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Full Length Research Paper

Molecular characterization of *Schistosoma mansoni* among schoolchildren in Côte d'Ivoire

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Abstract

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Schistosomiasis remains a great public health problem in several tropical and subtropical countries. Recently in Côte d'Ivoire, we have investigated hybrids between *S. haematobium* and *S. bovis* in human urine samples. Moreover, *S. haematobium* x *S. mansoni* hybrid has recently been identified in a French hospital in a migrant boy originated from Duekoué, western part of the country. The aim of this project was to molecularly characterize *Schistosoma mansoni* among schoolchildren in Côte d'Ivoire. A cross-sectional study was conducted in two localities of Côte d'Ivoire (Agboville and Duekoué). Stool samples were collected from schoolchildren and examined by Kato-Katz method to identify *Schistosoma* eggs. Miracidia were individually isolated and stored on Whatman® FTA card. We analyzed miracidia from stool samples using a Cox1 gene multiplex PCR. Nuclear ITS gene has not been analyzed. A total of 661 schoolchildren was included in this study. More boys than girls (61.9% vs. 38.1%) were included and the highest proportion of children was included in Agboville. The overall prevalence of *S. mansoni* infection was 9.8% (95% CI: 4.8-13.5%). Of the 239 miracidia molecularly analyzed, 166 yielded positive PCR signal (82.5% in Agboville and 17.5% in Duekoué). Only one case of *S. haematobium* Cox1 has been identified in Agboville. This case should be hybrid *S. haematobium* x *S. mansoni*. Our study reports that intestinal schistosomiasis remains prevalent even if the infection rate was low. Only one case of S. haematobium Cox1 was identified, suggesting the presence of S. haematobium x S. mansoni hybrids at very low prevalence.

Keywords: *Schistosoma mansoni*, Multiplex PCR, Cox1 gene, Côte d'Ivoire.

INTRODUCTION

Schistosomiasis is a chronic parasitic disease caused by a parasite of the *Schistosoma* genus. It is the most prevalent neglected tropical parasitic disease in sub-Saharan Africa (Colley et al., 2014; Lai et al., 2015; McManus et al., 2018). It remains a great public health problem in several tropical and subtropical countries.

The number of schistosomiasis cases in the world is considered to be greater than 250 million, mostly in Africa and the DALYs index ("Disability-Adjusted Life Years") was estimated at 1.4 million in 2017 (Hotez et al., 2014; GBD 2017 DALYs and HALE Collaborators, 2018). In Côte d'Ivoire, both *S. mansoni* and *S. haematobium* are endemic (Chammartin et al., 2014). The former is commonly predominant in the west (Utzinger et al., 2000; Assaré et al., 2015) and the latter in the central and

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southern parts (Coulibaly et al., 2013; Soumahoro et al., 2014; Angora et al., 2019). Molecular characterization using specific and highly sensitive PCR based assays have been developed for the detection of *Schistosoma* DNA for the diagnostic at all phases of clinical disease (Gomes et al., 2010; Oliveira et al., 2010). Two molecular markers are frequently used to identify parasites species: a nuclear marker (ITS2, ribosomal internal transcribed spacer 2), and a mitochondrial marker (Cox1, cytochrome oxidase subunit I) (Huyse et al., 2009; Boissier et al., 2016). Recently in Côte d'Ivoire, we have investigated the presence of hybrids between *S. haematobium* and *S. bovis* in schoolchildren urine samples. This latter hybrids has been found with high prevalence in the south (Angora et al., 2020). Data are sparse on molecular identification of *S. mansoni* in our country. Moreover, *S. haematobium* x *S. mansoni* hybrid has been identified in a French hospital in a migrant boy originated from Duekoué, western part of Côte d'Ivoire (Le Govic et al., 2019). In the present paper, in order to estimate the extent of this hybridization we molecularly characterized *Schistosoma mansoni* among schoolchildren, in the area of Duekoué as well as the Agboville area.

MATERIAL AND METHODS

Sampling sites and population

The study field work was conducted in two geographically areas such as Agboville in the South (N 6°3′29,928″N, W 4°5′42,882″) and Duekoué in the west (N 6°42′38,634″, W 6°46′22,2906″) of the country (Figure 1). The study population consisted of schoolchildren aged 5 to 14 years. These children must have lived in the study area for at least one year and be able to provide stool samples. These areas are well known for their high *S. haematobium* and *S. mansoni* endemicity (Agbaya et al., 2004; Raso et al., 2005; N'guessan et al., 2007).

