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Full Length Research Paper

Phenol-Chloroform-based isolation of *Schistosoma haematobium* DNA in urine samples at Institut Pasteur of Côte d'Ivoire

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Schistosoma haemtobium **infection remains an important place in neglected tropical diseases in sub-Saharan Africa. DNA detection of this parasite is never done in Côte d'Ivoire; so it is important to carry out this study which aims to perform phenol-chloroform method *S. haematobium* DNA extraction from urine in Parasitology Unit of Institut Pasteur. Thus, 107 urine samples positive for *S. haematobium* stored at -20°C collected from schoolchildren in Guebo 2 were used. Optical densities (OD) were measured by spectrophotometer at 260 nm and 280 nm wavelength and DNA concentrations were measured. The average of optical density was 0.780 ± 0.61 at 260 nm and 0.687 ± 0.53 at 280 nm. Most extracts DNA had OD260nm / OD280nm ratio lower than 1.8. DNA concentrations were high with an average of 234.0 ± 184.3µg / mL with extremes at 33.7µg / mL and 827.5µg / mL. Phenol-chloroform method gave relatively high concentrations of DNA. The PCR amplification of DNA will allow improving this method.**

Key words: *Schistosoma haematobium*, DNA, Extraction, Phenol-chloroform method.

INTRODUCTION

Schistosomiasis is a water-associated chronic parasitic infection caused by trematode worms of the genus *Schistosoma* and is ranked among the most neglected tropical diseases. It is a chronic disease caused by human contact with infected stream and larva being transmittable through the skin. The disease remains a great public health problem in several tropical and subtropical countries (Gryseels et al., 2006; Jordan, 2000; Southgate et al., 2005; Steinmann et al., 2006). About 230 million people are concerned with schistosomiasis in the world with an estimated 11000 deaths annually (Knowles et al., 2017)– around 90% of death cases are reported to be linked to SubSaharan countries (Colley et al., 2014; Mewabo et al., 2017).

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In Côte d'Ivoire, urinary schistosomiasis form caused by *Schistosoma haematobium* and the intestinal one caused by *S. Mansoni* are the most noticeable. There are often predominant in rural zone where hygiene and sanitation conditions are poor. Many studies showed high prevalence of urinary schistosomiasis in southern and western parts of Côte d'Ivoire (Adoubryn et al., 2006; N'guessan et al., 2007; Soumahoro et al., 2014).

The usual biological diagnosis of *S. Haematobium* is based on the microscopic detection of eggs in urine samples after a centrifugation or through a filtering on a multipore membrane and also on the biopsy of the rectum mucous membrane. Detection of specific antigens or antibodies is also available (Koukounari et al., 2009). However, the diagnostic performance of these parasitological methods decreases when infection intensities are moderate to low.

Molecular diagnosis, sensitive and specific, showed efficiency in detecting *S. haematobium DNA* in urine samples (Obeng et al., 2008). A Real-time PCR was designed to detect infections caused by schistosomes through urine and stool samples (Cnops et al., 2013). However, no data on PCR diagnosis of this parasitic disease is available in Côte d'Ivoire. This technique requires that DNA be obtained after isolation, hence the importance of this study which aims to develop a method for isolating genomic DNA of *S. haematobium* from urine samples at Institut Pasteur of Côte d'Ivoire.

MATERIAL AND METHODS

Study design and sites

This cross-sectional study was conducted in the village of Guebo 2, which is 6 km from KOSSIHOUEN in the department of SONGON (5° 19′ 0″ N, 4° 15′ 0″ W), in the district of Yopougon. The population lives on rubber and palm plantations, and on cassava business. Guebo 2 is a very hilly area with many moist hollows. Two different rivers where some household activities are done cross this village but one of them dried out at the time of the current survey. Nevertheless the water is more or less inaccessible to some inhabitants who hardly earn their living working on farms. The population fetches water from the wells and from the two rivers. A river drives through the primary school where there are some toilets yet not frequently visited, and pupils prefer going to the nearby bush to relieve themselves. Moreover, there is no healthy water inside the school. It was when there was an alarm of suspicion of urinary schistosomiasis epidemic by the regional health center in May 2012 that some urine samples were taken from schoolchildren in the primary school of Guebo 2 (Soumahoro et al., 2014).

