

Molecular Diagnostic of *Wuchereria bancrofti* Infection in Vector Populations from Endemic Regions of Burkina Faso: Preliminary Results

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Abstract

Since 2001, Burkina Faso had adopted a community-based treatment of lymphatic filariasis (LF) with annual mass drug administration (MDA) of Ivermectin-Abendazole. It remained some hotspots with active transmission. Therefore, in the elimination perspectives toward 2030, it is crucial to validate a diagnostic approach to assess the prevalence of *Wuchereria bancrofti* especially in vector populations for surveillance prospects. For the validation of diagnostic tool, we used samples from previous parasitological and entomological surveys conducted in August 2014 and September 2015 in hotspots where the transmission was still active. Mosquitoes were collected by human landing catches and indoor spraying but no diagnostic tool was used to assess *W. bancrofti* prevalence. Based on molecular technique using DNA of pooled mosquitoes successfully tested in other countries, we tried to validate such protocols from specimens collected during the previous surveys. The results confirmed the *W. bancrofti* infection in human populations as revealed by parasitological tests and also in vector populations tested for the first time by this molecular diagnostic. Within the *An. gambiae* s.l. populations, only *An. coluzzii* was found infected by *W. bancrofti* in the Centre-East and East regions, whereas *An. nili* was the most infected in the South-West region. Some specimens of *An. funestus* were tested positive to *W. bancrofti* with the LAMP PCR but failed to be confirmed by the conventional technique. Our preliminary data confirmed the validity of molecular technique to detect *W. bancrofti* infection within vector populations even additional calibration is still needed. In the South West, surprisingly *An. nili* was found positive to *W. bancrofti* infection by the two molecular techniques extending the list of LF potential vectors in the country. It raised the evidence that diagnostic tool is crucial to better define endpoints for the national LF elimination programme.

Keywords: *Wuchereria bancrofti*, Prevalence, Mosquitoes, Burkina Faso.

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Introduction

Lymphatic filariasis (LF) is the second parasitic disease after malaria, persisting in tropical and subtropical countries worldwide and recognized by World Health Organisation (WHO) as a Neglected Tropical Disease (NTD) [1,2]. It is caused mainly by *Wuchereria bancrofti* in Africa, transmitted by mosquitoes belonging to the genus *Anopheles*. In 1999 the Global Programme to Eliminate Lymphatic Filariasis (GPELF) had been initiated by the WHO with the overall objective to interrupt transmission by 2020 using mass drug administration (MDA) of Ivermectin in combination with Abendazole at least in 53 LF endemic countries worldwide [3]. In 2001, Burkina Faso implemented its National LF Programme committed to manage the elimination by 2015 in all health districts [4]. The first round of MDA occurring in 2001 was followed by several other rounds. By 2014 out of 70 health districts receiving >10 MDAs, only 39 health districts presented their respective microfilariae prevalence low than 10% reaching the interruption threshold [5]. In the other remaining districts, the microfilariae were still upper than 1% and did not match the interruption level. Specifically, the health districts in Centre-East, East and South-West parts of the country showed high and persistent microfilariae rates implying to pursue MDA until today in such districts which become the hotspots of LF in Burkina Faso [4,5]. Hence, in these areas many efforts are still making to cut down the prevalence until elimination threshold. Unfortunately, the monitoring and evaluation scheme to assess the impact of MDA in Burkina Faso is only focusing on antigen tests and also parasitological tests with microfilariae assays in the human populations which did not include yet the detection of microfilaria in vectors [5-7]. As in Ghana molecular approach permitted to detect *Wuchereria*-infected *An. gambiae* s.l. [8,9], integrating this technique can be a complementary way for the monitoring and the evaluation of post TAS (Transmission Assessment Surveys) validation in Burkina Faso. The objective of the current study is to validate a molecular diagnostic tools to assess the prevalence of *W. bancrofti* in mosquitoes. If successful, this molecular approach will benefit to the MDA programme for the future monitoring and evaluation of post LF validation in Burkina Faso as experimented in Ghana and Togo [10,11]. This molecular tool will complement parasitological investigations in human populations. It will serve as an important tool for the MDA programme for LF elimination towards 2030. For that we needed to exploit field samples already collected from areas where LF transmission was still active that explaining the use of collections of 2014-2015 from the hotspot areas where the infections were confirmed by parasitological data.

