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Susceptibility and compatibility of *Bulinus globosus* from different snails' population in north eastern Nigeria to *Schistosoma haematobium* obtained in Bauchi and its implication for control of Urinary Schistosomiasis

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Abstract

This study investigated the compatibility of *Bulinus globosus* populations from six states in northeastern Nigeria with local strains of *Schistosoma haematobium* under laboratory conditions. A total of 10 F_1 generation snails, bred in the laboratory from each state population, were individually exposed to 10 miracidia obtained from schistosome eggs collected in Piro village, Bauchi State. Following a prepatent period of 24 to 28 days, both sympatric (same geographical origin) and allopatric (different geographical origin) host-parasite combinations began shedding cercariae. The average cercarial output per snail per day was relatively low, ranging from 34 to 47 cercariae. However, the total cercarial output per 100 snails was high, ranging between 480,060 and 872,550 cercariae. The infection period per snail was short, lasting between 35 and 49 days. Infected snails exhibited a relatively low mortality rate (6.25–25%), suggesting a degree of tolerance to the parasitic infection. Notably, there were statistically significant differences ($P < 0.05$) among the states in both daily cercarial production and average infection duration. The combination of a short prepatent period, low mortality, and high total cercarial production indicates a strong compatibility between the local *S. haematobium* strain and *B. globosus* populations across northeastern Nigeria. These findings imply a high potential for the establishment and transmission of schistosomiasis in the region. Given this high compatibility, coupled with the extensive movement of people and livestock (transhumance) across the area, there is a significant risk for the spread and outbreak of human urogenital schistosomiasis. This underscores the need for region-wide surveillance and control strategies to mitigate transmission.

Keywords
Bulinus globosus
Snails' population,
North-eastern Nigeria,
Schistosoma haematobium

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INTRODUCTION

Schistosomiasis is and remains one of the most prevalent parasitic infections in the World. It is a disease endemic in 76 countries and territories. The disease, schistosomiasis is a global public health issue especially in the developing countries (World Health Organization, 2007). Current estimates shows that over 120 million have asymptomatic conditions, 20 million have severe disease while more than 600 million other persons are at risk of being infected (WHO, 2009)

Despite the disturbing public health challenge posed by schistosomiasis, it is not being given the priority attention it deserveS by both national and local health policies and programs in many countries of the World (Bruun and Aargard-Hansen, 2008). The disease is therefore considered to be one of the neglected tropical diseases (Bruun and Aargard-Hansen, 2007). Although the health significance and consequences of schistosomiasis have been appreciated and control measures initiated in a number of countries of the World (Guo, 2003; Chen, 1999; Katz, 1998; Tanaka and Tsuji, 1997), it still remains second only to malaria among all parasitic infections.

In Nigeria, the prevalence of schistosomiasis as shown in fig.1 indicates that it is endemic in all the 36 States and the Federal Capital territory, Abuja (Ngozie,et al.,2008). In spite of the disturbing level of prevalence in the country today, the public awareness about the disease even in endemic communities, morbidity pattern and pathological conditions caused are by far less appreciated. This can be attested to by the apparent lack of a coordinated control program. To put in place any deliberate and specific control program, data on epidemiology, biology of the schistosomes and their intermediate hosts together with the prevailing environmental factors are needed.

