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Original papers

Prevalence and molecular characterisation of *Schistosoma haematobium* among primary school children in Kebbi State, Nigeria

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ABSTRACT. Schistosomiasis is the major source of morbidity in Sub-Saharan Africa and Asia. It is estimated that 207 million people are infected, of which 97% are in Africa. The aim of this study was the determining of prevalence as well as the phylogeny of *S. haematobium* among school children in Argungu Emirate, Kebbi State Nigeria. A total of 325 urine samples was collected from school children between 7 to 14 years. *S. heamatobium* eggs was examined under dissecting microscope and DNA was extracted from urine sample and COX1 gene was amplified by nested PCR. The PCR products were purified, sequenced and analysed. This study showed a prevalence of 32.09%, with male pupils having the highest prevalence. *S. haematobium* infections in children who fetch water in the river have 24 times higher risk of being infected while those who bath in the river have 158 times higher risk of being infected. Our sequences were phylogenetically related to *S. haematobium* isolate U82266 from Kenya and consistence with the predominant species in Africa. This was the first *S. haematobium* and *S. mansoni* co-infection reported in Nigeria. *S. haematobium* infection is prevalent among school age and significantly associated with water contact.

Key words: *Schistosoma haematobium*, phylogeny, Nigeria, primary school children

Introduction

It is estimated that 779 million people are at risk of infection with schistosomes and 207 million people are infected, of which 97% are in Africa [1,2]. Human become infected through skin penetration when they come in contact with cercariae often during various water contact activities [3,4]. Five major species are known to infect humans: *Schistosoma haematobium*, *S. mansoni*, S. *japonicum*, *S. mekongi* and *S. intercalatum,* the first three are of most significant public health importance [5]. Other schistosomes that occasionally infect human causing dermatitis or insignificant infection includes *S. bovis* and *S. mattheei* together with some avian schistosomes [6]. These species differ biologically from one another and to their geographic distribution and the type of disease they cause [5]. Urinary schistosomiasis in Nigeria has substantial transmission occurring in almost all the state of the federation with high prevalence rate among children of school age [7,8].

However, some researchers highlighted the creation of freshwater sites via dam construction and irrigation as well as location of the human settlements near freshwater, makes the environment suitable for schistosomes to have truly flourished and disease prevalence has come to stayed [9].

Mitochondrial genes have been used to facilitate the molecular analysis of the relationship between different species, because they tend to have higher mutation rates than nuclear markers and exist in high numbers [10]. However, this study aimed at determining the prevalence as well as the phylogeny of *S. haematobium* among school children in Argungu Emirate, Kebbi State Nigeria for the first time.

Materials and Methods

Study area. This study was conducted within Argungu Emirate council area of Kebbi State Nigeria, involving three different districts namely Argungu, Gotomo and Yeldu (Fig. 1).

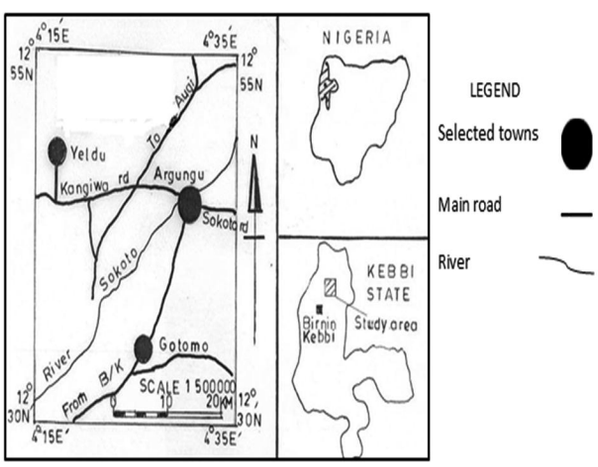

Fig. 1. Map of Nigeria showing location of the study area

Study population and design. A community-based cross-sectional study was conducted between 15 of July 2013 to 15 of July 2014 involving school children who are within the age of 7 to 14years attending primary school in the study area. Multi-stage sampling method was employed in obtained schools that participated in the study. Argungu Emirate has eight districts at the first stage of the sampling three districts namely Argungu, Gotomo and Yeldu were randomly selected. In the second stage of the sampling primary schools were randomly selected from the selected districts and finally the names of the school children obtained from the headmasters was used to enrolled the study participants in the respective schools selected following simple random sampling.

