social sciences (SPSS)

Results and Discussion

A total of 420 snail samples were collected from the streams in Adim and Abini communities as shown in Table 1. Out of the 420 snails, 105(25%) were collected from Adim and were identified as *Bulinus africanus* while others were *Limicolaria aurora* with a total of 105 (25%). Also, in Abini, 105 (25%) belonged to *Bulinus africanus* species, 50(11.9%), *to Limicolaria aurora* 50(11.9%) to *Archachatina marginatas*, and 5(1.2%) *Archachatina marginata suturalis.* The snails collected from Adim and Abini of Abi local government area were identified to belong to the genus Bulinus and species globossus. This is in line with studies carried out by Adie *et al*. (2015) on widespread occurrence of these snails as intermediate hosts of urinary Schistosomiasis in Biase and Yakurr Local Government Area of Cross River State, Nigeria. Akinwale *et al.,* (2010) also revealed 25.5% infection rate in *B. globosus*. This study is a benchmark for showing that infection rates in snails often fluctuate based on human-water contact patterns in the surrounding community. Table 2 shows summary of snails collected from streams in Adim and Abini communities during at all. Of the 420 snail samples collected, 210 (50%) were identified as *Bulinus africanus*. Of this figure, 12.9% (27/210) were infected with *Schistosoma haematobium* as snails from Adim was 105(50%) with a prevalence of 4.8% (10/105), while Abini had 8.1% (17/150). Also, of the 105 *Bulinus globosus* snail species found in Adim, only 10(9.5%) were found to be infested with *Schistosoma* spp. However, in Abini, *B. globosus* was the only snail species identified and 17(16.2%) was infested with *Schistosoma* spp. The same findings were reported for *Bulinus globosus* in Ogun State, Nigeria by Akinwale *et al*. (2010) who recorded 25.5% of infestation rate. Additionally, another study in Enugu State of Nigeria carried out by Oguanyi *et al.,* (2015) on the distribution of snail hosts revealed that *Bulinus globosus* was the most prevalent species in slow-moving streams, which is similar to our findings. They reported that snail density was closely linked to the presence of aquatic vegetation.

Table 3 shows breakdown of snails collected from streams in Adim and Abini communities during dry/rainy seasons. The prevalence rate for snails from Ette-oke stream during dry season was 0.4% (1/105),indicating a positive result, while the rainy season captured Anijak village stream with the highest frequency of snail vector of 7(6.6%) and Ette-oke community with 2(1.9%). Also, in Abini community stream during the dry season, only 1(0.47%) snail from Afifia stream was positive while 2(0.95%) from Emmorrow stream were positive while the rainy season recorded Emmorrow stream with the highest frequency of snail vectors 11(10.5%) and Afifia stream with 5(5.7%). According to Okeke *et al.,* 2014 work in Ibadan, Nigeria; snail populations often peak at the beginning and end of the rainy season as also observed in our study. Heavy mid-season rains can sometimes "flush out" snail populations, but the increased water volume and nutrient runoff in the rainy season generally support larger breeding colonies than the harsh dry season, supporting the higher percentage number of snails found during this season. Also, in Kware Lake, Sokoto state in Nigeria, Bello *et al.,* (2014) also observed that Bulinus species were more abundant during the rainy season due to the stabilization of water temperatures and the growth of algae, which provides both food and a substrate for egg-laying.

Table 1: Different types of snails sample collected in Adim and Abini communities

Villages	Snails species	No. of snails collected
Adim	*B. africanus*	105
	Limicolaria aurora	105
Abini	*B. africanus*	105
	Limicolaria aurora	50
	Archachatina marginatas	50
	Archachatina marginata suturalis	5
Total		420

Table 2: Summary of Snails collected from streams in Adim and Abini Communities

Villages	No. of snail collected	No. identified as *B. africanus*	No. (%) of snail infected
Adim	210	105(50)	10(4.8)
Abini	210	105(50)	17(8.1)
Total	**420**	**210(100)**	**27(12.9)**

Table 3: Breakdown of snails collected from streams in Adim and Abini Communities during rainy and dry season