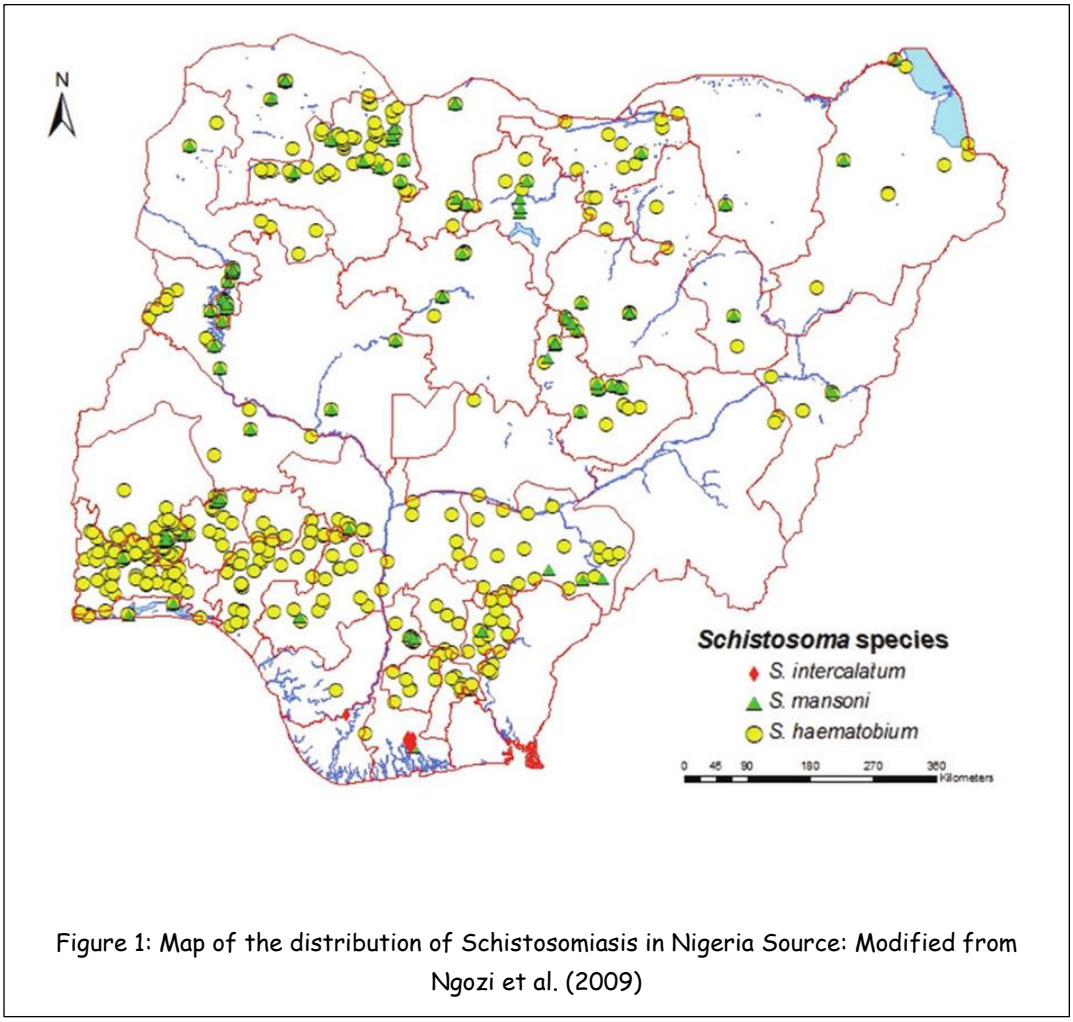

Figure 1: Map of the distribution of Schistosomiasis in Nigeria Source: Modified from Ngozi et al. (2009)

In an effort to contribute to providing some of these key information, this study investigates the interactions between larval *Schistosoma haematobium* collected from Piro village in Bauchi, Bauchi Local Govrnment Area of Bauchi State and *Bulinus globosus* the known major intermediate snail host in North Eastern Nigeria.

MATERIALS AND METHODS

Description of the Study Area

The North East geopolitical region of Nigeria is made up of Adamawa, Bauchi, Borno, Gombe, Taraba and Yobe States. The area lies between Latitudes 6°N to 14°N and longitudes 10°E to 14°E. It covers a land mass of 238,760km^2 with a total population of 16,791,053 (Anon, 2006).

North Eastern Nigeria shares international boundaries in the East by the Republic of Cameroon, in the north by Niger Republic, shares parts of Lake Chad with Chad Republic. The region has internal borders with the North Western States of Jigawa and Kaduna in the North West and Plateau and Benue States in North Central Nigeria (Fig. 2).