Material and Methods

Study sites

We worked on mosquitoes' specimens subsampled from entomological and parasitological collections realised in August 2014 and in September 2015 across six sites where LF was still actively recorded in human populations in Burkina Faso: Seiga (11°57'55.998"N; 0°51'34.956"W) and Koulpissy (12°4'41.228"N; 0°5'52.706"W) in the East

region; Renghin (11°50'46.057"N; 0°25'0.661"W) and Tensobtenga (11°40'53.72"N; 0°0'33.509"W) Tangonko (11°35'59.388"N; 0°0'41.166"W) in the Centre-East and Saptan (12°4'41.228"N; 0°5'52.706"W) in the South West (Figure 1).

Blood sample collection for parasitological diagnostic

Blood samples were nightly collected in August 2014 and September 2015 between 10 pm and 2 am from 100 individuals randomly selected per village and per month between 5 - 65 years old. A thick blood smear was performed from single finger-prick that allowed collecting 60 - 100 µL blood sample staggered in 3 drops. The thick blood films were air-dried and fixed in ethanol, then stained with 10% Giemsa (Sigma) in phosphate buffer (pH 7.0) for 30 minutes. Microscopic examination was then performed by two independent readers, using oil immersion magnification (100 x), for the presence of *W. bancrofti*.

Mosquito collections

The vectors were sampled consecutively during two years in August 2014 and September 2015 by human landing catches (HLC) as well as indoors and outdoors per village from 6:00pm to 6:00am. After collections, mosquitoes were identified using the key of Gillies and Coetzee [12]. The indoor resting mosquitoes were also collected by Pyrethrum Spray Catch (PSC) performed the next morning following each HLC into 10 houses around HLC houses between 6 - 9am. All mosquitoes collected were identified morphologically and stored into tubes for further laboratory analysis for species identification and the prevalence of *W. bancrofti* infections in the anopheline vector populations.

Laboratory analysis

The laboratory works were performed recently on July-September 2019 consisting to validate molecular diagnostic with mosquitoes collected in the field and kept in Eppendorf tubes on silica gel. They were identified by site, species, and collection date before being stored at -20°C together with thick smears samples.

Species identification within *An. gambiae* complex

Legs and abdomens of *An. gambiae* s.l. females from indoor and outdoor collections identified morphologically were analysed to species level using routine diagnostic PCR [13]. The DNA from heads and thoraces were kept for further analysis for the presence of L3 of *W. bancrofti*. As only *An. funestus* s.s. was reported in Burkina Faso as malaria and potentially LF vector, no PCR identification was needed for species identification [14,15].

Molecular detection of *Wuchereria bancrofti* from *Anopheles* mosquitoes

The detection of *Wuchereria bancrofti* L3 within mosquitoes was realized by conventional PCR using DNA from heads and thoraces of mosquitoes grouped by pool before PCR processes [8,16,17]. This technique permitted to analyze the *An. gambiae* s.l. collected in the Centre-Eastern and Eastern sites namely Seiga, Renghin, Tensobtenga and

