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MORPHOLOGICAL CHARACTERIZATION OF CERCARIA AMONG *Bulinus* SNAILS FROM INDIRE STREAM IN ARGUNGU, NIGERIA

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Abstract

Freshwater snails have demonstrated a high level of significance with respect to public health. This study identifies the morphological features of cercariae isolated from snails collected from Indire stream in Argungu area of Kebbi State. The snails were collected with the aid of a scoop net, transferred into labelled perforated plastic containers, and subsequently transported to the Biology Laboratory of Waziri Umaru Federal Polytechnic, Birnin Kebbi for identification. Individual snails were subjected to artificial bright light from a 100-watt electric bulb for one to four hours to stimulate the shedding of cercariae. Thereafter, the snails were examined under a light microscope. Out of one hundred and twenty-five (125) Bulinus forskalii snail species examined in this research, forty-nine (49) snails were infected with various species of larval trematodes (cercaria), three species of cercariae including Echinostome, Parapleurolophocercous, and virgulate cercariae were identified. The Echinostome cercariae had the highest prevalence, 523 (93.22%) followed by Parapleurolophocercous cercariae 25 (4.45%) while virgulate cercariae had 13 (2.32%). This study has made a valuable contribution by providing novel and improved insights into the prevalence of trematode

Keywords: Bulinus forskalii, Trematode, Identification, Cercariae.

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infections within the study region. The findings of this research have established an initial basis for understanding the epidemiological context and formulating a control strategy to tackle trematode infection in human and animal communities. Efforts aimed at managing trematode parasite illnesses should be strategically focused on several levels, with specific emphasis placed on the areas bordering river ecosystems.

INTRODUCTION

Freshwater snails received considerable attention as they are intermediate hosts of several trematodes that cause diseases in humans and domestic animals (Mohammed *et al*., 2016) the snails serve as the intermediate host for the development of several larval stages such as sporocysts, rediae, and cercariae an infectious stage of most trematode or metacercaria form (Tigga *et al*., 2014 and Abdulkadir *et al.,* 2018). The proportion of snails that release cercariae (prevalence of infection) and the number of cercariae released from each infected snail (intensity of infection) play important roles in the transmission of trematodes from the snail host (Combes, *et al*. 1994 and Abdulkadir *et al.,* 2018). Tigga *et al*., 2014 reported that only *Lymnaea* group of snails are involved in establishing of life cycle in at least 71 species of trematodes while Bulinus species of snail has being established to transmit schistosomiasis a second most widely spread parasitic disease after malaria (WHO, 2015). Notably among the *Bulinus species* was *Bulinus forskalii Snails* which was predominantly identified in this research, these groups of freshwater snail has been reported to live in a wide variety of habitats but most commonly occur in small shallow and seasonal seepages and pools. Some members of the group transmit *S. Haematobium* as well as non-human schistosomes (Rabone *et al*., 2019). This tally with earlier report made by De Kock and Wolmarans (2005) that *Bulinus forskalii* was found ubiquitously throughout sub-Saharan Africa.

Important in the transmission of trematodiasis is the infective larval stage, the cercariae (Abdulkadir *et al*., (2018), this results into infection in human either directly by penetrating the human skin or indirectly by transforming into encysted metacercaria in vegetable and consumed along with food. Cercarial emergence from the snail intermediate host is a key phase in the reproductive success of trematodes, and this step is directly related to the life-span of the cercariae (Mohammed *et al*., 2019). The cercarie has a tapering head with large penetration

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glands. It may or may not have along swimming "tail" depending on the species. The motile cercaria finds and settles in a host where it will become either an adult, or a mesocercaria, or a metacercaria, according to species. Mesocercaria: They are involved in an encysted stage either on vegetation or in a host tissue on the second intermediate host. They have a hard shell and are also involved in the trophic transmission. This is where the parasite is able to infect the definitive host because it consumes the second intermediate host that has metacercaria on/in situ (Abdulkadir *et al.,* 2018).

Several studies of larval trematodes have been reported and studied by many researchers (Abdulkadir *et al*., 2018). On the contrary, in Kebbi State there is a lack of information related to trematode larvae. Most studies are restricted mainly to diseases caused by trematode species such as schistosomiasis and fasciolisis. This suggests the need to study the morphology and characterization of cercariae released from snails in Kebbi State. The river Indire is a shallow water body with a slow water-current, which provides a favourable environment for the breeding of intermediate-hosts snails. Furthermore, the intensive population movement from other agricultural schemes was observed, where Bilharzia is an attendant health problem. In addition, the situation was further aggravated by the monitored considerable water-contact activities as well as the contamination by heavy urination and defecation. Thus, prone this research work to identify the trematode larvae (cercaria) was pivotally needed to identify morphological and characterise the trematode larvae and to throw light on the factors influencing the transmission pressure of trematodiosis in the study area.