In terms of climate, North Eastern Nigeria is described as the hot dry zone of Nigeria. Mean daily temperature, during the dry months of January to March equals or exceeds 35°C while relative humidity does not exceed 40% during the same period (Nigeria Building and Road Research Institute. NIBRRI, 1983). The wet period experienced in the area is usually short spanning from May to September while the dry period is longer, extending from October to April (Natural Resources Conservation Council of Nigeria-NARESCON, 1992).

Owing to the peculiar climatic nature of this region, its vegetation characteristics corresponds to the following ecological groups: Guinea and Sudan Savanna and the Sahel or semi-arid vegetation types

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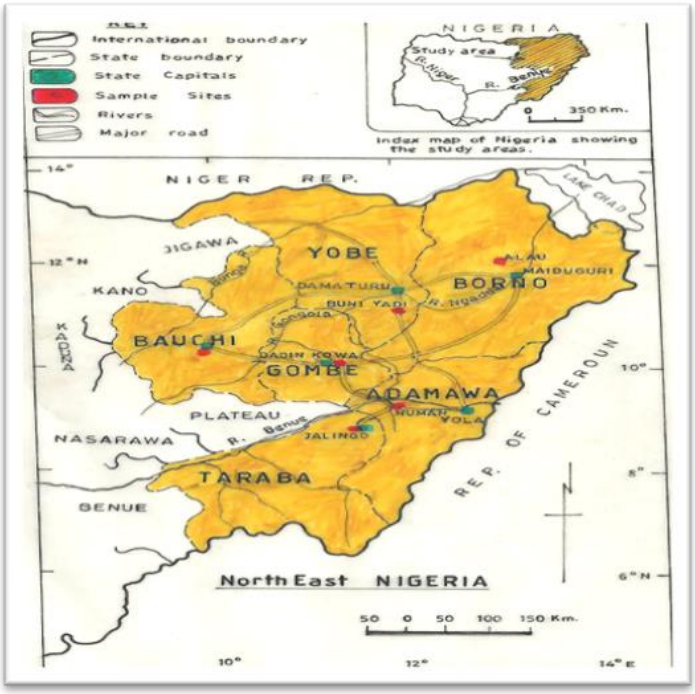

Figure 2: Map of North Eastern Nigeria Showing Study States and Sites

The Sahelian landscapes of Borno, Yobe and that of the Northern extremities of Bauchi and Gombe States with their short raining period and an annual precipitation ranging from 250-500mm, supports the cultivation of early ripping of crop varieties like Sorghum, Maize, Sesame and hardy three plants like Gum Arabic. The Southern parts of these states which have the Sudan-Guinea Savanna Vegetation type, supports the cultivation of many crops such as Beans, Cotton, Groundnuts, Maize etc.

Adamawa and Taraba States occupy the Guinea Savanna areas and the Mambila highlands. This part of North Eastern Nigeria experiences a fairly longer rainy period (April-October), with showers in November. With an annual rainfall of 960 to over 1000mm (James *et al.*, 2009) a wide variety of both tree and other crops are cultivated. In addition to farming, the vast network of seasonal rivulets either as offshoots of the River Benue or originating from the Mambila hills, encourage communities along such rivers to also engage in fishing activities (Anon, 2006). The Southern parts of Bauchi State, the central and Southern parts of Gombe State significantly occupy the Sudan Savanna. A wide variety of crops (Groundnuts, Beans, Cotton Rice, Millet, Sorghum) are cultivated. The abundant variety of grasses in the area support the extensive animal husbandry practiced in this area.

Selection of Sampling Sites

The study sites were selected in North Eastern Nigeria as shown in Fig. 2 above. These sites were selected to reflect the broad ecological stratification of North Eastern Nigeria as described in Section 3.1 which are Guinea, Sudan and Sahel Savannas. Five different fresh water habitats consisting of ponds, streams, ditches were selected with the aid of guides based on information on the availability of snail intermediate hosts, accessibility, and nature of water body (permanent or temporary).