Study design and data collection

We carried out a cross- sectional study in Agboville and Duekoué across Côte d'Ivoire. Two or three primary schools (depending on the school size) of each study area were selected for the recruitment of schoolchildren. The number of children per school was proportionally allocated according to population size in each school. Only schoolchildren aged 5–14 years who had lived in the study area for at least one year prior to the survey were included. During registration, children were assigned to a unique identification code to allow anonymity. The stool samples were collected in sterile pots in primary schools and transferred to the nearest health center for further examinations. This study was integrated into a cross-sectional survey of the prevalence of schistosomiasis among school-aged children from January to April 2018 in which *Schistosoma* miracidia and eggs were collected from stool and stored on Whatman-FTA® cards at -20°C (Angora et al., 2019).

Laboratory analysis

Stool samples were used to identify and quantify *Schistosoma mansoni* eggs with its lateral spine by the Kato-Katz method (Katz et al., 1972; Bärenbold et al., 2017). Two thick smears from each stool sample were microscopically examined to identify and quantify eggs of *S. mansoni*. After homogenizing with saline and sifting the stool samples on a series of four sieves, the stool of the last 40µm meshed one has been used to capture 15 to 20 individual miracidia or eggs by patient on Whatman-FTA® cards (GE Healthcare Life Sciences; Amersham, UK) and stored at -20°C.

For molecular analysis, A 2.0 mm disc containing the sample was removed from the FTA card with a Harris-Micro-Punch (VWR; London, UK) and incubated in 50 µL of double-distilled water for 10 min. Genomic DNA from each miracidium or egg was extracted using Chelex method (Beltran et al., 2008). Molecular identification was performed by PCR amplification of a mitochondrial gene named Cytochrome Oxidase subunit I (Cox 1) as the component of the respiratory chain in mitochondrion. We performed a rapid multiplex PCR method (RD-PCR) discriminating between *S. haematobium*, *S. bovis* and *S. mansoni*, as described in our previous work (Angora et al., 2020). Briefly, the PCR amplification consists on using a single reverse primer and 3 forward primers in the same tube. Each of the forward primer is species-specific and will amplify a specific cox1 DNA region (differing in length) for *S. bovis/S. curassoni* (260 bp), *S. mansoni* (215 bp) and *S. haematobium* (120 bp). Primers employed were an universal reverse (Shmb.R: 5-CAA GTA TCATGA AAY ART ATR TCT AA -3) and three species-specific forward primers (Sb.F: 5-GTT TAG GTA GTG TAG TTT GGG CTC AC-3; Sm.F: 5-CTT TGA TTC GTT AAC TGG AGT G-3; and Sh.F: 5-GGT CTC GTG TAT GAG ATC CTA TAG TTT G-3). Each PCR was performed in a total reaction volume of 10 µL, comprising 2 µL of the DNA extract, 5 µL of GoTaq® G2 Hot start green Master Mix 5X (Promega; Madison, Wisconsin, USA), 1 µL of 10X primer mix (4 µL of 100 µM reverse primer, 4 µL of each 100 µM forward primer and 84 µL of molecular water) and 2 µL of molecular water. The reaction conditions included an activation step of 95 °C for 4 min, followed by 45 cycles of 95 °C for 10 s, 52 °C for 30 s and 72 °C for 10 s, and a final extension at 72 °C for 2 min. The PCR products were visualized on 1% agarose gel. These molecular analyses were performed at the Malaria Research and Control Center (MRCC) of the National Institute of Public Health (NIPH) in Abidjan,