Collection and processing of sample. Data comprising age and gender were collected using class register containing the list of the school children (pupils) obtained from respective school headmasters. A total of 325 urine samples were collected from school children between 7 to 14 years of age from the participating schools. Labelled, sterile, wide mouth screw capped plastic containers were distributed to the selected children with the instruction to deposit midstream urine between hours of 10:00–14:00 for optimum egg passage.

Parasitological examination. Approximately 50ml urine sample was passed through a 12.5cm Whatman No.3 (Whatman international, Maidstone England) filter paper and filtered urine was collected. The filter paper was stained with ninhydrine (0.2%) in ethanol solution and was allowed in the dark for at least 15 minutes to increases the visibility, thereafter, the paper was examined under the dissecting microscope for egg detection as described previously [11].

Genomic DNA extraction and PCR Amplification. DNA from *S. heamatobium* egg was extracted from 200µl of urine sample following concentration of the sample. The DNA was precipitated and concentrated using Qiamp DNA blood mini kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The DNA was finally eluted with 50µl of elusion buffer and stored in –20°C. Self-design primers were used for PCR amplification of Schistosoma Cox1 gene (Forward 5'-CCTATGGGTGGTGATCC-3' and reverse primers 5'-ACACGAGACCCACAGCTT TT-3'). All samples were handled and treated aseptically and under ice condition, onward PCR 25µl reaction mixture containing 2.0µl of DNA

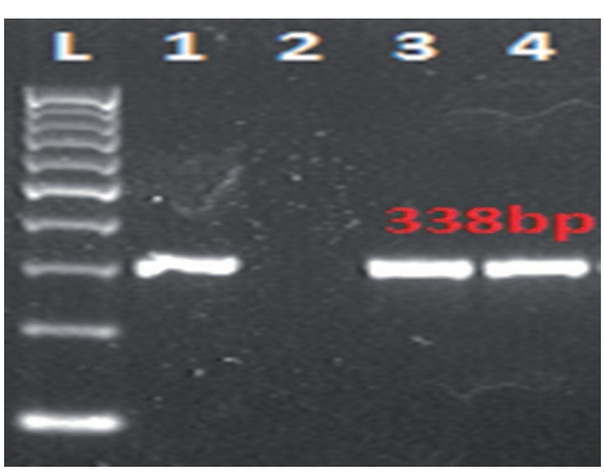

Fig. 2. Detection of 338 bp of Cox1 sequence of S. *haematobium*. L = 100 bp size markers; lane 1 = Positive control; lanes 2 = Negative control; lanes 3 and 4 = egg-positive samples from Kebbi State Nigeria.

Table 1. Reference sequences of different *Schistosoma* species

Species	Cox1 with accession numbers
S. japonicum	U82264 [10]
S. malayensis	U82262 [10]
S. mekongi	U82263 [10]
S. mansoni	U82265 [10]
S. haematobium	U82266 [10]
S. haematobium present study	KJ606959
S. haematobium present study	KJ606957

samples, 12.5µl Go Taq^R Green Master Mix, 0.5µl of each forward and reverse primer, and 8.5µl double distilled water. The thermocycling condition began with initial denaturation at 95°C for 15min, 30 cycles of 95°C for 30 seconds, and 57.5°C at 1.5 min for Cox1 extension at 72°C for 1min and final extension 60°C for 5min. The PCR products were used for electrophoresis and bands corresponding with the size of the targeted gene were considered positive (Fig. 2).

