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Cercarial shedding of trematodes and their associated snail intermediate hosts in Borno State, Nigeria

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PEER REVIEW

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Comments

This is a good study in which the authors evaluated the distribution of cercariae of trematodes and their associated snail intermediate hosts in their own country (Borno State, Nigeria) in order to provide insight into better understanding of the epidemiology of trematode infections e.g. schistosomiasis and fascioliasis.

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ABSTRACT

Objective: To determine the distribution of cercariae of trematodes and their associated snail intermediate hosts in Borno State, Nigeria.

Methods: Handpicking and scooping by the aid of scoop nets were used to collect a total of 1 700 snails. They were examined for cercarial release using emergent method of cercarial release.

Results: About 16 (0.94%) of the 1 700 snails were positive for cercariae of trematodes based on morphology and standard measurement. Three species (*Bulinus forskalli*, *Bulinus globosus* and *Lymnae natalensis*) of snails were identified based on shell morphology and standard criteria. Distribution of the infection among the sampled snails showed that, 10 (1.75%) of *Bulinus globosus* and 6 (0.55%) of *Lymnae natalensis* released cercariae of *Schistosoma haematobium* and a distome cercaria of *Fasciola* spp. respectively. The intensity of infection among the examined snails varied significantly ($P < 0.05$) based on senatorial zones of sampling.

Conclusions: The present study has demonstrated the presence of some important trematodes and snail species that are possible intermediate hosts in Borno State. This study will further provide insight into better understanding of the epidemiology of schistosomiasis caused by *Schistosoma haematobium* and other trematodes and the role of snails (*Bulinus globosus* and *Lymnae natalensis*) as intermediate hosts.

KEYWORDS

Distribution, Snails, Cercariae, Intermediate hosts, Trematodes, Borno State

1. Introduction

Snail transmitted diseases constitute an integral part of parasitic diseases transmissible to humans as many fresh water snails serve as intermediate host in the trematode-parasite transmission cycle[1]. Equally, many species of freshwater snails belong to the class of highly infective flukes of veterinary importance that cause severe debilitating

illness in millions of animals[2]. The spatial distribution of intermediate hosts determines the presence and prevalence of trematodes infections transmitted by the intermediate hosts. Most regions of the world have specific snail hosts responsible for transmission of trematodes infection, for example, *Biomphalaria* spp, *Oncomelania* spp and *Bulinus* spp transmit the parasites *Schistosoma mansoni*, *Schistosoma japonicum* and *Schistosoma haematobium* (*S. haematobium*)

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respectively, in Africa[3]. Freshwater snails involved in the transmission of trematode species belong to the superfamilies Schistosomatoidea, Fasciolioidea, Clinostomoidea, Paramphistomoidea, Ehinostomatoidea, Diplostomoidea and Pronocephaloidea[4].

Previous workers have shown the abundance of bulinids and lymnaeids and the degree of their cercarial release in Africa and Europe[5,6]. Similar investigation was made by Chontanarath and Wongsawad in freshwater snails from Chiang Mai Province, Thailand[7]. Recently, Born-Torrijos *et al.* comparatively evaluated cercarial release and single-step PCR methods in determining degree of cercarial shedding by snails collected from New Zealand and Spain[8]. The use of such techniques (molecular method) may open new frontiers in the study of cercarial shedding among snails. In Nigeria, Ofozie showed that, the population dynamics of snails under both natural and experimental conditions, and their cercarial release patterns, may be depended on the vegetation cover, physical and chemical properties of the environment[9].

Most previous works on release of cercariae by freshwater snails have been mainly restricted to America, Asia and Europe with little reports from other regions especially Africa. In Nigeria, especially Borno State, there is still gap in knowledge particularly on the pattern of cercarial shedding of trematodes within the region. The role of snails in the epidemiology of important infections such as schistosomiasis, fascioliasis, paragonimiasis, and clonorchiasis deserves attention. Therefore, the current study was aimed at determining the distribution of snail(s) of possible medical and veterinary importance and the cercarial release pattern in Borno State.

