Malaria in Burkina Faso from 2000 to 2019: Assessment of Diagnostic Tools

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Keywords: Burkina Faso, Sub-microscopic malaria, PCR, Microscopy, Rapid Diagnostic tests

Abstract: Malaria elimination depends on the potency of surveillance tools. We assessed the efficacy of rapid diagnostic tests (RDTs) and polymerase chain reaction (PCR) in sub-microscopic malaria detection. Cross sectional or screening studies realized in Burkina Faso from 2000 to 2019 were found in PubMed using keywords "malaria", "PCR" and "Burkina Faso" and specific inclusion criteria. Malaria prevalence and sub-microscopic (SM) were calculated from PCR, LM, and RDTs results. Overall, 6 studies (4 in Nanoro, 1 in Bourasso, and 1 in Bobo-Dioulasso) fit the inclusion criteria. The prevalence by PCR in Bourasso (before 2009) was the highest compared to Nanoro (92% vs 27.3%, $p<0.001$) and Bobo-Dioulasso (92% vs 34.5%, $p<0.001$). From PCR results it seems that SM prevalence is relatively stable over the last 20 years independently from the location (11.4% in Bourasso, 10.3% in Nanoro, 19.9% in Bobo-Dioulasso). Except in Nanoro where SM_{HRP2} was higher than SM_{PCR} (12.8% vs 10.3%, $p=0.04$), RDT HRP2 and RDT pLDH failed to compete with PCR in SM detection. Although implementation of RDTs have triggered the reduction of malaria cases, they are not suitable for sub-microscopic malaria detection. Therefore, novel diagnostic tools as sensitive as PCR and as easy to perform as RTDs are needed.

1 INTRODUCTION

From 2010 to 2015, the number of infected and death cases of malaria have reduced by 21% and 29% among all age groups. Despite that, malaria is still a public health issue especially in Sub-Saharan Africa. This region is the most affected where Children under 5 years and pregnant women are the most vulnerable population in terms of mortality and morbidity (UNICEF and WHO, 2000). An early diagnostic of malaria is essential to prevent the fatal outcomes such as anemia, low bird weight, and mother and/or child death (Steketee et al., 1996; Luxemburger et al., 1997). In 2015, 90% of cases and 92% of malaria deaths were reported in the same African region although the death rate felt by 35% among children under 5 years. Recent estimations suggest that 91 countries and areas had ongoing malaria transmission (WHO, 2016).

In Burkina Faso, malaria represents 63.2% of hospitalizations and 49.6% of deaths among children under 5 year-old. Noticing the reduction of malaria cases worldwide, the National Malaria Control Program of Burkina Faso aims to end the disease by 2030. Thus, political commitment, implementation of Artemisinin Combination Therapy (ACT), better access of population to diagnostic and vector control strategies (insecticide treated bed nets) are already implemented ((PNLP), 2014). However, the success of malaria surveillance depends on the performance of existing surveillance tools (Breman and Holloway, 2007) especially on asymptomatic individuals who can exhibit low-density malaria infection or submicroscopic malaria (Cheng et al., 2015). The major tools so far used are light microscopy (LM), rapid diagnostic tests (RDTs) and polymerase chain reaction (PCR). Microscopy has been the main tool for more than two decades as it is cheap (Siala et al., 2010); RDTs recently introduced

Zaongo, S., Sam, W., Ouedraogo, B., Ouedraogo, J. and Ji, D.
Malaria in Burkina Faso from 2000 to 2019: Assessment of Diagnostic Tools.
DOI: 10.5220/0008788001490155
In *Proceedings of the 2nd Syiah Kuala International Conference on Medicine and Health Sciences (SKIC-MHS 2018)*, pages 149-155
ISBN: 978-989-758-438-1

as an alternative to microscopy weakness (Makler et al., 1998) are implemented in Burkina Faso since 2009 (Natama et al., 2017); and PCR, introduced in malaria regions in the 1990s, showed increased prevalence of malaria in communities screened. Herein, we proposed to assess these malaria diagnostic tools efficacy in sub-microscopic malaria detection over the last 20 years in Burkina Faso.

2 MATERIALS AND METHODS

2.1 Literature Search and Inclusion Criteria

Were considered as relevant, all articles identified in PubMed using search terms "malaria", "PCR" and "Burkina Faso". The literature research was done on April 10th, 2019. The studies were eligible only if they had the inclusion criteria that are: (a) the articles were written in English and published within 2000 to the 4th of March 2019, (b) the study participants consisted of a population sample of individuals in, an endemic area who were not chosen on the basis of malaria symptoms or test results, (c) cross sectional studies or screening studies, (d) Data of light microscopy (LM), RDTs [Histidine Rich Protein 2 (HRP2) and/or Plasmodium Lactate Dehydrogenase (pLDH)] and/or PCR/ quantitative PCR (qPCR) / Retro-Transcription PCR (RT-PCR) / direct blood PCR (db-PCR) should be presented, (e) at least *Plasmodium falciparum* infection was detected. For the screening of cohorts, only the data at the inclusion (before any intervention) were considered.