Urine samples collection and parasitological examination

Urine samples collected from schoolchildren in sterile pots were transferred to the Parasitology unit in Institut Pasteur of Côte d'Ivoire. After centrifugation of the urine samples *S. Haematobium* eggs were identified by microscope. The centrifuge pellets were stored at -20°C in order to perform *S. haematobium* DNA extraction by phenol-chloroform method. Only, *S.haematobium* positive urine samples were used in this study and a total of 107samples were included.

DNA extraction

Phenol – chloroform method was used for DNA extraction from urine samples. 500 µL of lysing solution (NaCl 100 mM, Tris 10 mM, EDTA 25mM, SDS 0.5 %, pH 8), subsequently, 5 µL of proteinase K (0.2 mg/mL) was added to 200 µL of urine pellet (Damien, 2005). The obtained mixture was pulse vortexed and incubated for 3 hours at 55°C.On the lysate 500 µL of phenol solution were added and the mixture was centrifuged at 10.000 rpm for 10 minutes at 4 °C in order to separate proteins from nucleic acids. The floating aqueous phase was collected in a new microcentrifugetube and 500 µL of phenol-chloroform- isoamyl alcohol (25v/24v/1v) was added. After shaking by vortexing, the mixture was centrifuged at 10.000 rpm for 10 minutes at 4 °C. Then, 500 µL of chloroform-isoamyl alcohol (24/1) was added to the floating in a new 1.5mL microcentrifuge tube. After centrifugation in the same conditions, the floating got poured in a new microcentrifuge tube. In the next step, 50 µL Sodium acetate (3M, pH 5.4) and 1.25 mL of absolute ethanol was added in order to precipitate out nucleic acids. The tubes were set at -20 °C for 30 minutes then centrifuged at 10.000 rpm 10 minutes at 4°C.At the end, 1 mL of 70°ethanol was added to the obtained residue andcentrifuged at 10.000rpm for 5 minutes at 4°C. The floating was getting rid of again and the pellet was dried using Speed Vac (Thermo Scientific™ Savant™) and stored at -20°C.

Purity measurement and DNA quantification

The DNA extract was made soluble in some TE 1 X (Tris-HCl 10 Mm, pH 8.0 andEDTA 1 mM) and diluted at 1/6. The optical density (OD) was determined at 260 nm and 280 nm through spectrophotometer (Eppendorf AG, Hamburg, Germany). The OD_{260}/OD_{280}ratio was used to estimate the purity of DNA. The extract is said to be 'pure' when the ratio is between 1.8 and 2.0. In case of contamination by proteins, the OD_{260}/OD_{280} ratio is inferior to 1.8. The presence of RNA in the specimen results in a OD_{260}/OD_{280}ratio more than 2. The DNA concentration (µg/µL) was obtained by multiplying the 260nm optical density by the dilution factor, and a ratio of 50 corresponding to an optical density of 1.

Data analysis

Data analysis was performed using SPSS21 software (Statical Package for Social Science, IBM SPSS Statistics). The Student test t was used to compare the averages of DNA concentration. P-value < 0.05 was considered significant.

RESULTS

Socio-demographic characteristics of population

The table 1 shows the socio-demographic characteristics of the targeted population whose urine samples were tested *S. haematobium* positive. Male (53.6%) were more found than female (53.6%) with sex-ratio of 1.14. The most represented age groups were 5 to 8 years old and 9 to 12 years old with respective percentages of 43.0% and 41.1%. The average age was 9.94 years (SD =2.5 years) with extremes of 6 and 16 years old. 42.1% of schoolchildren population displayed as major symptoms of a terminal haematuria.

Table 1. Socio-demographic characteristics of population.

Variable	Total (N=107)	Percentage (%)
Gender *(sex- ratio=1,14)*		
Male	57	53.3
Female	50	46.7
Age groups (years)		
5-8	46	43.0
9-12	44	41.1
13-16	17	15.9
School level		
CP1	24	22.4
CP2	27	25.2
CE1	13	12.1
CE2	14	13.1
CM1	18	16.8
CM2	11	10.3
Haematuria		
Yes	45	42.1
No	62	57.9
Swimming in fresh water		
No	86	80.4
Yes	21	19.6

Prior swimming in river was a recurrent issue in 19.6% of the cases.