2. Materials and methods

2.1. Study area

Borno State, the location of the present study is situated within latitude 10° N and 14° N and longitude 11°30' E and 14°45' E. It has an area of 61435 km² and is the largest state in the federation (Nigeria) in terms of land mass. It is located in the north-eastern corner of Nigeria and occupies the greatest part of the Chad Basin, and shares borders with the republics of Niger to the north, Chad to the north-east and Cameroon to the east. Within the country, its neighbors are Adamawa to the south, Yobe to the west and Gombe to the southwest. Based on the 2006 provisional census figures, Borno State has a population of 4 151 193 and population density of approximately 60 inhabitants per square kilometer. It has a climate, which is hot and dry for a greater part of the year, although the southern part is slightly milder. The period of wet season varies from place to place due to the influence of the various climatic factors such as the direction of the rain-bearing winds and topography. Generally, the rainy season is normally from June to September in the north and May to October in the south with relative humidity of about 49% and evaporation of 203 mm per year. The State has two major vegetation zones *viz.* Sahel in the north with severe desert encroachment covering most of the Chad Basin areas and Sudan savannah in the south, which consists of scrubby vegetation, interspersed with tall tree woodlands[10].

Five local government areas distributed across the three senatorial zones of the State were randomly selected and sampled for the purpose of this study. The southern and central zones each had two local governments, while the northern zone had one (Figure 1).

2.2. Sampling sites and collection of samples

The sampling sites were water sites that frequently had contact with either humans or animals such as fishing communities along riverbanks, irrigation canals, washing sites, drinking

points for animals and sites used for other domestic activities by humans. Ecological sites sampled in the study consisted of water bodies (ponds, dams, lakes, water pools, streams) and terrestrial environment (plain land and rocks). Vegetation cover, irrigation canals, and debris were also sampled for the presences of snails. Two of the local government areas (Maiduguri and Jere) are located within urban settlement, while the other three are rural settlements.

Three trained collectors sampled in the months of August and September 2011. Samples from aquatic environment (lakes, streams, ponds, water pools and dams) were collected using improvised scoop nets made from wire mesh (2 mm in diameter) attached to a long metal handle (1.4 m long) as described by Sharif *et al.*[11], while those on plain land, vegetation cover and debris were handpicked after wearing protective hand gloves. During all collections, the collectors wore protective aprons with Wellington boots. Collection was done in the morning between 07:30 h to 09:30 h and 100 m² was sampled per each sampling site for a period of 1 h every day. A total of 400 snails were collected per local government area of study except for Monguno local government, where only 100 were sampled. All samples were collected within a period of 5-14 d of sampling. Collected snails were kept in a lid-perforated wide-mouthed plastic container containing dechlorinated water and labeled appropriately[12,13]. The containers were immediately transported to the laboratory where the snails were rinsed by passing through several changes of dechlorinated water (Faro®). The snails were fed with lettuce throughout the duration of the experiment[13].

2.3. Identification of snails

Collected snails were identified according to the method of Brown who provided keys for the identification of African freshwater snails of medical and veterinary importance[14]. Similarly, shell morphology and measurement of shell together with some soft parts of the snails helped in identification[15].

2.4. Release pattern and identification of cercariae

The emergent method of cercarial release was adopted as described by Sharif *et al.* for the confirmation of the presence or absence of cercarial shedding[11]. Snails identified using the above procedure were placed individually into wide mouth glass specimen bottle containing 30 mL of dechlorinated water and exposed to sunlight (no direct sunlight) between 9.00 am-1.00 pm daily for possible cercarial shedding[11]. A volume of 15 mL of the water in each specimen bottle was poured into Petri dish and examined for the presence of cercariae under a stereoscopic microscope as described by Uthpala *et al.*[12]. Snails that did not release cercariae on the first day of exposure were re-exposed every subsequent day until the seventh day before being discarded. Absence of cercarial shedding on the 7th day of exposure to sunlight was indicative of the absence of infection. Water in the specimen bottle housing the snails was changed every day to avoid build up of toxicity arising from the snail's metabolic wastes.

Cercariae released by the snails were mounted on grease free slide using the single staining procedure in borax carmine[12]. Identification of the cercariae to species level was done using standard morphological characteristics and measurements[14]. This involved narcotizing the cercariae with 0.35% of NaCl solution and measuring them with a calibrated eyepiece graticule[12]. The length of the body, tail, furcae and the distance between the oral and ventral sucker were measured in the identification as described by Smyth[16].