Sequencing and phylogenetic analysis. The PCR products were purified using PCR clean-up system (Promega, USA). The products were commercially sequenced (First base, Malaysia). The sequences were submitted to GenBank under accession numbers KJ606956, KJ606957. Molecular Evolutionary Genetics Analysis version 6 (MEGA6) software [12] was used for sequences, alignment via Clawstal W and phylogenetic analysis was inferred using Maximum likelihood (ML) based on the Jukes-Cantor model. Reference sequences of different Schistosoma species were selected from NCBI database [http://www.ncbi.nih.gov] based on published sequence [13] (Table 1).

Data analysis. Mega7 software was used for phylogenetic analysis and data from questionnaires were coded and entered into SPSS version 22.0 software. Chi square and logistic regression was used for statistical analysis.

Ethical approval and informed consent. Ethical approval to carry out this study was obtained from UPM research ethics committee at Faculty of Medicine and Health Sciences (UPM/TNCP/RMC/1.4, 18.1 (JKEUPM)/F1). Permission was also given by Ministry of Education in Kebbi State (KESUBEB/PRS/P&S/156). Informed consent from parents/guardians of the study subjects as well as that of parent's teachers association (PTA) of various respective schools was sought.

Results

From a total of 325 school children who participated in the study 107 were found to be positive for *S. haematobium* infection, with the prevalence rate of 32.09%. It was also found that, out of 196 male pupils, 69/190 (32.25%) were infected with *S. haematobium*, while out of 129 female pupils screened, 38/129 (29.00%) cases were recorded. Therefore, the result showed that male pupils had higher prevalence rate. Prevalence of 36.80% was found among pupils that are within the age range of 10 to 12 years. On the other hand, Gotomo town was found to have the highest prevalence rate – 60.0% of school pupils were infected with *S. haematobium.* While, no infection was found in Yeldu town. Logistic regression showed significant association between *S. haematobium* infections and some risk factors such as: age, fetching water, playing/bathing and swimming (Table 2). It was also revealed that children who fetch water in the river/stream had 24 times higher risk of being infected with *S. haematobium* compared to those who do not, with adjusted OR = 24.5, p = 0.002. Also, showed that children who play/bath in the river/stream have 158 times higher risk of being infected with schisto-

Table 2. Logistic regression predicting association between studies risk factors and *S. haematobium* infection

Variables	Wald χ^2	P - value	Adjusted odd ratio	95% CL	β	SE
Age	5.059	0.024*	1.43	1.047-1.964	0.361	0.16
Fetching water	9.301	0.002*	25.0	3.137 -191.495	3.199	1.049
Playing/bathing	42.36	0.001*	157.6	34.336-723.248	5.06	0.777
Swimming	11.072	0.001*	33.9	4.254-269.931	3.523	1.059