Collection of Snails

Snail samples were collected from each site using long handled scoop net according to the methods of Madsen *et al.* (1987). Samples of both live and dead snails were collected and then separated; the dead ones were packaged in clean empty 500ml specimen bottles. Live specimens were however wrapped in damp cotton wool before they were kept in specimen bottles. Both lives and dead specimens were then transported to the laboratory in Abubakar Tafawa Balewa University, Bauchi for experimentation. In the laboratory, snail samples collected from sites were separated and *B. globosus* were identified according to the standard keys of Brown, (1994).

Rearing of Snails: Live specimens collected from each site were reared separately and maintained in the laboratory according to the methods described by Madsen (1985)

Preparation of Snail Food

Snail feed was prepared from fresh lettuce leaves (*Lettusa sativa*) purchased locally from markets metropolis. The Leaves were first washed so as to clear off soil particles and any other debris using tap water. Thereafter the leaves were immersed in warm water for 10-20 seconds to soften the tissues after which they were spread in between the pages of papers and placed in an oven for about 10 minutes at 60°C. The leaves were then cooled and used for feeding the snails.

Susceptibility Studies:

Experimental infection of snails were conducted using miracidia of *S. haematobium* from schistosome eggs obtained from urine of infected persons identified in an earlier prevalence survey in Piro Village near Gubi Dam, a suburb of Bauchi town.

Collection and Hatching of *Schistosome* eggs

Clean labeled 50mls screw capped specimen bottles were issued to already identified schistosomiasis patients. They were instructed on how to collect

midstream urine samples. Samples thus collected were transported to the laboratory in Abubakar Tafawa Balewa University, Bauchi for further treatment. Urine samples were then allowed to stand in test tubes overnight in a dark cupboard in order to prevent hatching and to allow schistosome eggs to sediment by gravity. The next day the supernatants were carefully drained away and fresh water added to the sediment so that the eggs could hatch into miracidia which were subsequently harvested following the method of Manning *et al.* (1995) and Stothard (2000);

Snail Infection with miracidia

First generation (F1) snails that hatched from the parent stock, collected from field with shell heights of 3-6mm (i.e. ≅ 3 weeks old) were used in the study. Snails of the same size were consistently used from each population, to control for any effect of size on rates of infection. 10 snails per population were then challenged by being exposed individually to ten (10) Miracidia in 15ml of dechlorinated water for 2 hrs at ambient temperature (26°C-30°C) as recommended by Brown, 1994, Manning, 1995 and Stothard, *et al.* 2000.

Cercariae Production and Counting

At 21 days post infection after the commencement of cercarial shedding, infected snails from the six populations were separated and kept in 2l of snail water in 50ml beakers. All snails were exposed for 3hrs to the same light intensity in accordance with the method of Webster and Southgate (2003) in order to ensure cercarial emergence. Before cercarial counts, beakers were gently swirled round by hand to ensure even dispersion of the cercariae in the water then 1ml aliquot was immediately taken by use of pipette and put in a watch glass.

Two drops of lugols iodine solution were added to the 1ml sample and viewed under a binocular dissecting microscope in order to count the cercariae shed. This was repeated three times for each snail and the average taken. This average was used to calculate the total number of cercariae per 20ml sample and this procedure was repeated for all the snails in a group. This procedure was repeated 3 times for each snail and the average taken. The water in the 50ml beakers were changed after each set of counts so that the cercarial production of each snail host per population was carried out weekly. The total cercarial count per 100 expose snail was determined according to the method of Frandsen (1979b) as follows:

TCP/100snails =

$$\left(\sum_{1}^{n} \text{Total cercarial production per test group at each time of counting.} \quad \text{x} \quad \text{Number of days between counts} \right) \frac{100}{\text{N}}$$

n = number of countings

N = number of snails in each exposed group.

Statistical analysis

Weekly cercarial production for the various locations were analysed using One way Analysis of Variance using the SPSS software computer version 21 of 2012 while total cercariae production was analyzed by use of Chi square test using the same version of SPSS/PC software.