2.2 Malaria Prevalence by PCR, LM, and RDTs

Based on the results of each diagnostic tool, malaria prevalence (P) was estimated by dividing the total number of positive individual by the total number of screened population. Therefore, the prevalence by PCR (P_{PCR}), Microscopy (P_{LM}), HPR2 (P_{HRP2}), and pLDH (P_{pLDH}) were used to estimate the sub-microscopic malaria ongoing for 20 years in Burkina Faso.

2.3 Burkina Faso Map and Study Sites Location

The map was drawn in RStudio software (Version 1.0.153, RStudio, Inc) using the packages "map and mapdata". To represent the study sites location, their geographic coordinates were integrated before the map generating.

2.4 Sub-microscopic Malaria Evaluation

The sub-microscopic (SM) infection was calculated either based on PCR or RDTs results. When the PCR results were considered:

$$SM_{PCR} = P_{PCR} - P_{LM}.$$

Considering the RDTs as reference:
$SM_{HRP2} = P_{HRP2} - P_{LM}$; or $SM_{pLDH} = P_{pLDH} - P_{LM}$.

2.5 Data Analysis

Data were entered on Microsoft Excel 2013 then subsequently transferred and analyzed on SPSS (Version 20, IBM Corporation). The Fisher exact test was used to compare the proportions as previously described (Campbell, 2007; Richardson, 2011). Therefore, the prevalence based on diagnostic tools or location were compared using α=0.05 as significance level.

3 RESULTS

3.1 Descriptive Statistics

From PubMed using the specific keywords, 92 articles were found and 6 studies fit the inclusion criteria (Figure 1).

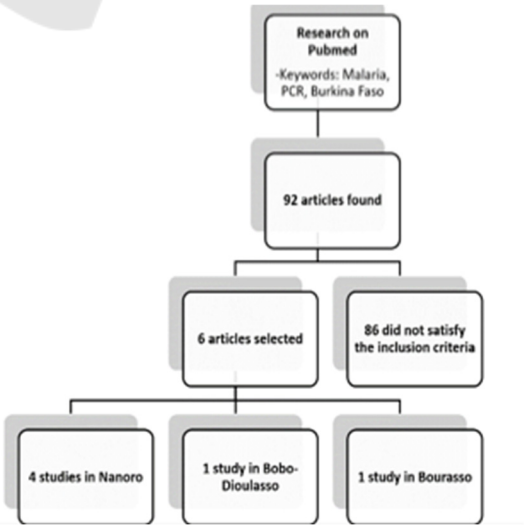

Figure 1. Paper mining flowchart

They were predominantly conducted in Nanoro (4/6, 66.7%). Among the 2 remaining studies one was performed in Bourasso (1/6, 16.7%) and the other in Bobo-Dioulasso (1/6, 16.7%). The geographical location of Nanoro (in Center-West, located at 85 km of Ouagadougou the capital city) and Bobo-Dioulasso (in West, the second largest town of the country) are represented in Fig 2. Bourasso is a rural village located between Bobo-Dioulasso and Ouagadougou; its location is closer to the Malian border of the country (see Figure 2).

Figure 2. Burkina Faso map with study sites location

The most targeted populations were pregnant women and infants (4/6, 66.7%) as shown in Table 1

Location	Target population	Positive test				Total sample	Ref
		PCR	Microscopy	RDT HRP2	RDT pLDH		
Nanoro	Infants	16	0	3	-	400	(Natama et al., 2017)
Bobo-Dioulasso	Pregnant women	224	95	134	102	650	(Kyabayinze et al., 2016)
Nanoro	All inhabitants	139	122	136	-	283	(Mens et al., 2012)
Nanoro	Pregnant women	201	112	178		380	(Kattenberg et al., 2012)
Nanoro	Infants	120	62	-	-	678	(Natama et al., 2018)
Bourasso	All inhabitants	185	162	-	-	201	(Stich et al., 2006)

Table 1. Details of the selected studies

3.2 Sub-microscopic Malaria Prevalence

From the selected articles, a total of 2592 people were screened by PCR and LM. The number was reduced when we split the screened populations into before 2009 (201 screened people) and after 2009 (2391 screened people). However, considering RDT HRP2 and RDT pLDH, which were not used in every study, the screened populations were 1713 and 650 respectively.