Purity measurement and DNA concentration

The OD260nm mean was 0.780 (SD= 0.61) with extremes of 0.122 and 2.758, those of the OD280nm was 0.687±0.53 with extremes of 0.091 and 2.670.

The figure 1 shows that the optical densities with precise wave lengths had a similarly curve of an uneven development. In comparison, the wave length average of optical densities showed no significant statistical difference (p=0.54).

The purity of DNA after extraction was estimated by comparing the ration OD260/OD280 to a range of 1.8-2.0. In 99.1% cases the ratio was lower than 1.8 the minimal value of purity. One sample just resulted in a ratio of OD260nm/OD280nm between 1.8 and 2.0 which is the DNA purity margin (figure 2). No value of this ratio was superior to 2.0, making it to suspect any presence of other nucleic acid.

The mean of DNA concentration was 234 µg/mL (SD=184.3) with extremes of 33.7µg/mL and 827.5µg/mL. The figure 3 shows the DNA concentration in the different samples.

DISCUSSION

The target population of the survey is a group of schoolchildren between 6 and 16 years old with a mean age of 9.94 years (SD=2.5). Studies fulfilled in Senegal in 2007 showed the presence of urinary schistosomiasis in this population (Seck et al., 2007). We found in this study that boys were more infected by the parasite than girls. On the contrary, results from a study in the Southern part of Côte d'Ivoire reported that the girls were the most infected (Coulibaly et al., 2013). The high prevalence among girls could be explained as there are in contact with fresh water bodies for longer periods of time during household activities (Diakité et al., 2017). The haematuria as main symptoms of urinary showed by some studies (Yapi et al., 2005) has been reported from schoolchildren in the current study.

Phenol-chloroform method used for DNA extraction in our study is a cheap technique but has high risks linked to the phenol and need to be used on hood. That is why phenol and chloroform residues were collected in special flasks to be destroyed. Some other techniques with DNA extraction kits are used for isolation. But the preference for either DNA extraction kits or phenol chloroform depends often on the downstream use of the DNA extract. Usually, phenol-chloroform method is performed for subsequent experiments which require a large quantity of DNA. Extraction kits can also be used, QiagenQIAmp mini-kit (Qiagen Sciences, MD) was used in Niger and allowed to amplify the DNA *Dra1*regionof *S. haematobium* (Ibironke et al., 2011). However, this method requires sometimes a forehand collection of urine samples on Whatmancard.

The optical density measurement was done in a two wavelengths UV, i.e 260 nm and 280 nm. The ratio OD260/OD280 was used to estimate the purity of DNA isolated through phenol-chloroform from urine samples. Most of the samples had OD260/OD280 ratio lower than 1.8. Nevertheless, higher values were documented