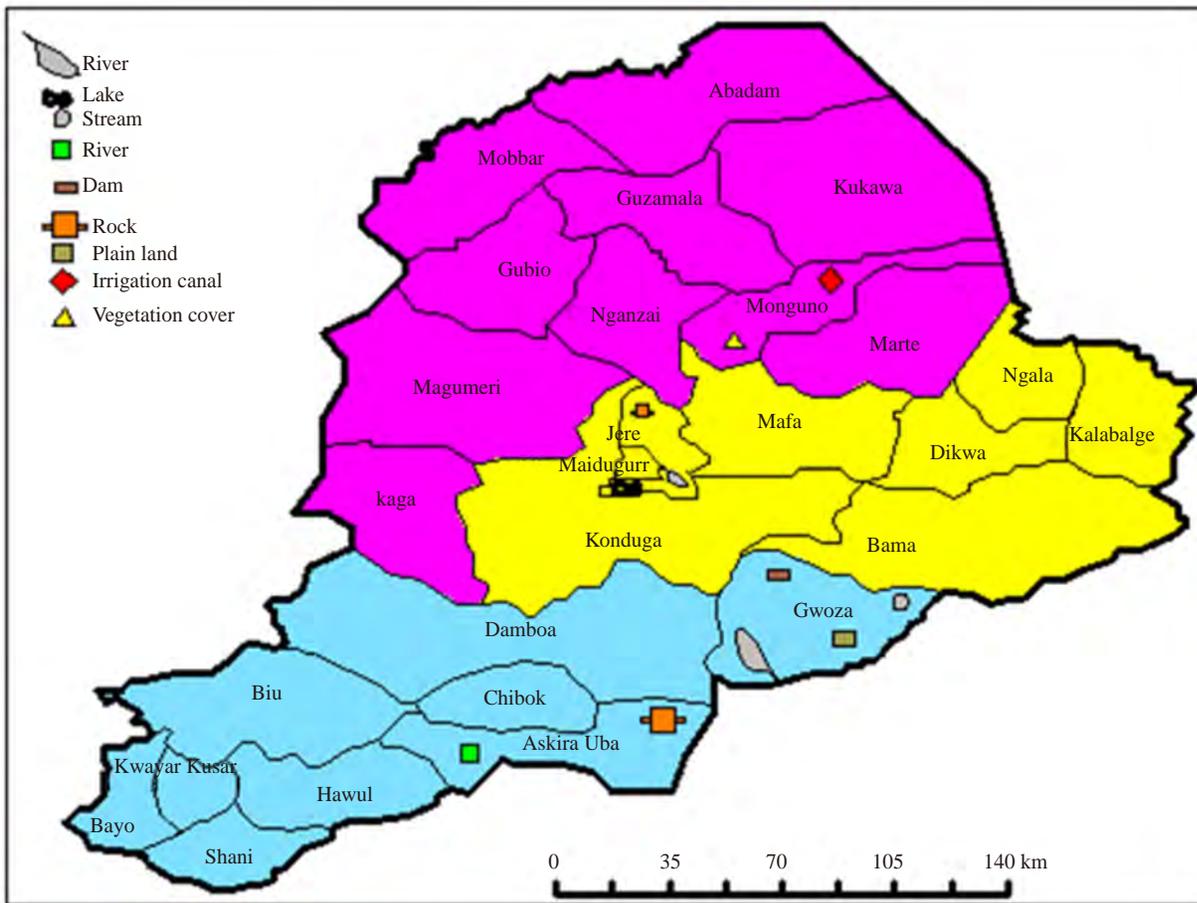


Figure 1. Map of Borno State showing the local government areas and ecological sites of sampling.

2.5. Statistical analysis

The results generated were subjected to *Chi*-square analysis for test of significance using statistical package for social sciences (SPSS version 17, 2003). $P < 0.05$ was considered significant at 95% confidence limit.

2.6. Ethical consideration

Ethical clearance was obtained from the research and ethics committee of the Faculty of Veterinary Medicine, University of Maiduguri prior to the commencement of this research.