The aim of the study was to characterize and identify various cercarial on bases of their morphology in *Bulinus forkalii* (freshwater snails) harvested from Indire stream of Argungu Local Government Area of Kebbi State.

MATERIALS AND METHODS

Study Area

Indire is situated in Argungu, Kebbi State, Nigeria, its geographical coordinates are 12° 34' 0" North, 4° 25' 0" East, and its original name (with diacritics) is Indire, Kebbi state located within the Sudan/Sahel Savannah, climatic and vegetation zone. It experiences three dominant seasons. The wet season (damuna) extends from June to September. Starting from August, the numerous streams, lakes, and ponds, notably the Gulbin Kebbi overflow their banks. The cool season (dari) extends from October to January and is usually characterized by a relatively long

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spell of dryness and dust (Harmattan) from the Sahara. The hot season (bazara) starts in February and lasts until May. This period is marked by intensive heat with temperatures of over 42° C. The environment is agriculturally viable since it is endowed with high soil fertility, vast farmlands, and economically viable rivers sheltered by fine tropical climates. Owing to these factors, agriculture has remained the major source of revenue and indeed the backbone of the economy of the state. Major food crops in the area are millet, guinea – corn, maize, cassava, potatoes, rice, beans, onions, and vegetables, while cash crops including wheat, soya beans, ginger, sugarcane, groundnuts, and tobacco are also produced in the state.

Study Site

A pre-survey was carried out at Indire Stream to identify the site for snail collection. Five site were selected based on obvious water contact activities and places where water was stagnant or slowly moving/since snails are mostly found around these places as described by Gautam and Kakulte (2014).

Snail Collection

The collection of snail samples was conducted using either a scooping net or manual forceps between the time period of 8:30 a.m. to 11:00 a.m. The collection took place within an approximate area of 5m2 at each designated collection point. This process was repeated for five consecutive weeks, specifically from November to December of 2019. The snails obtained from each location were individually placed in distinct plastic containers that were appropriately identified. The containers were filled with water sourced from the respective collection sites, and lettuce or cabbage was provided as food for the snails. The snails that were gathered were subsequently transported to the Biology laboratory at Waziri Umaru Federal Polytechnic in order to undergo identification. The freshwater snails were subjected to a cleaning process in the laboratory to eliminate any dirt present. Afterward, they were classified into different species based on their physical features, employing identification keys as outlined by Frandsen and Christensen (2004) and the "Field Guide to African Freshwater Snails" published by the Danish Bilharziasis Laboratory (2004). The snails were also quantified during this procedure.

Cercariae Collection and Identification

A modified method of Abdulkadir *et al.,* (2018) was used, snails were placed separately in plastic container containing water filtered from their sites of

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collection. The snails were exposed to artificial bright light from a 100 Watt electric bulb for one to four hours to induce shedding of cercariae. Each snail was carefully observed under microscope and examined for possible emergence of cercariae at regular intervals of one hour for twenty four (24) hours. Live cercariae shed by each snail species in a Petri dish were transferred in water solution onto a microscopic slide, covered with a cover slip and observed under X40 objective of the light microscope. The observed cercariae were then stained with lugol's iodine (0.1%). A cover slip was carefully placed on the stained slide and observed under the X40 objective of the light microscope. The movement and the morphological details of the live and stained cercariae observed under the microscope, respectively, were recorded and identified using standard key of Frandsen and Christensen (1984).

Prevalence and intensity of infection

Prevalence of infection was determined by counting the proportion of snails that released cercariae while the intensity was determined by counting the number of cercariae released from each infected snail (Combes, *et al*. 1994).

The results generated were subjected to Chi-square analysis for test of significance using statistical EpiInfo statistical package. $P<0.05$ was considered significant at 95% confidence limit.

RESULTS

The overall prevalence of the infection recorded in this study is presented in Table 1. Out of 125 snails of *Bulinus forkalii* identified forty nine 49(19.6%) were found to be infected. Weekly prevalence of the infection recorded for the consecutive weeks 1-5 revealed that snails collected during week 4 had the highest prevalence followed by those collected during week 2 11 (4.4%), similar prevalence of the infection 9(3.6%) was recorded among snails collected during the week 1 and week 5 respectively, least prevalence of the infection was recorded among snails harvested in week 3 8(3.2%) however, there is no significant relationship in the prevalence of infection between number of snails examined and those infected across the week p-value = 0.1991. the mean cercarial burden of the infected snails was 11.4 larval cercariae per snail likewise the observed differences observed in the mean intensity was not statistically significant P-value = 0.3023 (Table 1).