Results:

Snail Infectivity

The results of infecting *B. globosus* from different populations with *S. haematobium* miracidia from Piro village near Gubi Dam are sumarised in Table 1. Where it can be seen that the prepatent periods in the study ranged from 24-28 days. The shortest period of patency of 24 days was observed between *S. haemotobium* and *B. globosus* from Bauchi town of Bauchi State while the longest was observed between *S. haematobium* and *B. globosus* from Jalingo town of Taraba State. The variation observed during the study in pre-patent periods among the various snail populations was not significantly different from each other (P>0.05).

Infectivity of *S. haematobium* in Bulinid Snails from Different Populations

Results obtained in this study showed that all snails challenged with equal doses of *S. haemotobium* miracidia became infected as shown by shedding of cercariae at the end of the pre -patent periods as also indicated in Table1. Such infections led to varying levels of mortality of snails. Overall mortality from infection ranged from 6.25% to 25.00% among the different snail populations.

Table 1. Infectivity of *S. haematobium* miracidia to Different Populations of *B. globosus* from Different Parts of North East Nigeria.

Origin of snail	Pre-patent period (days)	No. of dead snails	Averaged Cercarial production per 100 snails	Cercarial production/snail/day	Averaged duration of infection (days)
Borno (Alau)	26	04(25.00)	626,850	34	42
Yobe (Y/Buni)	27	02(12.50)	607,950	34	35
Bauchi (ATBU)	24	01(6.25)	872,550	41	49
Gombe (D.Kowa)	26	03(18.80)	480,060	47	49
Adamawa (Numan)	25	02(12.50)	671,580	34	35
Taraba (Jalingo)	28	04(25.00)	532,980	47	49
Total(where applicable)		16	3,791,970	237	259
Col. Chi-square	5.445^{ns}	4.849^{ns}	0.008^{ns}	21.223^{***}	17.868^{***}

Figures in parenthesis are percentage mortalities.

*** = significant at (P< 0.005)

Duration of Infection and Cercarial Production per Day per Snail

The cercarial production/snail/day ranged from 34-47 (Table 1). The least value (34) was observed in snails from Borno, Yobe and Adamawa States while the highest value (47) was recorded for *B. globosus* from Taraba and Gombe States.The shortest duration of cercarial production was observed in *B. globosus* from Yobe and Adamawa States while the longest duration was from *B. globosus* from Taraba State. Chisquare analysis did not revealed any significant differences in the duration of infection ($p>0.05$) but cercarial production per snail per day was significantly different among the 6 snail populations ($p<0.05$).

Cercarial Production/100 Snails

The Total Cercarial Production of the populations sampled are as shown in Table 1. While *B. globosus* from Gombe had the least Total Cercarial Production (TCP) of 480,060, *B. globosus* from Bauchi had the highest total cercarial production of 872,550. Chisquare analysis showed no significant differences among snail populations from the different populations sampled ($p>0.05$).

Weekly Cercarial Production.

Results of weekly cercarial production of different *B. globosus* infected with *S. haematobium* miracidia are presented in Table 2. It can be seen that the mean count of cercariae from snails of the various geographic locations was 150±107.2. The minimum mean count was 120±116.7 while the highest count of 197.9±60 was recorded from Taraba and Bauchi States respectively.

Analysis of variance of the weekly production of cercariae among the different populations of *Bulinus globosus* tested were significantly different ($P<0.05$).

Table 2: Mean ± Standard Deviation of Cercarial Production in Six Populations of *Bulinus globosus*

Variable	N	Mean count of cercariae
Overall	42	150.5±107.2
Location:		ns
Borno	7	142.1 ± 99.5^{ns}
Yobe	7	137.9 ± 141^{ns}
Bauchi	7	197.9 ± 60^{ns}
Gombe	7	151.7 ± 130.5^{ns}
Adamawa	7	152.3 ± 102.1^{ns}
Taraba	2	120.9 ± 116.7^{ns}
Week:		***
1	6	182.8 ± 46.5^{b}
2	6	296.7 ± 38.0^{a}
3	6	273.7 ± 20.4^{a}
4	6	118.0 ± 72.4^{cs}
5	6	93.2 ± 54.4^{cd}
6	6	58.5 ± 49.8^{de}
7	6	35.5 ± 44.5^{e}

*** = Mean cercariae production between weeks are significant at P < 0.0.5

*Means with the same superscripts are statically similar.