Streams	No of snail collected	No identified as Bulinus (%)	Snail sp	No (%) of snail infected
		Adim		
		Dry season		

Ette-Oke	105	50 (47.6)	Bulinus africanus	1(0.4)
Anijak	105	55 (52.4)	Bulinus africanus	0
Total	210	105		1(0.4)
Adim -		**Rainy season**		
Ette-Oke	105	50 (47.6)	Bulinus africanus fcafrafricanus africanus	1(1.9)
Anijak	105	55 (52.4)	Bulinus africanus	7(6.7)
Total	210	105		10(8.6)
		Abini Dry season		
Afifia	105	50 (47.6)	Bulinus africanus	1(0.47)
Emmorrow	105	55 (52.4)	Bulinus africanus	2(0.95)
Total	210	105		3(1.42)
		Rainy season		
Afifia	105	50	Bulinus africanus	5(5.7)
Emmorrow	105	55	Bulinus africanus	11(10.5)
Total	210	105		17(16.2)

Table 4 shows the morphometric parameter of *B. africanus* snail collected. Height 25 ± 3.7, width 4.3 ± 1.2, aperture height 14.3 ± 1.7, and aperture width 7 ± 1.6. In studies across Southwestern Nigeria Akinwale *et al.,* (2011), Bulinus species often show high morphological plasticity, our records show a significantly larger height-to-width ratio than those found in some Northern studies (e.g., River Wudil, Kano), where average heights were closer to 8-12mm. This suggests that the environment in Cross River (likely higher humidity and nutrient availability) supports larger snail growth. Table 5 shows the distribution of *S. haematobium* infections among respondents examined in Adim and Abini communities. A total of 40 urine samples were collected (20 for each community) with a general prevalence of (17.5%). The prevalence of Urinary Schistosomiasis (Schistosoma haematobium) for Adim and Abini were 4(20%) and 3(15%), respectively. These figures (15–20%) are highly consistent with recent findings in the same region. Adie *et al.* (2021) found a prevalence of (19.13%) in Abini and (19.05%) in Adim among school-aged children. Our results confirmed that these communities remain high-risk "hotspots." Temperature readings were takings were taken on site using mercury in glass thermometer; pH and conductance readings of the samples were quickly determine. Table 6 shows the physicochemical parameters of water obtained from sites of sampling in Adim and Abini during dry/rainy season. For Adim community during the dry season, highest records showed temperature (28^0C), pH (6.0), Total Dissolved Solids (36.9), Conductivity (55), Dissolved oxygen (3.4) was recorded, and the raining season had highest temperature of (28^0C), pH (6.1), Total Dissolved Oxygen (36.9), Conductivity (53), Dissolved oxygen (37). Also sites of sampling in Abini during dry season indicates highest temperature of (29^0C), pH (5.7), Total Dissolved Solids (33.5),

Conductivity 50 and Dissolved oxygen 3.8, whereas the raining season recorded highest temperature was (28^0C), pH (6.5), Total Dissolved Solids (50), Conductivity (67) and Dissolved oxygen (4.2). The observation of peak snail density at (28^0C) is a inline for Njoku-Tony (2011) and Bello *et al*. (2014), who noted that temperatures above (30^0C) or below (20^0C) significantly inhibit snail respiration and egg-laying. The slightly acidic to neutral pH (4.9-6.5) you recorded is common in forest-stream ecosystems in South-South Nigeria. Most literature suggests *B. globosus* prefers a pH of (6.0-7.5), implying that our lower pH values might explain why the populations aren't even higher (REF). Finally, Table 7 shows water velocity recorded in streams in the communities. Figure 1 shows molecular analysis of *Bulinus africanus* species from Adim and Abini communities with 680bp. The snail samples were subjected to DNA extraction for *Bulinus* sp using Taq polymerase. The agarose gel electrophoresis showed amplified cox gene in lanes 2, 3 and 5 while L represents the 100 bp. Thus a distinctive banding pattern was obtain for *B. globosus*. Rollinson *et al.* (2001) typically recorded bands around 600bp for certain Schistosoma markers. The use of the cox1 gene (Cytochrome Oxidase Subunit 1) aligns with modern "DNA Barcoding" standards. Studies like Kane *et al.* (2008) highlight that while the ITS2 region usually yields bands around 500-600bp, the cox1 fragments often range between 650-70bp depending on the primers used. Our 680bp result is a very strong match for this specific mitochondrial marker. In this study the overall prevalence of *Bulinus globosus* screen for Schistosome cercaria was 25.7%. This is however close to the prevalence rate reported in studies carried out by Emini and Enogiomwan (2019) in Evaoke, Enomi, Ibietroma, Ibieoke, and Efukpaba villages of Abi Local Government Area of Cross River State where a prevalence of (28.7%) was recorded. These studies carried out also uses physical and microscopical Method.