Overall, in 20 years the prevalence of malaria by PCR, and microscopy was 33.1 % (885/2592), and 21.3% (553/2592). Considering that RDTs were implemented after 2009, the Prevalence of malaria by PCR, LM, HRP2, and pLDH from 2009 until now is at 29.3 % (700/2391), 16.4% (391/2391), 23.3% (451/1713), and 16% (102/650). Thus, the sub-microscopic $SM_{PCR,}$ $SM_{HRP2,}$ and SM_{pLDH} are estimated at 12.9%, 6.9% and -0.4% (considered as 0) respectively.

In Nanoro, the malaria prevalence with PCR (P_{PCR}) was 27.3% (476/1741). It was the highest estimated prevalence in comparison to microscopy (P_{LM}= 17%, 296/1741), and HRP2 (P_{HRP2}=29.8%, 317/1063). Thus, SM_{PCR}= 10.3%, and SM_{HRP2}= 12.8%. In Bobo-Dioulasso, P_{PCR} was at 34.5% (224/650) whereas P_{LM} (14.6% (95/650), HRP2 (P_{HRP2}=20.6%, 134/650), and pLDH (P_{pLDH}= 15.7%, 102/650) were showing lower prevalence. The estimated SM_{PCR}= 19.9%, SM_{HRP2}= 6%, and

SM_{pLDH}= 1.3%. In Bourasso, malaria prevalence using PCR (P_{PCR}) was estimated at 92% (185/201). Therefore, SM_{PCR} was at 11.4% as the prevalence by microscopy (P_{LM}) was 80.6% (162/201).

Interestingly, we noted from Fig. 3 that malaria prevalence by PCR in Bourasso was the highest in comparison to Nanoro (92% vs 27.3%, p<0.001) and Bobo-Dioulasso (92% vs 34.5%, p<0.001). The same observation was noted when we compared the prevalence in Bourasso to the overall prevalence after 2009 (92% vs 29.3%, p<0.001). Except in Bourasso, the investigations conducted in Nanoro and Bobo-Dioulasso were performed after 2009. After 2009, the overall prevalence vs Nanoro (29.3 vs 27.3%, p= 0.16) was relatively similar while overall prevalence vs Bobo-Dioulasso (29.3% vs 34.5%, p=0.01) was statistically different. Moreover, compared to Nanoro the prevalence in Bobo-Dioulasso was higher (34.5% vs 27.3%, p<0.001).

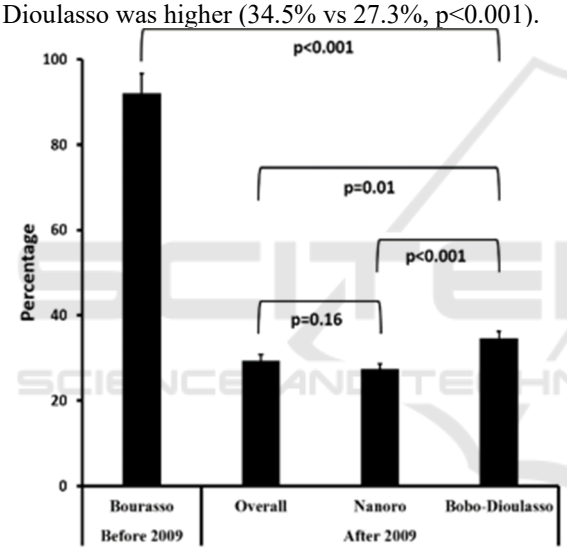

Figure 3. Prevalence by PCR before and after the implementation of RDTs in Burkina Faso

In comparing the SM calculated from PCR and RDTs, we noted that PCR had the highest detection rate. Moreover, considering PCR results it seems that SM is relatively stable over the last 20 years independently from the location (11.4% in Bourasso, 10.3% in Nanoro, 19.9% in Bobo-Dioulasso, and 12.9% overall). Except in Nanoro where SM_{HRP2} was higher than SM_{PCR} (12.8% vs 10.3%, p=0.04) the general trend is showing that RDT HRP2 failed to compete with PCR in terms of SM diagnostic. However, RDT HRP2 showed a relatively higher sensitivity for SM diagnostic compared to RDT pLDH (6.9% vs 0% Overall, p= and 12.8 vs 1.3 in Bobo-Dioulasso). The details of the aforementioned results are presented in Figure 4.