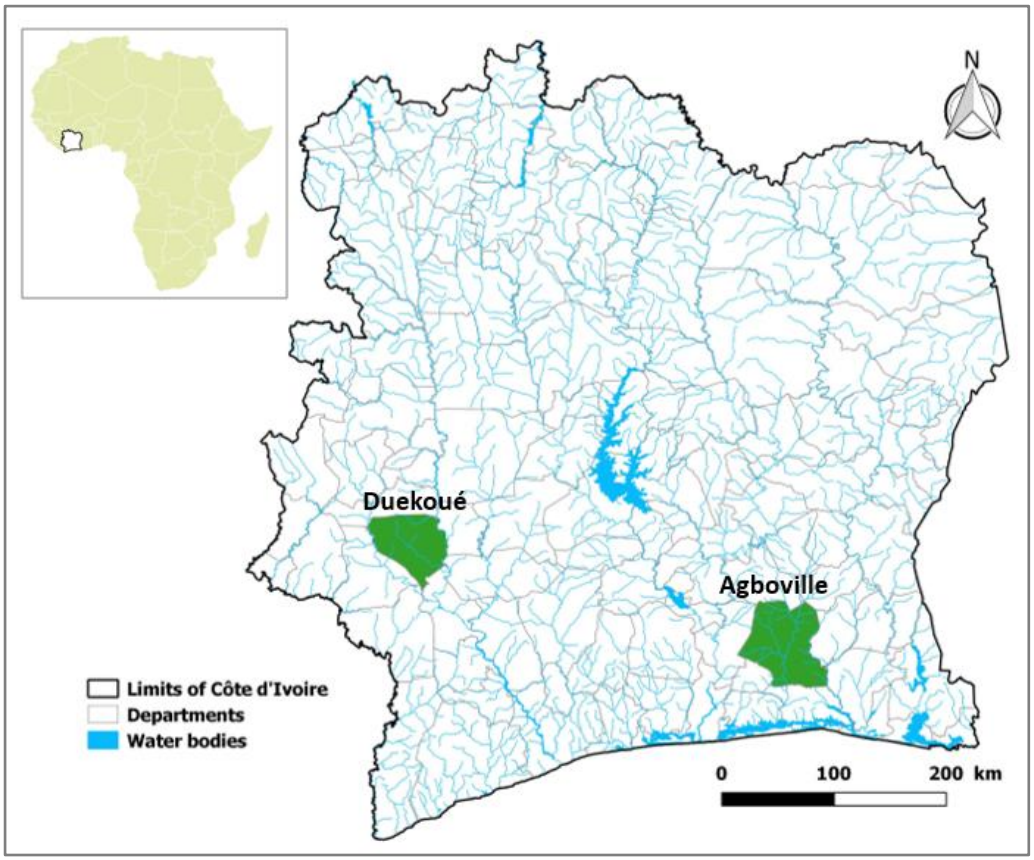

Figure 1: Study sampling sites identified by green color.

Côte d'Ivoire.

Statistical analysis

Statistical analyses were performed with STATA version 15.0 (Stata Corporation; College Station, TX, USA). Univariate analysis (χ^2 and Fisher's exact test, as appropriate) was used for comparison between groups. Children were stratified into three age groups (5–8, 9–11, and 12–14 years). Parasitic infections were defined as positive for *S. mansoni* when at least one egg was identified in a stool sample. Associations between parasitic infections and sociodemographic were assessed. We also computed the arithmetic and geometric means of *S. mansoni* eggs per gram of stool (EPG). The differences with a p-value below 0.05 were considered statistically significant.

Ethical consideration

This study received approval from the National Committee of Ethics and Research [Comité National d'Ethique et de Recherche] (certificate number: N° 003-18/MSHP/CNER-kp). The research was conducted in accordance with the principles outlined in the Helsinki Declaration. School authorities, teachers, participating children and their parents/guardians were informed about the objectives, procedures, and potential risks and benefits of the study. Written informed consent was obtained from children's parents/guardians, while children provided oral assent.

RESULTS

Sociodemographic characteristics

A total of 661 schoolchildren were included in the study. There were more boys than girls (61.9% vs. 38.1%) and the highest proportion of children was from Agboville. The mean age was 10.1 years (standard deviation = 2.5 years) with a median age of 10 years. Children aged 5–8 years were the less common age class in the population (29.0%). Table 1 shows the sociodemographic characteristics of the study population, stratified by sites.

Table 1: Sociodemographic characteristics of the study population, stratified by Sites.

Variable	Number of children	Percentage (%)
Study sites		
Agboville	387	58.6
Sikensi	274	41.6
Gender		
Girls	252	38.1
Boys	409	61.9
Age (years)		
5-8	191	29.0
9-11	235	35.5
12-14	235	35.5

Table 2: Prevalence of *S. mansoni* infection, stratified by study sites, sex and age.

Characteristic	***S. mansoni***	
	Total	**Positive n (%)**
Study sites		
Agboville	387	45 (11.6)
Duekoué	274	20 (7.3)
p-value		*0.084*
Gender		
Girls	252	27 (10.7)
Boys	409	38 (9.3)
p-value		*0.60*
Age (years)		
5-8	191	15 (7.8)
9-11	235	23 (9.8)
12-14	235	27 (11.5)
p-value		*0.46*

Parasitological and molecular analysis

The overall prevalence of *S. mansoni* infection was 9.8% under microscope (95% CI: 4.8-13.5%). *Schistosoma mansoni* was most commonly found in Agboville (11.6%) compare to Duekoué (7.3%). Age and sex were not associated with *S. mansoni* infection (p=0.60). Table 2 shows the prevalence of *S. mansoni* infection, stratified by study sites, sex and age.

The arithmetic mean of *S. mansoni* eggs per gram of stool (EPG), including standard error (SE) from positive samples was 91.1 EPG (SD: 11.3 EPG) with a minimum and maximum of 20 and 400 EPG, respectively. The geometric mean of *S. mansoni* eggs from positive stool samples was 4.1 (SD: 0.9 EPG).