*Significant at $p \leq 0.05$; χ^2: Chi square test; β: Beta; SE: standard error

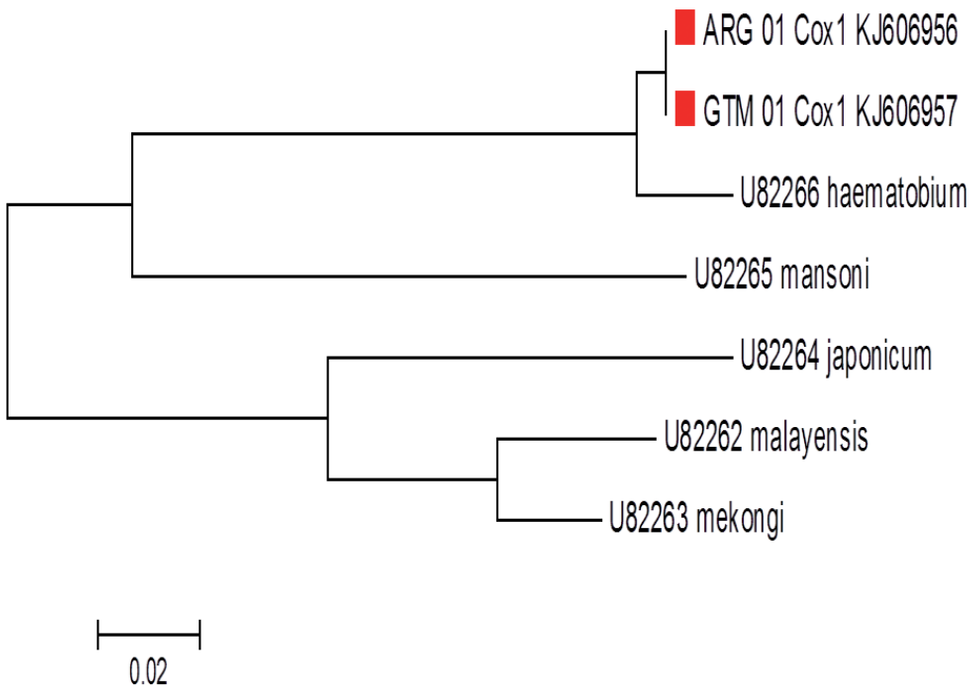

Fig. 3. Phylogenetic tree depicting relationships among *Schistosoma* species inferred from Cox1 nucleotide data. The tree constructed using Maximum likelihood (ML) based on the Jukes-Cantor model in MEGA6 software. The data set was bootstrapped 500 times and appropriate bootstrap values placed on each branch. No out-group taxon was used.

somiasis compared to those who do not with adjusted OR = 157.6, p = 0.001. In addition, it was also observed that children who swim in the river/stream had 34 times higher risk of being infected with urinary schistosomiasis compared to those who do not with adjusted OR=33.9, p = 0.001.

Phylogenetic analysis revealed that our samples placed in a distinct cluster with *S. haematobium*. Members of this group were phylogenetically related to *haematobium* – U82266; an isolate from Kenya classified under *S. haematobium* (Fig. 3). On the other hand, these sequences (KJ606956 and KJ606957) were grouped together in a cluster and phylogenetically closer to other sequences from Mali, Tanzania, Cameroon, Malawi, Ghana, Gambia and South Africa (Fig. 4). Regardless of the phylo - genetic method used, the topology pattern of the resultant trees was observed to be similar.

Discussion

The results obtained from this study showed that out of 325 school children who participated in the study 107 were found to be positive for *S. haematobium* infection, with the prevalence rate of 32.09%, this concur with findings from previous study that reveals high transmission rate of *S. haematobium* infection in south western Nigeria [7]. The rate of *S. haematobium* infection with respect to sex shows that males have higher prevalence 35.2% than females 29.5%. However, this study shows no significant gender difference (p>0.05) which is similar to what was reported in a previous study [14] which shows prevalence of *S. haematobium* did not differ between gender except when gender influence water use and contact. Therefore, persons who have greater contact with the infested water harbouring cercariae stage of the parasite may have high prevalence regardless of gender [15] this is also supported by other researchers [14,16]. However, the high prevalence found among 10–12yrs in this study suggested that children within the said age bracket visit river, streams and ponds more often compared to other groups, this is closely related to 13–15yrs age group reported in previous studies [17,14].

Despite the fact that Argungu Emirate is endowed with abundant bodies of water which seems to be a major factor that provides a breeding site for snail vector [5]. Our findings revealed the presence of *S. haematobium* infection in two of the district with Argungu having higher prevalence (66.36%) followed by Gotomo (33.64%). Whereas there was no infection recorded in Yeldu district which might be as a result of the low sample size in this district. However, individuals who are closer to