Table 4: Morphometric parameter of *B. africanus* snail collected

Shell morphometric	Maximum	Minimum	Mean ± SD value (mm)
Height	30	21	25±3.7
Width	6	3	4.3±1.2
Aperture height	16	12	14.3±1.7
Aperture width	9	5	7±1.6

Table 5: Distribution of *S.haematobium* infections among respondents examined in Adim and Abini communities.

Communities	No of samples	No infected (%)
Adim	20	4(20)
Abini	20	3(15)
Total	**40**	**7(17.5)**

Table 6: Physical characteristics of water samples obtained from sites of sampling in Adim and Abini during dry/rainy season

Places	Temperature (°C)	pH	Total dissolved solid (mgL^{-1})	Conductivity (μ^5 $Cm^{-1)}$	Dissolved oxygen	Density
	Adim Community Dry season					
Ette-Oke						
Stream	27.8	5.8	21.44	32.0	3.0	1
Swamp	27.2	5.4	18.76	28.0	2.8	1
Irrigation Canal	26.8	5.0	16.08	24.0	3.2	1
Anijak						
Stream	2.8	6.0	36.85	55	3.4	1
Swamp	27.4	5.2	27.47	41	2.8	1
Irrigation Canal	27.8	4.9	32.16	48	3.0	1
	Rainy season					
Ette-Oke						
Stream	28.1	6.06	35.78	53.4	3.4	1
Swamp	28.2	5.9	32.16	48	3.0	1
Irrigation Canal	27.8	5.6	30.0	58.2	2.9	1
Anijak						
Stream	28.0	5.8	39.0	58.2	3.7	1
Swamp	27.8	5.2	30.0	45.6	3.1	1
Irrigation Canal	28.7	6.0	36.85	55.0	3.0	1
	Abini Community Dry season					
Afifia						
Stream	28	5.2	33.5	50	3.8	1
Swamp	28.7	5.0	32.8	49	2.7	1
Ricefield Irrigation Canal	29	4.9	30	45.6	3.0	1
Emomorrow						
Stream	28.4	5.2	18.76	28	3.0	1
Swamp	28.3	4.9	21.44	32	3.4	1

Ricefield Irrigation Canal	29	5.7	32.16	48	2.8	1
	Rainy season					
Afifia						
Stream	27.8	6.5	44.9	67	4.0	1
Swamp	28.2	5.7	37.5	56	3.8	1
Ricefield Irrigation Canal	27.4	5.4	32.8	49	2.9	1
Emomorrow						
Stream	28	5.8	33.5	50	4.2	1
Swamp	27	5.4	31.49	47	3.2	1
Ricefield Irrigation Canal	26	6.2	21.44	32	3.0	1

Table 7: Stream Velocity in Adim and Abini Villages

Village		**Stream length (m)**	**Time taken for cork to travel the entire stream length (s)**	**Stream velocity (ms^{-1})**
Adim	Ette-oke	8	80	0.10
	Anijak	6	40	0.20
Abini	Afifia	6	55	0.11
	Emomorow	5	48	0.10