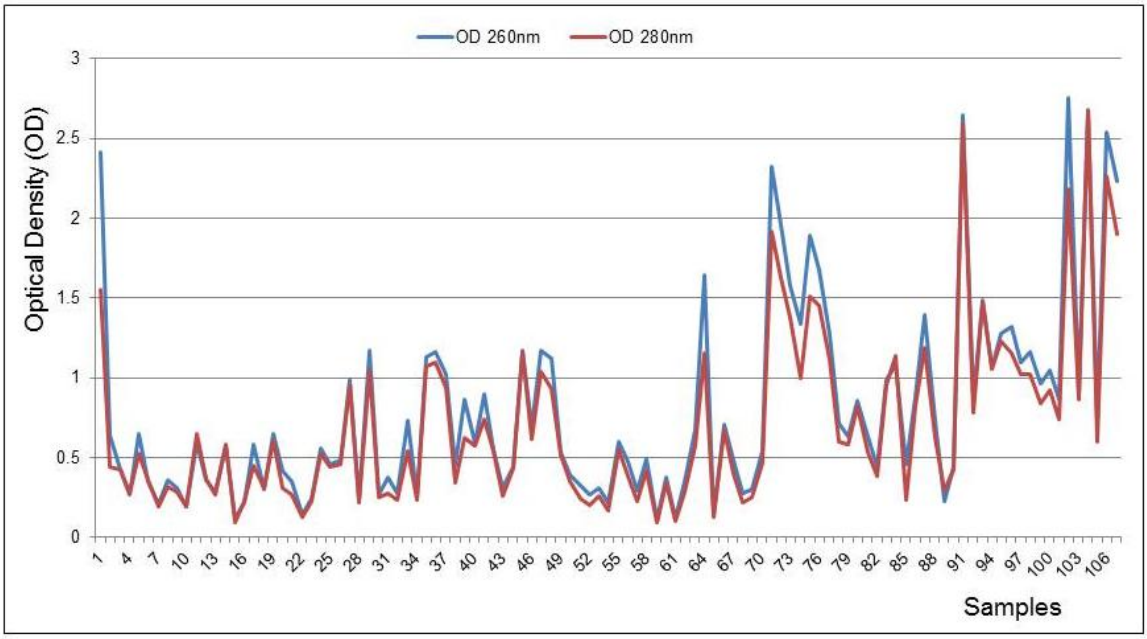

Figure 1. Comparison between OD260nm and OD280nm of DNA according to samples.

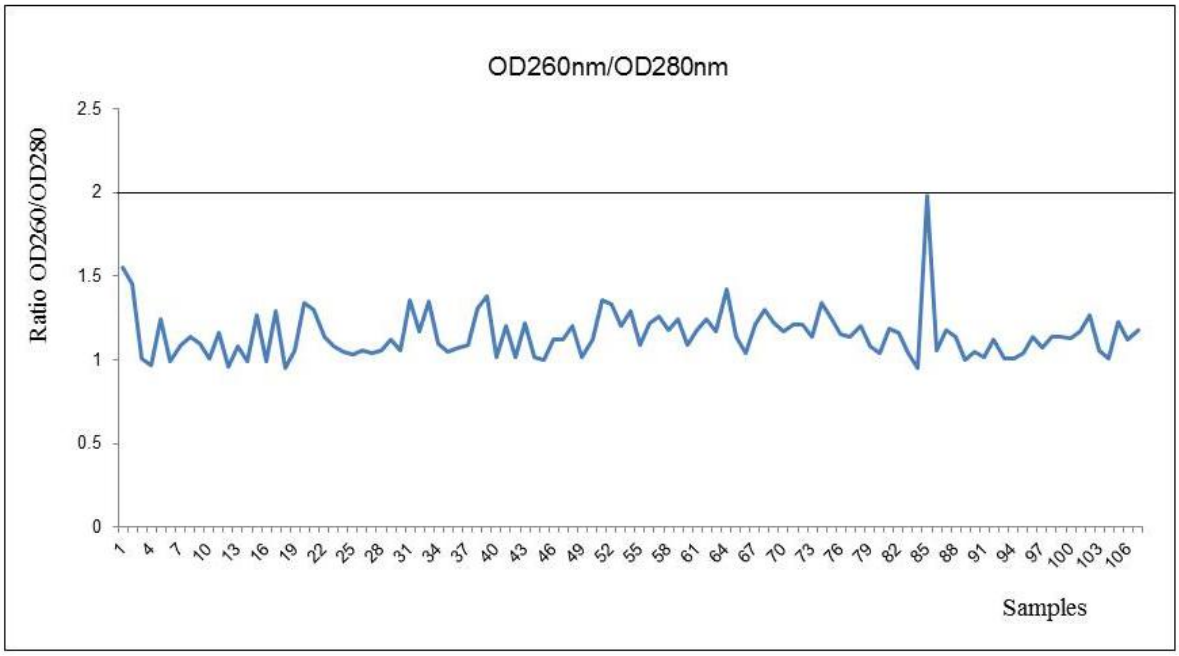

Figure 2. Comparison between ratio OD260/OD280 of DNA according to samples.

in other surveys (Ibironke et al., 2011; Lodh et al., 2014). These low values could eventually be explained by the presence ofproteins in the samples after isolation and show that there is less contamination of type RNA. In fact proteinase K is an enzyme which hydrolyses proteins via peptide bonds and these proteins are subsequently captured by chelating agents in order to be eliminated in insoluble form by phenol and chloroform (Rasmussen and Morrissey, 2008). Due to a weak proteinase K concentration used (0.2mg/mL), the chelating agents didn't have enough substrate to deal with and could give a ratio OD260/OD280 lower than 1.8. *S. Haematobium* eggs hatch out easily by the influence of hypotonic solutions or when temperature changes. They discharge their cytoplasmic content which can be other protein capable of contaminations.