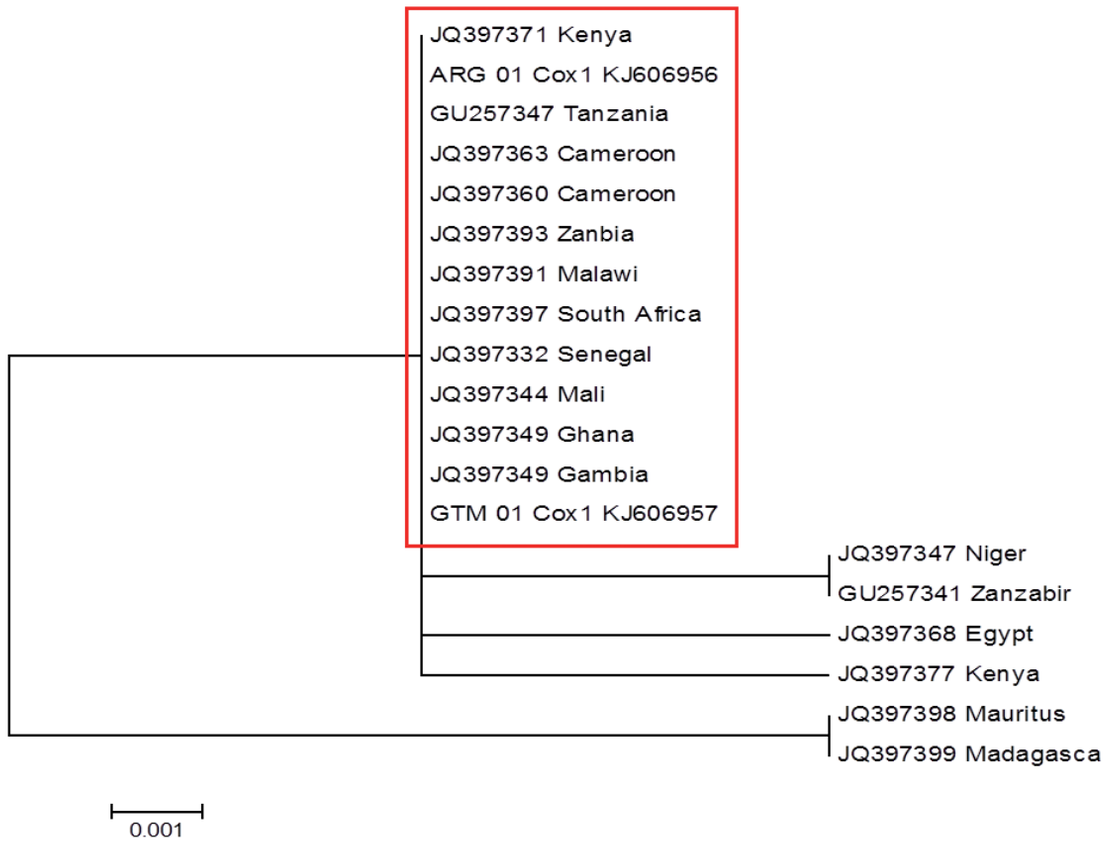

Fig. 4. Phylogenetic tree depicting relationships among *Schistosoma heamatobium* species inferred from Cox1 nucleotide data. The tree constructed using Maximum likelihood (ML) based on the Jukes-Cantor model in MEGA6 software. The data set was bootstrapped 500 times and appropriate bootstrap values placed on each branch.

the river have higher chance of becoming infected with urinary schistosomiasis compared to those away from water body [7]

Association between *S. haematobium* and risk factor such as age, fetching water, playing/bathing and swimming activities revealed a significant predictor of schistosomiasis outcome with the likelihoods of being infected with schistosomiasis increasing by 1.4 times for each unit increase in age, with adjusted odds ratio =1.43 ($p<0.05$) with the swimming group having longer exposure time than other groups. Similar trends were observed among other factors as seen in Table 2. The odds ratio observed among children who play/bath, fetches water, and swim indicate that the more the children visit the infested water surface with cercariae the higher the chances of becoming infected compared to those who do not. However, this study indicate that the transmission of schistosomiasis takes place where fresh water snail vector is present and where there is frequent contact between the population and infested water which concur with similar study in western part of Nigeria [18], Though this finding is in contrast with earlier reports from Senegal [19] and Tanzania [20], several geographical analyses