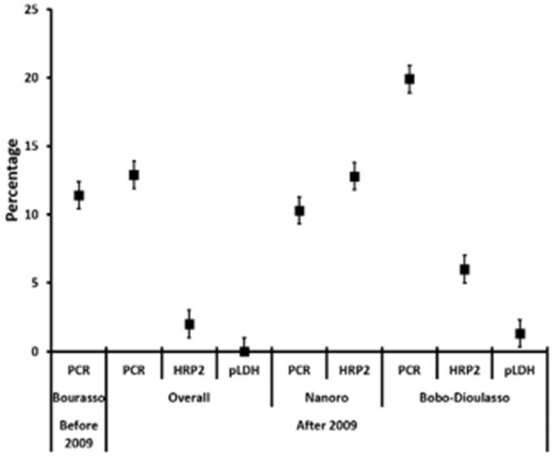

Figure 4. Submicroscopic malaria prevalence based on PCR, RDTs, locations and period.

4 DISCUSSION

From PubMed, few articles fitting the selection criteria were found. 3 regions were of interest: Bourasso (before 2009), Nanoro and Bobo-Dioulasso (after 2009) (Figure 1 and 2). Bourasso is a village belonging to the rural area located near the Malian border. Like Bobo-Dioulasso, malaria transmission in Bourasso is holoendemic with a peak during the rainy season May to October (Soma et al., 2018). Thus, the prevalence in Bourasso before 2009 could be assimilated to the one observed in Bobo-dioulasso during the same period. Nanoro and Bobo-Dioulasso are well-known for their health research centers of reference. That is probably why most of the studies were conducted in Nanoro (Figure 1) which in contrary is a hyperendemic transmission area from July to November (Natama et al., 2018).

We found that prevalence by PCR in Bourasso (before 2009) was the highest (92%). After 2009, the prevalence was estimated at 27.3% in Nanoro and 34.5% in Bobo-Dioulasso. From 92% the prevalence was reduced to 29.3% (Overall) after 2009 (Figure 3). This suggest that after the usage of RDTs, malaria cases were somehow divided by 3. This is parallel with earlier studies addressing the positive impact of RDT as they enhanced the surveillance for malaria elimination (Linn et al., 2015; Donald et al., 2016). However, malaria elimination program also depends on the ability of diagnostic tools to detect SM.

We evaluated the SM rates by PCR, and RDT. Considering PCR results it seems that SM is relatively stable over the last 20 years independently from the study site (between 10.3% and 19.9%, see

figure 4). In contrary, RDTs HRP2 and pLDH failed to detect SM. This could be explained by the high sensitivity of PCR which is able to detect very low levels of parasitemia (de Monbrison et al., 2003); while (a) Antigen-based detection RDTs are not efficient for the infection detection during the early stages (Siala et al., 2010), (b) their problems of storage are well-known (Gamboa et al., 2010; WHO et al., 2011) and (c) they are not quantitative enough to distinguish between levels of infections (Murray and Bennett, 2009). Golassa and *coll*. after comparing PCR, and RDT had concluded that only PCR was able to detect low-density malaria infection also called asymptomatic malaria (Golassa et al., 2013). In areas where PCR cannot be performed daily, this finding represents a threat to elimination programs. Actually, SM infections only rarely provoke acute diseases (Rogier et al., 1996), but they are capable of infecting mosquitoes and continuing the transmission (Muirhead-Thomson, 1957; Coleman et al., 2004). Moreover, it is known that SM can persist for several months without any symptoms that would prompt treatment seeking (Roper et al., 1996); which is not appropriate for pregnant women in high transmission areas as they are exposed to anemia, placental malaria, and low-birth weight (Steketee et al., 1996). Considering our findings suggesting there are lot of SM ongoing in Burkina Faso, and knowing the risks of this form of malaria, we could therefore assume that it is an urge to promote more sensitive diagnostic tests.

Plasmodium feeds on hemoglobin and excrete iron under toxic form for the parasite that converts it into hemozoin. Hemozoin behaves like little magnets that are detectable and measurable. In the past few years, Magnets detection technique is becoming prominent (Kim et al., 2010; Mens et al., 2010; Castilho Mde et al., 2011; Yuen and Liu, 2012). From research published, RDTs limit of detection was estimated at 100 parasites/ul while the threshold of the device detecting hemozoin was equivalent to ≤ 30 parasites/ul (Butykai et al., 2013). The technic is faster than RDT and more than 3 times as accurate as current kits. To our knowledge, despite Sub-Saharan African countries like Burkina Faso are majorly exposed to malaria, the magnet detection technique has not been used yet.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests

ACKNOWLEDGEMENTS

Thanks to N. Aida N. Ouedraogo for her help with the proofreading of this paper.

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