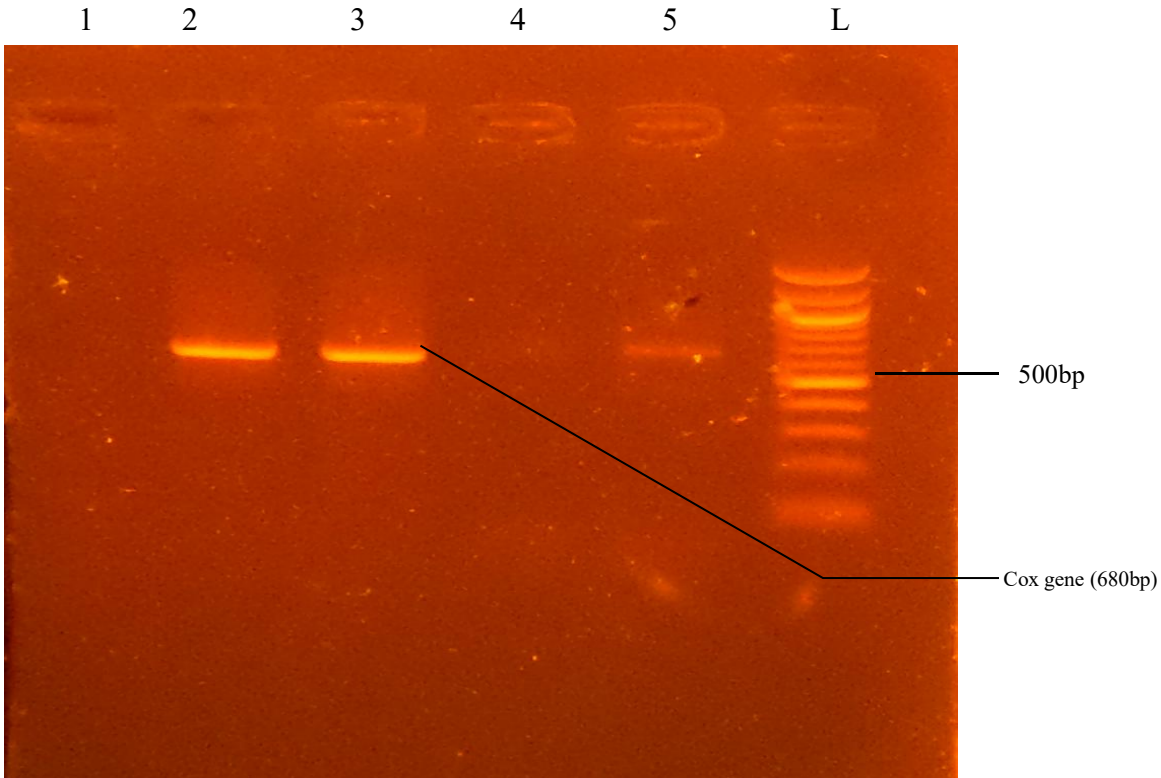

Plate 2: Agarose gel electrophoresis showing the amplified Cox gene in lanes 2, 3 and 5 while lane L represents the 100bp ladder

Conclusion

Identified freshwater snails in Adim and Abini villages of Biase Local Government Area is *Bulinus africanus* of which some of the *Bulinus are* infected with *Schistosoma haematobium*. Both season and habitat type have clear influence on the abundance of the snails, prevalence and transmission of cercariae, a knowledge which can help in the study of the epidemiology of the schistosomiasis infection in the study area. This study has identified *B. africanus* snail as intermediate host of urogenital *Schistosomiasis* in Adim and Abini communities of Biase LGA. It has also provided information on the infestation rate of snails with the cercaria of *S. haematobium* in Adim and Abini communities.

Recommendations

The following recommendation are necessary to curb this great menace; there is need to provide health education on avoidance of improper water contact activities in infested water using appropriate behavioural change and communication strategies, provide potable, safe water for domestic use to affected communities, there is need to carry out chemotherapy of schistosomiasis using praziquantel in affected communities in order to reduce the morbidity of the disease as part of the National Schistosomiasis Control Programme, in line with WHO recommendations.

Declaration of Competing Interest

All Authors have declared that no competing interests exist.

Authors Contributions

This work was carried out in collaboration between all authors. Authors EIE & IP: Project conceptualization, design, writing, literature searches, field work and overall supervision, Author ALE: Writing, editing and manuscript first draft and Authors AS & AIO: Review, Resources, molecular analysis, statistical analysis.

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