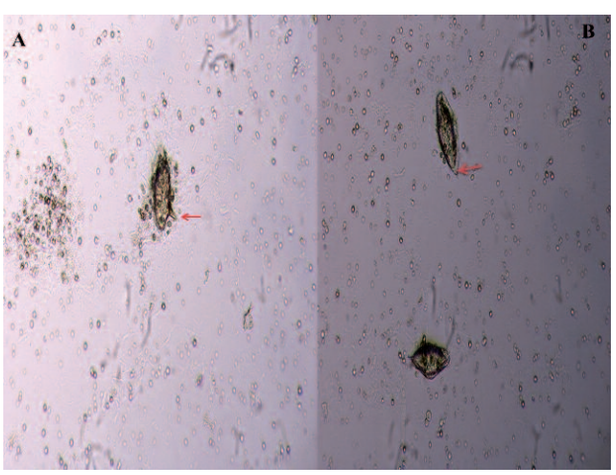

Fig. 5. Urine sediment microscopy from patient with both *Schistosoma mansoni* **(A)** and *Schistosoma haematobium* **(B)** as seen under a light microscope using 40× magnifications. *S. haematobium* eggs have a prominent terminal spine (arrow) while *S.mansoni* eggs have a large, lateral spine (arrow).

and reports on water-contact patterns and immunity to schistosomal infection have indicated that 'proximity to the transmission site' can be a strong predictor of both infection and moderate to heavy infection with *S. haematobium* [21]

Phylogenetic tree is an evolutionary relationship among a set of organisms or group of organisms [22]. In this study mitochondrial gene (Cox1) was used to construct phylogenetic tree which provide an insight on molecular epidemiology of the parasite from the study area. Our result showed that, all isolates belonged to *S. haematobium* species forming cluster with *S. haematobium* reference sequence and different from other *Schistosoma* species which is supported by previous study where the dendrogram constructed from African species and the Asian species sequences, the isolates of schistosomes split into two distinct group, the African and the Asian [13]. Our result also provide information on the genetic relationship of socioeconomically important schistosomes, in which our isolates *S. haematobium* and *S. mansoni* were closely related in accordance with previous studies using both mitochondrial and nuclear DNA markers [13,23].

This study also demonstrates for the first time in the study area, the occurrence of *S. haematobium and S. mansoni* co-infection in one of the pupil with no renal involvement (Fig. 5). This seems to be consistent with the earlier study that reported the possibility of infection with more than one schistosome haplotype or even species at a time among school children [24]. However, in this study both were seen in urine sample this might be as a result of contamination of the urine sample by the stool of the same child. Isolates from two different districts seems to be indistinguishable as revealed from the Cox1 tree, these might be due to the fact that all isolates were coming from the same geographical area, the two districts were very close about 25 kilometre. The genetic hyper variability of human schistosome infections is influenced by a number of genes, overlapping contact sites, snail and human movements, thereby supporting the coexistence of a great number of genotypes in a particular region. They can also stimulate the development of new parasitic strains or families through genetic exchange and recombination between local and introduced genotypes

We concluded that *S. haematobium* infection is prevalent among school age children in the Argungu Emirate, Kebbi State, Nigeria, which is significantly associated with several risk factions related to water contact. The isolated *Schistosoma* was phylogenetically identified as *S. haematobium* and found to be phylogenetically related to *haematobium* isolate from Kenya. The need for urgent interventions is necessary in order to save the entire people from the socioeconomic effects of the burden of the disease. There should be simultaneous implementation of control measures in all the communities, especially those close to the water surface area. Morbidity control should be given more emphasis, especially chemotherapy and environmental management putting emphasis on health education by the relative health authorities. There is also need for therapeutic prophylactic intervention for childrent between the age of 10 to 15 both in school and out of school.

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