A total of 265 miracidia or eggs (216 for Agboville vs 49 for Duekoué) were collected from 14 positive schoolchildren in FTA cards. Of 239 miracidia molecularly analyzed, 166 yielded positive bands for *S. mansoni* Cox1 at 82.5% in Agboville and 17.5% in Duekoué. Only one case of *S. haematobium* Cox1 bands was found in Agboville (Table 3). This case should be hybrid *S. haematobium* x *S. mansoni*.

Table 3: Distribution of *Schistosoma* Cox1 profile by study site.

Sites	Total analyzed	*S. mansoni* Cox1 n (%)	*S. haematobium* Cox1 n (%)
Agboville	194	137 (82.5)	1 (100)
Duekoué	45	29 (17.5)	0
Total	239	166 (100.0)	1 (100)

DISCUSSION

The current study investigated the prevalence of intestinal schistosomiasis in schoolchildren two settings of Côte d'Ivoire, and also molecularly characterize *S. mansoni* infection using the mitochondrial Cox 1 gene. This study has shown the low overall prevalence of intestinal schistosomiasis at 9.8%, while the district specific prevalence rates were 7.3% and 11.6% Duekoué and Agboville, respectively, in schoolchildren. Despite being low, this prevalence, indicates that intestinal schistosomiasis is still endemic in the two regions of Côte d'Ivoire. Similar results were found in study conducted in the northern part (Kouadio et al., 2023). The low prevalence of intestinal schistosomiasis could be explained by climatic factors and the use of a single dose of praziquantel in mass drug administration (MDA). This strategy has been widely used in many parts of the country to reduce the disease occurrence.

Others studies reported a high prevalence among adult populations in the Southern Côte d'Ivoire (Coulibaly et al., 2013; Bassa et al., 2022). The arithmetic mean of *S. mansoni* egg counts (91.9 EPG) recorded was low and would be classified as a light infection.

However, the results of our study show the reality of this disease extension in schoolchildren, which reveals a real public health problem. Thus, efforts to prevent and control the disease in children need to be stepped up, including, for example, the detection of hybrid parasites. The increasing of molecular data from schistosome miracidia in the disease control has resulted in many studies' report on hybridization of these parasites (Huyse et al., 2013; Webster et al., 2013; Angora et al., 2020). *Schistosoma haematobium* and *S. mansoni* are two prevalent species of schistosomes in humans in Côte d'Ivoire (Assaré et al., 2015; Tian-Bi et al., 2018; Angora et al., 2019). In the present study, the molecular analysis of *S. mansoni* Cox1 indicates that isolates yielded positive bands for *S. mansoni* Cox1 at 82.5% in Agboville and 17.5% in Duekoué. Previously, *S. mansoni* was found at a high frequency from Agboville area in epidemiological study (Angora et al., 2019). Therefore, the current study confirms the actual presence of this species. Only, one case of *S. haematobium* Cox1 was found. Aberrant elimination of *S. haematobium* eggs in the stool and *S. mansoni* eggs in the urine is widely observed, especially in endemic areas. Studies have shown cases in West Africa (Meurs et al., 2012; Le Govic et al., 2019). This phenomenon could be explained by the advent of parasite hybridization in the wild. Hybrids resulting from crosses between *S. mansoni* and *S. haematobium* have been reported in Senegal and Côte d'Ivoire (Huyse et al., 2013; Le Govic et al., 2019).

Hybridization of closely related *Schistosoma* species has already been shown in the laboratory (Mansour et al., 1984) an in nature (Huyse et al., 2013; Tian-Bi et al., 2019) for *haematobium* group. However, hybridization between *S. mansoni* and *S. haematobium* is very rarely observed. This could be explained by the significant phylogenetic distance between the haplotypes of these two species (Young et al., 2012). This crossbreeding between adults of *S. haematobium* and *S. mansoni* may occur due to possible cases of co-infection observed in certain regions. The presence of hybrids challenges our current understanding of parasite transmission and host ranges, which in turn may affect the effectiveness of current control strategies.

This study has limitations in terms of the molecular markers used. Indeed, the identification of hybrids considers only one mitochondrial marker, considering that an egg derived from faeces is a parasite with a genetic background of *S. mansoni* which has hybridized with *S. haematobium*. This could lead to underestimation or misidentification of hybrids. To complete the analysis, a nuclear molecular marker such as ITS2 should be used, or even better, wider molecular screening on a whole genome scale (e.g. SNP).

CONCLUSION

The results of this study indicate that *S. mansoni* infection is still endemic in the rural areas peri-urban of Côte d'Ivoire. The identification of *S. haematobium* x *S. mansoni* hybrids indicates a potential risk to public health. It is therefore imperative to integrate molecular diagnostics to search for hybrids in order to optimize control strategies, and to prevent and control the disease, particularly in communities where a high prevalence has been reported.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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