

Research Article

Comparison of the Loop-Mediated Isothermal Amplification (LAMP) and the Kato-Katz Techniques in the Diagnosis of *Schistosoma mansoni* in Burkina Faso

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Abstract

Intestinal schistosomiasis or intestinal bilharzia, mainly caused by *Schistosoma mansoni*, is one of the most common parasitic diseases in the world, and a neglected tropical disease (NTD). It ranks first among water-borne diseases and is the 2nd most endemic parasitic disease after malaria and according to the World Health Organization (WHO), schistosomiasis is transmitted in more than 78 countries and territories in tropical and subtropical regions, and more than 250 million people are infected, mainly in Africa. Kato Katz (KK) remains the standard technique for diagnosing this disease. A promising new approach, loop-mediated isothermal amplification (LAMP), may be needed in developing countries such as Burkina Faso. Thus, the aim of this study was to compare the LAMP technique and the Kato-Katz technique in the diagnosis of *Schistosoma mansoni* in Burkina Faso. 52 stool samples were collected from patients in the town of Bobo Dioulasso and examined using the KK technique, which corresponds to microscopy and the LAMP technique, to assess the sensitivity and specificity of this molecular technique. The results showed a prevalence of intestinal schistosomiasis of 8% in the study, and the Kappa coefficient obtained between the 2 techniques was 0.99, roughly equal to 1. The sensitivity and specificity of the LAMP molecular test was 100%.

Keywords

Intestinal Schistosomiasis, Molecular Diagnosis, Comparison, LAMP, Kato Katz

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1. Introduction

Human schistosomiasis (bilharzia) is a parasitic disease that occurs almost exclusively in tropical or subtropical regions [1]. It is caused by non-segmented flatworm trematodes living in the larval state in a freshwater mollusc and as an adult in the human venous circulatory system [2]. A more discreet and less known parasitic disease than malaria, it is still endemic in many countries in Africa and Asia and can cause serious digestive or genitourinary lesions. This infection is endemic in 78 countries mainly in tropical and subtropical areas, although it predominates in sub-Saharan Africa where more than 80% of cases occur, leading to approximately 280,000 deaths per year [3]. Of the 23 species of schistosomes described to date, only five (5) infect humans and they are: *Schistosoma mansoni*, *Schistosoma haematobium*, *Schistosoma intercalatum*, *Schistosoma japonicum*, *Schistosoma mekongi* [4]. In Burkina Faso, parasitological surveys have highlighted the hyper-endemicity of *Schistosoma mansoni* and *Schistosoma haematobium* [5]. These studies also confirmed that on the malacolic level, Burkina Faso presents itself as a crossroads of molluscs intermediate hosts of schistosomes in West Africa [6]. The serious consequences, particularly in socio-economic terms in Burkina, led to the establishment in 2004 of a national control program whose strategy is the mass treatment of school-age children and high-risk communities in 22 sites. sentinels with praziquantel [7]. This strategy has made it possible to reduce the prevalence of this condition in Burkina Faso. However, this disease remains relevant in Burkina Faso with a prevalence of 81.1% in 2021 for *Schistosoma mansoni* [8]. Early diagnosis of infections, especially in cases of low parasitemia, remains a challenge in the fight against this disease. The reference technique for the diagnosis of intestinal schistosomiasis is the Kato-Katz technique [9]. However, the capacity of this technique for the early detection of infections and cases of low parasitaemia would be limited [10]. The polymerase chain reaction (PCR) technique allows a better diagnosis but requires more equipment and expertise [11, 12]. The amplification technique loop-mediated isothermal (LAMP), a molecular technique would be an alternative to classical PCR [13, 14]. It is a molecular technique that has a one-step amplification reaction that amplifies a target DNA with high specificity, efficiency, and rapidity under isothermal conditions, ideal for low-income countries and disease diagnosis in field conditions [1-15]. Indeed, this technique requires less convenience and the interpretation of the results is accessible [16]. Previous studies in other countries have shown the feasibility of this technique. In Burkina Faso, there are no studies on the diagnosis of schistosomiasis using this technique. It is in this context that this study was initiated to compare the LAMP technique and the Kato-Katz technique in the diagnosis of *Schistosoma mansoni* in Burkina Faso.