Discussion

Susceptibility to *S. haematobium* by *B. globosus* from Sampled Sites

In this study, the pre-patent periods for snail infections were relatively short lasting only (24 – 28 days in both sympatric and allopatric combinations. This observation is similar to those of Capron *et al.* (1965) in Malawi and Lo (1972) in Egypt who also reported short pre-patent periods in their studies. The short pre-patent periods reported in this study may be attributed to environmental temperatures. It is known that variation in tropical temperatures

from moderate to high tend to favour the intra-molluscan development of schistosome Thus, the temperature regimes in the study area provided suitable internal environment within the snail intermediate host, thereby aiding rapid establishment of the miracidia and subsequent efficient asexual generation of primary and secondary sporocysts. This situation was similarly observed by Shiff, *et al.* (1975) during their work on seasonal influence on the production of *S. haematobium* and *S. mansoni* cercariae in Zimbabwe and further noted that low winter temperatures interrupted the intra-molluscan development of Schistosomes.

The finding the present study that all exposed F1 generations of snail intermediate hosts from the different sites became infected with miracidia harvested from infected local urine samples is noteworthy and gives a very good index of susceptibility *Bulinus* snails from different parts of North Eastern Nigreria to a local Schistosome Strain. It however contrasts with the finding of Stothard *et al.* (2000) in Zinzibar Island.

Compatibility of *S. haematobium* with *B. globosu*

The occurrence of very high compatibility levels between local schistosome strains and *B. globosus* from different parts of North Eastern Nigeria is an interesting finding and has potential implications on the epidemiology of schistosomiasis in the sub region. One of the main sources of introduction of schistosome species in an area is through travel by infected persons especially children. There are good network of roads linking the six States that constitute the north eastern Nigeria as well as good intra-State road networks. This has undoubtedly made travel to be easier. Thus, when an infected person from Bauchi State where the schistosome was obtained travel to any of of the other five States in the sub-region, there is a high risk of spreading infection to the visited area since the Snails have shown remarkable compatibility with the Bauchi schistosome strain.

Compatibility between snail-schistosome combinations had been reported to depend on the "matched" or "mismatched" status of a host and parasite phenotypes (Rollinson *et al*, 2001., Theron and Coustau, 2005) this idea poses that if a particular schistosome has a genetic constituition that appropriately matches that of a snail of a particular constitution, a successful susceptibility and compatibility will take place. On the other hand, if a genetic "mismatch" is the order between host and parasite, there won't be any infection in the snail host thus, leading to resistance to infection. Therefore, it would appear that there is a perfect match between the local Bauchi schistosome strains with *B. globosus* from all parts of north eastern Nigeria. Further work on compatibility between both sympatric and allopatric combinations of snails and schistosomes from the other parts of the sub-region is desirable in order to fully appreciate the dynamics of the epidemiology of schistosomiasis in north eastern Nigeria.

The current findings have great implication on the control of schistosomiasis in the sub-region. Thus, it is advocated that control measures involving chemotherapy directed at the parasiteis the most feasible strategy to be adopted for the area. Owing to the varied ecological setting a well as the diverse nature of fresh water habitats in the sub-region, an effective snail control would be a very difficult task to achieve.

CONCLUSION

It's concluded from the present study that Bulinus snails obtained from different sites in the study area (North Eastern Nigeria) are all susceptible to the local schistosome strain harvested in urine samples collected from infected persons in Bauchi. The current study has further demonstrated that *Bulinus* species from different parts in the study area are all compatible with *S. heamatobium* obtained locally from Piro village in Bauchi, Bauchi Local Government Area, Nigeria.

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