Results in Table 2 showed the three (3) cercariae identified based on their morphological features these include Echinostome cercariae, seen as round oval without tail and had the highest prevalence 523 (93.22%) followed by Parapleurolophocercous cercariae 25 (4.45%) which posses a large, oval shape head with Long tail the least occurred was virgulate cercariea13 (2.32%) this had a small, oval shape head and short tailed .

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Table1: Prevalence and Intensity of Cercariae Infection among *Bulinus forskalii snails* Examined

Number of weeks	Number of snail examined	Number of snail infected	Prevalence (%)	Total cercarieal count	Mean intensity
1	25	9	3.6	41	4.5
2	25	11	4.4	149	13.5
3	25	8	3.2	69	8.6
4	25	12	4.8	246	20.5
5	25	9	3.6	56	6.2
Total	125	49	19.6	561	11.4

χ^2=6.0000, df=4, p= 0.1991 ; H=4.8171, df=4 P=0.3023

Table2: Frequency and Morphological Features of Cercariae Identified from Infected *Bulinus forskalii* snails

Cercariae	Morphological Features seen under microscope	Frequency	Percentage (%)
Echinostome	Round oval without tail were seen	523	93.22
Parapleurolophocercous cercariae	large, oval shape head with Long tail	25	4.45
virgulate	Small, oval shape head and short tailed were seen	13	2.32
Total		**561**	100

DISCUSSION

The overall infection rate of 19.6% recorded in the current study differed from the results obtained in similar studies from other parts of the country. For instance, Luka and Mbaya (2015) recorded a much lower prevalence of 0.94% among snail intermediate hosts examined in Borno State, Nigeria and Abdulkadir *et a.l* (2018) Nigeria where they recorded 10.55% prevalence among snails examined, 0.2% in the Niger River valley by Rabone *et al.,* (2019). The result obtained was also higher than 14.1% Prevalence of trematode infections in snails recorded in by Mohammed *et al.,* (2016) Khartoum, Sudan. This indicated prevalence of trematode infection among snail examined in Indire stream is high this could be attributed to the period of sample collection which was dry season which tend to favour snail aboundance stability of aquatic medium and increased water contact activities thus favouring transmission and infection of snail intermediate host (Tigga *et al*., 2014; Abdulkadir *et al*., 2018 and Rabone *et al*., 2019).

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Echinostome cercariae are the highest observed cercariae type in the snail studied. This cercaria has been reported to be produced by digenic trematodes and its presence in the freshwater bodies studied implied that there is a likelihood of the spread of trematodiosis amongst their respective definitive hosts (man and other animals). They are intestinal parasites of birds, reptiles, and mammals of the family Echinostomidae. (Abdulkadir et *al*., 2018)

Parapleurolophocercous cercariae have long tails, large, and oval shapes. The cercariae can develop into intestinal trematodes in the family Heterophyidae, Chai *et al* (2009) found that the metacercarial and adult stages of these trematode species were frequently found in second intermediate hosts and definitive hosts in Thailand. Mohammed *et al*., (2016) also reported the occurrence of this type of cercariae in Khartoum Sudan and it accounted for second most prevalent release of the cercarial type released by 29.4% of the snails.

Virgulate cercariae are small, oval, and short tails. They are the least shed cercariae encountered in this study. This result corroborates the findings of Ibrahim and Ahmed (2019) in Egypt where this type of cercariae was the least shed among the six types of cercariae identified. It was however, reported that this type of trematode is produced by species of the family Lecithodendriidae (intestinal parasites of bats, birds, and amphibians) and by species of the genera Lymnaea and Melanoide (Ibrahim and Ahmed, 2019).

Conclusion

Morphologically characterized cercariae identified from the *Bulinus forskalii* snail harvested from Indire River using artificial light stimulation include Echinostome cercariae, Parapleurolophocerous and Virgulate cercarieae. Freshwater snails have displayed a high level of importance with regard to the health of the public. This study has contributed in the way of new and more accurate information on the trematode infection that is present in study area. The findings have served as an initial step for understanding the epidemiological situation and establishment of control program of trematode infections in humans and animals. This study also provided important information regarding how each cercarial type is developed into a specific trematode family or genus.

Recommendations

Based on the study the following recommendations were made;

1. Control of trematode parasitic infections should be targeted at all levels with emphasis on the areas bordering the rivers.
2. Communities using water bodies in Indire should be educated and made aware of potential risk to them and their livestock of contracting trematode infections.

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3. People who frequent and get in contact with water from Indire streams should be advised to use protective gear like gum boots and gloves, especially for those with open wounds and skin lesions.
4. Water from potentially infected sources should be treated before domestic use.
5. Community members should avoid consuming raw vegetation and washing fruits or vegetables using water from study areas with a high possibility of infection.

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