3. Results

One thousand and seven hundred snails were collected from 11 different ecological sites distributed across the five local government areas for investigation. Three species of freshwater snails consisting of *Bulinus globosus* (*B. globosus*) (570), *Bulinus forskalli* (*B. forskalli*) (40) and *Lymnaea natalensis* (*L. natalensis*) (1090) were identified based on taxonomic keys provided. About 16 out of 1700 investigated snails released cercariae consisting of 10 *B. globosus* and 6 *L. natalensis* which released cercariae of *S. haematobium* and distome cercaria of *Fasciola* spp, respectively. No snail released more than one cercarial type during the study. Cercarial release was checked among the investigated snails within a period of 7 days and discarded thereafter. There was no mortality among the snails throughout the period of investigation. Tables 1 and 2 present the results of the current study. The identified cercariae of *S. haematobium* had long elliptical body [(396±14) μm] terminating with a tapering and forked tail (Figures 4 and 5). Pair of large eyespots is located anterior to the intestinal bifurcation. Granulated

dark color penetration glands were located in the anterior region. Ventral sucker was smaller than the oral sucker and was located at the posterior 2/3 of the body at a distance of (310±09) μm from the oral sucker. The tail bifurcated distally [(666±14) μm], producing short furcae [(252±14) μm]. Cercaria released by *L. natalensis* is a distome cercaria of *Fasciola* spp. It is small with an oval body [(120±5) μm] connected to a large simple tail [(350±5) μm] (Figure 6). The oral sucker is larger than the ventral sucker, which is located in the middle of the body.

The distribution of snails based on the snail species sampled and their respective infection rates showed that, 1090 lymnaeid snails were collected, constituting 64.11% of the total snails examined and yielding 0.55% infection rate (distome cercariae of *Fasciola* spp.) followed by 570 *B. globosus* snails and an infection rate of 1.75% *S. haematobium*, while the least investigated snails belonged to the specie, *B. forskalli*. A statistically significant variation in infection ($P < 0.05$) was observed among the species of snails examined.



Figure 2. Photograph of *B. globosus* (3684 2736) showing the apex (A) and the globose whorl (W).



Figure 3. Photograph of *L. natalensis* (3684 2736) showing the spire (S) and the body whorl (BW).

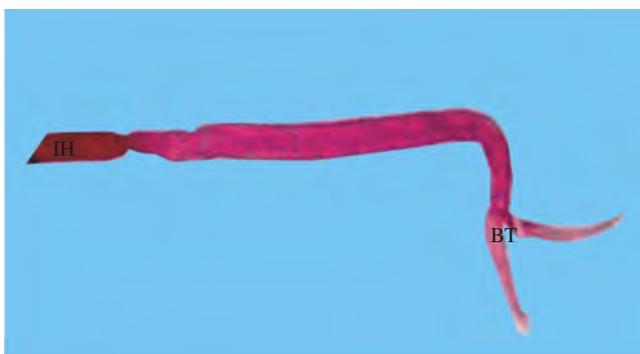


Figure 4. *S. haematobium* (875 476) cercaria showing an incomplete cercarial head (IH) and a bifurcate tail (BT).



Figure 5. *S. haematobium* (875 476) cercaria showing the cercarial head (CH) and the bifurcate tail (BF).



Figure 6. A distome cercaria of *Fasciola* spp. (875 476) showing the head (H) and the tail (T).

The distribution of various snails based on the species sampled, number examined and the respective infection rates across the local government areas of sampling are presented in Table 1. No cercaria was released by snails collected from Askira-Uba, Gwoza and

Monguno local government areas of the State. Twelve snails from those sampled in Maiduguri were positive for cercarial shedding giving an infection rate of 3.0%, while those from Jere local government had four snails positive for release cercaria accounting for 1.0%. The variations in the intensity of infection were not statistically significant ($P>0.05$) based on the local government area of sampling. Table 2 presents the distribution of the sampled snails based on the ecological sites of sampling. In addition, the table contains the number examined from each ecological site and their respective infections rates. Eleven different ecological sites were examined with each site contributing varied number. Six of the ecological sites were inland habitats (ponds, dams, lakes, water pools, streams and irrigation canals), while the remaining four (vegetation cover, plain lands, debris and rocks) were terrestrial in origin. There was variation in the density of snails collected per unit area (100 m^2) during the collection period. Sites such as plain land, water pools, ponds and dams had 15-30 snails per every 100 m^2 sampled, while the rest had 2-10 snails per every 100 m^2 sampled.

Table 1

Distribution of sampled snails based on local governments and their respective infection rates.