2. Materials and Methods

2.1. Study Area and Population

Data collection took place in the district of Dô in December 2022. The district of Dô represents one of the three districts of the city of Bobo Dioulasso and is located in the northeast part of the commune with an area of 201,962 km². It has a population of 122,536 inhabitants according to the latest census of 2019. Precipitation in this district is on average 900.8 mm. It is a rice growing area and agriculture being one of the main activities of the population hence the conduct of the study in this area due to the mode of contamination of schistosomiasis. The analysis of the samples and the processing of the data for our study took place in the city of Ouagadougou over a period from February 2023 to April 2023. As part of our research, we looked at school-aged children aged 5 to 17. Subjects are assessed for eligibility and physical, clinical, and laboratory examinations are performed to diagnose schistosomiasis.

2.2. Collection of Parasitological Data and Examination of Stools

We used stool samples from subjects fulfilling the inclusion criteria. As soon as the sample was collected, the macroscopic appearance of the stool was recorded and then sent to the laboratory for microscopic examination. For microscopic analysis of stools from patients infected with schistosomiasis, we used the Kato Katz technique [17] which is a stool lightening technique that allows the eggs of *Schistosoma mansoni* contained in the stool to be distinguished at low magnification.

2.3. Extraction of Schistosome Eggs from Stools

Extraction of eggs from stools was done by adding cold 1.2% NaCl to the jar containing the stools and keeping in the refrigerator overnight. The next day, the stools were homogenized for 30 seconds until a homogeneous mixture was obtained. Then, the feces were filtered through 4 stainless steel sieves of different mesh sizes and rinsed with 1.2% NaCl. For best results, re-homogenate the homogenate trapped on the upper sieve of the mixer and pass the homogenate through the sieve again. Then we removed the first 3 sieves and the liquid obtained on the last sieve containing the eggs and was spilled in a 15 mL falcon tube and centrifuged at 1000 X G for 5 minutes and the supernatant was removed then the eggs were frozen in the form of dry pellets to be observed later under a microscope.

2.4. Extraction of DNA from Schistosome Eggs

To extract schistosome egg DNA from patient stools, we used the Chelex 100 extraction technique [18]. Briefly, we aspirated 200 µL of an egg-containing NaCl solution to

which we then added 1.4 mL of previously prepared lysis buffer (TEN-9) and vortexed the mixture. We then incubated the mixture at 95 °C for 10 min (for lysis of hard-to-lyse bacteria and cells) and then vortexed the mixture again for 15 sec. The mixture was then centrifuged at 13,000 incubated at 95 °C for 5 minutes. Finally, we centrifuged at 13,000. After extracting the DNA from the stool samples, we proceeded to quantify the DNA extracts from the positive samples using Nanodrop obtained to have the initial DNA concentration of its samples.

2.5. LAMP testing

This technique is based on a generation of artificial

stem-loop DNA structures flanking the target sequences. The cyclic movement of the strands is carried out at a constant temperature, approximately 65 °C, at which the double-stranded DNA remains in dynamic equilibrium. This allows the primers to hybridize to the complementary DNA sequence, so that DNA polymerase can begin DNA synthesis. It has two (2) phases: cyclic and non-cyclic phases [16]. The LAMP assay was performed using a mixture of some standard protocol reagents (Table 1). Briefly, the reaction was carried out with a total volume of 25 µL reaction mixture and tested using a heating block set at 63 °C for 60 min and then heated to 80 °C for 5 min to complete the reaction. Negative controls (ultrapure water or DNA from uninfected stool samples) were included in the LAMP reaction. These samples have never been amplified.

Table 1. Composition of the Mix of the LAMP test reaction mixture.