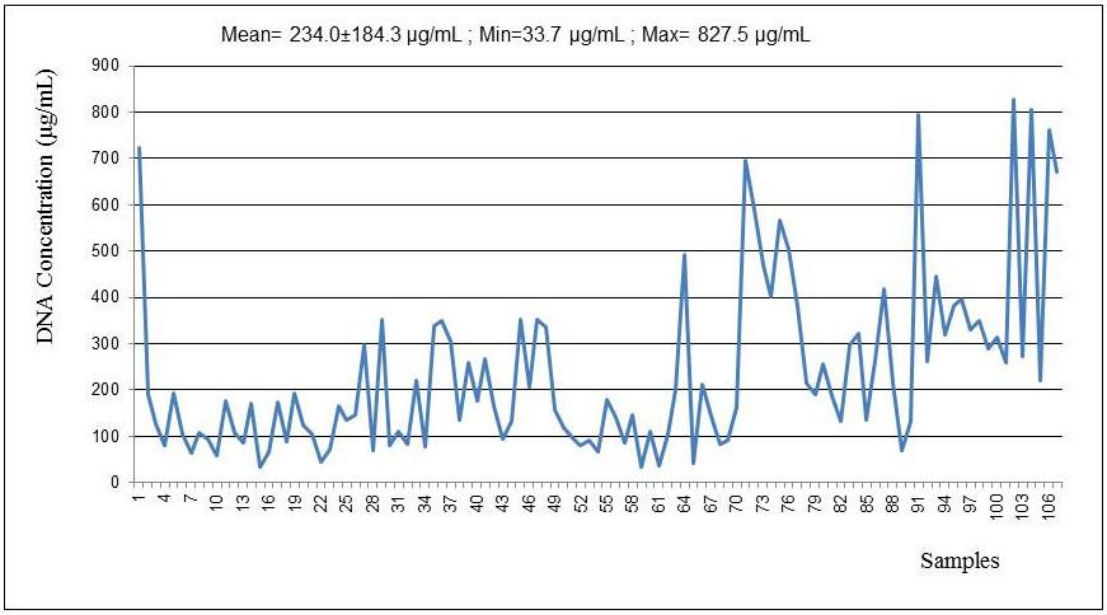

Figure 3.DNA concentration from samples.

The use of purifying DNA extraction kits to improve the purity of the extracts could be a corrective way.

DNA concentrations varied in each sample among the phenol-chloroform based withmean concentration of 234µg/mL. This method gave a high quantity of DNA but the purity was insufficient because of the contamination by proteins as demonstrated by the optical density transmission ratio lower than 1.8.

The PCR based amplification of the *Dra1*gene by using specific primers allows to confirm the real presence of *S. haematobium* DNA which can be observed at 121 bp (Ibironke et al., 2012). However, the DNA *Dra1* gene of this parasite was spotted in urine samples where eggs were not seen by microscopes (Ibironke et al., 2011)**.**

CONCLUSION

The biological diagnosis of urinary schistosomiasisis customarily based on a microscopic detection of *S. haematobium* eggs in urine samples. This survey helped to phase in phenol-chloroform based method for genomic DNA isolation, which is open to be improved.

Conflict of interests

No conflict of interests was reported by the authors of this manuscript.

Authors' Contributions

Angora KE and Ako AB are the principal investigators of the study. Vanga-Bosson AH and Ira-Bonouman AV helped to carry out the writing of the manuscript. Touré OA, Menan HE and Djaman AJ supervised the study. All authors contributed to the drafting of the paper.

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