Zone	Local government	Snail species	Number examined	Number infected [n (%)]
Southern	Askira-Uba	<i>B. globosus</i>	20	0 (0.00) ^a
		<i>L. natalensis</i>	380	0 (0.00) ^a
		<i>B. forskalli</i>	00	0 (0.00) ^a
	Gwoza	<i>B. globosus</i>	10	0 (0.00) ^a
		<i>L. natalensis</i>	390	0 (0.00) ^a
Central	Maiduguri	<i>B. globosus</i>	240	10 (4.17) ^a
		<i>L. natalensis</i>	140	2 (1.43) ^a
		<i>B. forskalli</i>	20	0 (0.00) ^a
	Jere	<i>B. globosus</i>	300	0 (0.00) ^a
		<i>L. natalensis</i>	80	4 (5.00) ^a
		<i>B. forskalli</i>	20	0 (0.00) ^a
Northern	Monguno	<i>B. globosus</i>	00	0 (0.00) ^a
		<i>L. natalensis</i>	100	0 (0.00) ^a
		<i>B. forskalli</i>	00	0 (0.00) ^a
Total			1700	16 (0.94)

Same superscripts within columns do not differ significantly ($P>0.05$).

Table 2

Distribution of the sampled snails based on ecological sites and their respective infection rates.

Ecological habitat	Snail species	Number examined	Number infected [n (%)]
Ponds	<i>B. globosus</i>	50	4 (8.0) ^a
	<i>L. natalensis</i>	120	0 (0.0) ^a
	<i>B. forskalli</i>	00	0 (0.0) ^a
Dams	<i>B. globosus</i>	180	2 (1.1) ^a
	<i>L. natalensis</i>	10	2 (20.0) ^a
	<i>B. forskalli</i>	20	0 (0.0) ^a
Lakes	<i>B. globosus</i>	20	0 (0.0) ^a
	<i>L. natalensis</i>	20	0 (0.0) ^a
	<i>B. forskalli</i>	20	0 (0.0) ^a
Rivers	<i>B. globosus</i>	00	0 (0.0) ^a
	<i>L. natalensis</i>	18	0 (0.0) ^a
	<i>B. forskalli</i>	00	0 (0.0) ^a
Streams	<i>B. globosus</i>	00	0 (0.0) ^a
	<i>L. natalensis</i>	14	0 (0.0) ^a
	<i>B. forskalli</i>	00	0 (0.0) ^a
Vegetation cover	<i>B. globosus</i>	00	0 (0.0) ^a
	<i>L. natalensis</i>	140	0 (0.0) ^a

Table 2, continued

Distribution of the sampled snails based on ecological sites and their respective infection rates.

Ecological habitat	Snail species	Number examined	Number infected [n (%)]
Plain land	<i>B. forskalli</i>	0	0 (0.0) ^a
	<i>B. globosus</i>	0	0 (0.0) ^a
	<i>L. natalensis</i>	276	0 (0.0) ^a
Irrigation canal	<i>B. forskalli</i>	0	0 (0.0) ^a
	<i>B. globosus</i>	10	0 (0.0) ^a
	<i>L. natalensis</i>	60	0 (0.0) ^a
Water pool	<i>B. forskalli</i>	0	0 (0.0) ^a
	<i>B. globosus</i>	310	4 (1.3) ^a
	<i>L. natalensis</i>	260	4 (1.54) ^a
Debris	<i>B. forskalli</i>	0	0 (0.0) ^a
	<i>B. globosus</i>	0	0 (0.0) ^a
	<i>L. natalensis</i>	42	0 (0.0) ^a
Rocks	<i>B. forskalli</i>	0	0 (0.0) ^a
	<i>B. globosus</i>	0	0 (0.0) ^a
	<i>L. natalensis</i>	130	0 (0.0) ^a
Total		1700	16 (0.94)

Same superscripts within columns do not differ significantly ($P>0.05$).

4. Discussion

In the present study, we reported the prevalence of *S. haematobium* cercariae and a distome cercaria of *Fasciola* spp. Three different species of snails were identified in this study based on the morphology of the shell, and they consisted of *B. globosus*, *B. forskalli* and *L. natalensis* with the last being the most abundant, almost twice the population of *B. globosus* sampled and several times the number of *B. forskalli* examined. However, *B. globosus* released cercariae far more than *L. natalensis*, with no cercaria released by *B. forskalli*. Cercarial shedding was limited to only snails from the inland habitats (ponds, dams and water pools). Similarly, snails from water pools were more infected accounting for 1.40% infection rate than those from other sites among the 570 snails investigated. Infection did not show any significant ($P>0.05$) variation based on the various ecological sites of sampling. The prevalence of the infection among snails from stagnant water agreed with those of Devkota *et al.*[17], who reported lowest prevalence of infection from rivers among other water bodies sampled. This may be explained by the fact that, fast water currents that displace snails or at least keep them from congregating in large numbers, make snail-miracidia contacts a rare occurrence.