Reagents	Concentration	Volume in µL
WarmStart Colorimetric LAMP 2	2	12.5
H ₂ O	-	To be completed
FIP	40 pmol	4
BIP	40pmol	4
F3	5 pmol	0.5
B3	5 pmol	0.5

*BIP: Backward Inner Primer

*FIP: Forward Inner Primer

The LAMP reaction results were visually inspected by colorimetric change by adding 2 µL diluted 1:10 of the fluorescent dye SYBR Green I (Invitrogen, Carlsbad, CA, USA) to the reaction tubes. Also, 5 µL of the LAMP reaction products were also checked by electrophoresis on a 2% agarose gel stained with ethidium bromide, visualized under UV light, and then photographed using the ultraviolet (UV) image system (Gel documentation system, UVitec, UK) [19].

2.6. Data Analysis

During our study, our data were entered using Excel 2016 software, this same software was used to generate figures and tables. The comparison of the 2 techniques was carried out by calculating the Kappa Cohen Coefficient.

3. Results

3.1. Socio-Demographic Characteristics

Among the 52 patients included in our study, we recorded

29 females and 23 males with a sex ratio of 1.26. The average age was 8.29 years and participants aged between 6 and 10 years represented 84.62% of the study population.

3.2. Prevalence of Intestinal Schistosomiasis by the Kato Katz Technique

Parasitological analysis of stools using the Kato Katz technique showed a prevalence of 8% (4/52) subjects carrying *Schistosoma mansoni* eggs while from the other 48 patients, no presence of schistosome eggs was observed.

3.3. LAMP Test and Prevalence of Schistosomiasis Using the LAMP Technique

The LAMP test results were detected visually by the colorimetric change of the samples. Red coloring indicated a negative result and orange or yellow coloring indicated a positive result (figure 1).

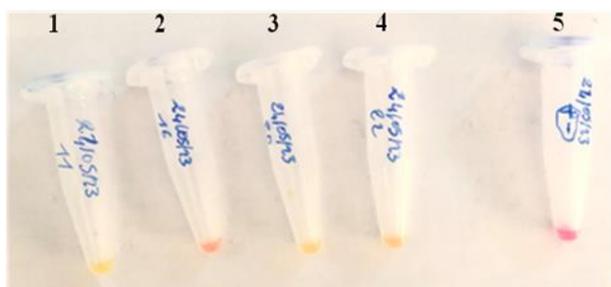


Figure 1. LAMP Test Positive Samples.

These results were also visualized on the 2% agarose gel stained with ethidium bromide (Figure 2).

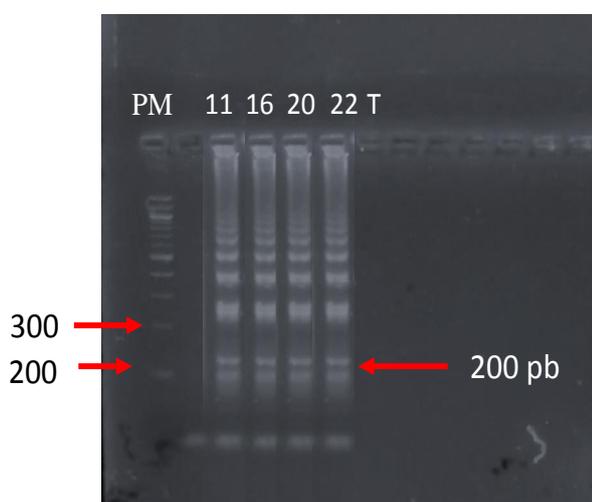


Figure 2. Samples visualized on agarose gel.

The analysis by the LAMP test revealed the presence of mitochondrial minisatellite DNA of *S. mansoni* in 4 patients, resulting in a prevalence of 4% in our study.

3.4. Comparison of LAMP Results Versus the Kato Katz Technique

This comparison during our study showed us an equal overall prevalence of the Kato Katz technique and the LAMP technique (4%). We also obtained 100% sensitivity and specificity of the 2 techniques in our study.

3.5. Calculation of the Cohen Kappa Coefficient

The Kappa coefficient which is an index of concordance between 2 methods was determined during our study by the following formula:

$$K = \frac{OA-AC}{(1-AC)} \quad (1)$$

where OA is the observed agreement and AC is the agreement

by chance [20]. Thus, the Kappa coefficient during our study was 0.9, therefore a perfect agreement between the Kato Katz technique and the LAMP technique.