The distribution of the sampled snails among local government areas of sampling is indicative of the variations in the ecological sites. Similarly, the relative abundance of snails' species such as *Bulinus* and *Lymnaea*, which are known intermediate hosts for diseases like fascioliasis and schistosomiasis, is in part a justification for the higher prevalence earlier reported of these diseases in Borno State[18,19].

The overall infection rate of 0.94% recorded in the current study differed from the results obtained in similar studies from other parts of the world. For example, less than 16% prevalence rate was reported by Uthpala *et al.* who sampled four genera of snails from irrigation canals in three climatic zones of Sri Lanka and 7.33% was reported by Tigga *et al.* among snails from canals, tanks, ditches, ponds and crop fields[12,20]. However, this is similar to the result obtained by Kigadye and Nkwengulila who reported

an overall prevalence of 1.3% among snails sampled at a pond in Tanzania[21]. Similar study conducted in Jos, Nigeria by Okpala *et al.* indicated a higher (8.13%) infection among the examined snails compared to the present study[22]. The result of the present study also agrees with Anderson and May who attributed the low prevalence of cercarial shedding among natural snail population to direct consequence of parasites induced host mortality[23]. The season of sampling may have contributed to the low prevalence of cercarial shedding among the examined snails. This corroborates earlier result reported by Chandiwana *et al.* who observed the effect of season on the rate of cercarial shedding among snails[24]. Snails shed more cercariae during the dry season because of high number of snails to reduced volume of water. In addition, increased volume of water in water canals due to rainfall and the effect of temperature are other factors known to affect population of snails and particularly cercarial shedding which is dependent on light and temperature. Increased water velocity was reported to dislodge snails from their natural habitats, thereby reducing their production and infection potentials[8,21]. Besides factors discussed above, the relative short duration of sampling in this study is a limitation that further studies need to improve on.

This study has demonstrated that the mollusks, *B. globosus* harbored *S. haematobium* cercariae and *L. natalensis*, the cercariae of an unidentified trematode in Borno State, Nigeria and may therefore serve as intermediate hosts to the trematodes. The prevalence of the infection among the investigated snails though low may pose serious danger to humans and animals within the study area unless efforts are made to limit contact between definitive hosts and the snails carrying the infective forms of the parasites. The use of molluscicides to control snails is an important control measure. Government should direct its efforts towards the provision of potable water for both human and animal use. Public enlightenment will help restrict herdsmen/nomads from grazing in potentially dangerous sites.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Many fresh water snails serve as intermediate host in the trematode-parasite transmission cycle. Abundance of freshwater

snails (Bulinids and lymnaeids) and the degree of their cercarial release and shedding had been reported in Africa, Europe, New Zealand and Spain, America, Asia and Europe. In Nigeria, Borno State, the snails, the pattern of cercarial release and shedding might have some roles in the trematodes infections.

Research frontiers

Studies are being performed in order to identify snails, cercarial release pattern and shedding to demonstrate the intermediate hosts and parasitic infections in the ecological sites such as water bodies and terrestrial environment.

Related reports

The overall infection rate of 0.94% recorded in the current study differed from results obtained in similar studies from other parts of the world *i.e.* 16% prevalence rate reported by Jayawardena *et al.* (sampled four genera of snails from irrigation canals in three climatic zones of Sri Lanka); 7.33% reported by Tigga *et al.* (snails from canals, tanks, ditches, ponds and crop fields).

Innovations & breakthroughs

Determining the distribution of snail(s) of possible medical and veterinary importance, cercarial release pattern and shedding in parasitic infections have impact on human and animals.

Applications

The study has demonstrated the presence of some important trematodes and snail species that are possible intermediate hosts in Borno State.

Peer review

This is a good study in which the authors evaluated the distribution of cercariae of trematodes and their associated snail intermediate hosts in their own country (Borno State, Nigeria) in order to provide insight into better understanding of the epidemiology of trematode infections *e.g.* schistosomiasis and fascioliasis.

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