4. Discussion

In our study, we obtained more women during the examination for the diagnosis of schistosomiasis. Indeed, the female gender predominated with a percentage of 55.80% of our study population. Our results obtained are similar to those found by [8] who obtained a prevalence of 51.70% among women during their study. This could be justified by the fact that females would be more in contact with waterways for activities such as laundry, washing dishes, rice growing, market gardening, etc. Also, during our study, children whose ages were between 6 and 10 years old were the most recorded. These results are similar to those obtained by [21] who found that children aged between 5 and 10 were the most numerous during their study and could be explained by the fact that children of this age play more in waterways (swimming) and are therefore more exposed to schistosomiasis. Among the positive samples obtained in our study, it appeared that men were the most affected by intestinal schistosomiasis. These results are similar to those obtained by [22] who also found in their study that the male gender was the most affected by intestinal schistosomiasis. During our study, the prevalence of intestinal schistosomiasis was 8%. The studies carried out by [22] showed a prevalence of 39%, therefore higher than that obtained during our study. This drop in prevalence in our study could be explained by the effectiveness of mass treatment with Praziquantel for the elimination of schistosomiasis. We also found a prevalence of 8% by the LAMP technique in our study. This prevalence is lower than that found by [20] who reported a 46 % prevalence of LAMP during their study in Kenya. This low prevalence of schistosomiasis by LAMP in our study could be explained by the fact that the intensity of transmission was different in our study areas. In our study, the determination of sensitivity and specificity also made it possible to compare the Kato Katz technique and the LAMP technique. The results obtained were almost similar. We obtained 100% sensitivity and specificity in our study. These results are also similar to those obtained by [23] who obtained a sensitivity of 92.86% between the two techniques. In addition, studies carried out by [24] demonstrated a sensitivity of 97% and a specificity of 100%; substantially equal to the results of our study. The Kappa Coefficient during our study was approximately equal to 1; which means that the two techniques used are in perfect agreement. Studies carried out by [20] demonstrated a Kappa coefficient value that is equivalent to 1 during their studies, and therefore similar to that obtained during our study. The results obtained are also similar to those found by [24] who found a Kappa coefficient of 0.9 showing that the LAMP technique and KK are in perfect agreement. This coefficient allowed us to understand to what extent the LAMP technique is very sensitive and specific while remaining accessible for our developing countries including Burkina Faso. The results of our study suggest that the

LAMP technique would be an asset in the fight for the elimination of schistosomiasis. Indeed, this technique detects *Schistosoma mansoni* DNA even during an early infection and is significantly simpler to perform and does not require any specific expert personnel. In addition, LAMP products can be visualized with the naked eye without electrophoresis, giving it the advantage of being used in field conditions.

5. Conclusion

A real public health problem, schistosomiasis requires reliable diagnosis and good management for its control, especially in developing countries such as Burkina Faso. This comparative study between these two methods of diagnosing the disease made it possible to demonstrate the sensitivity and specificity of the LAMP molecular technique compared to the Kato Katz technique which is the standard technique for the diagnosis of schistosomiasis. This study showed that the LAMP test would therefore be well suited to diagnose intestinal schistosomiasis in endemic regions with limited resources such as Burkina Faso due to its speed, ease of handling, effectiveness, and high specificity and sensitivity of detection in ground conditions. Thus, for the continuation of the work, we plan to extend our study area to increase samples size and geographical sites to better characterize the disease prevalence in Burkina Faso and to further compare the LAMP technique with the PCR technique for the diagnosis of intestinal and urinary schistosomiasis in the context of the country.

Abbreviation

LAMP	Loop-Mediated Isothermal Amplification
PCR	Polymerase Chain Reaction
NTD	Neglected Tropical Disease
KK	Kato Katz
DNA	Deoxyribonucleic Acid
WHO	World Health Organization
BIP	Backward Inner Primer
FIP	Forward Inner Primer

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Conflicts of Interest

The authors declare no conflict of interest.

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Research Field

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