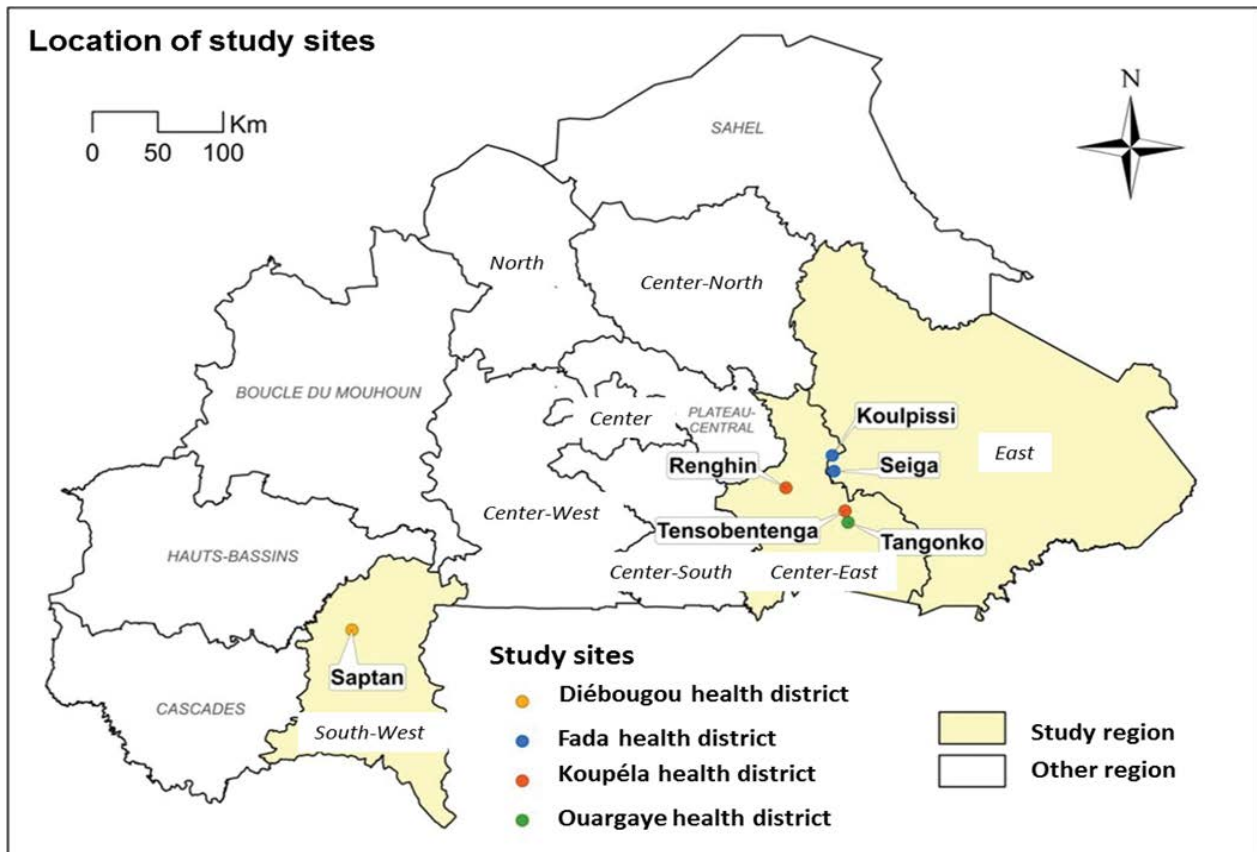


Figure 1: Study sites' location in Burkina Faso.

Tangonko. Furthermore, during this process, we received the primers for the LAMP-PCR that we applied for the detection of *W. bancrofti* from Saptan mosquitoes which presented a diversity of species such as *An. gambiae* s.l., *An. funestus* s.l. and *An. nili*. This technique was performed according to the Loop-mediated isothermal method (LAMP-PCR) which final PCR products were revealed by electrophoresis gel [18]. Then all positive pools for the LAMP-PCR were systematically analyzed with the conventional PCR technique as described above just to confirm the results in accordance with those tested for the other five sites (Koulpissy, Seiga, Renghin, Tangonko and Tensobentenga).

Data analysis

Statistical analysis was performed with the software R version 4Ri386. The "Pool Screen® 2.0" software using a statistical model to estimate vector's infection from the number of positive pools with 95% CI served to determine the *W. bancrofti* infection rates. The microfilaria vectors infections rates (expressed over about 1000 mosquitoes) have been estimated by the algorithm of Katholi et al. [19].

Results

Prevalence of microfilariae in human populations

Out of 1985 thick blood smears analyzed, 652 were from August 2014 and 1333 from September 2015 including all the six study sites (Table 1). In 2014, *W. bancrofti* was detected in four villages (Seiga, Koulpissy, Renghin, Tensobentenga and Saptan) revealing that its prevalence was

still relatively higher in human populations in such sites. The related microfilariae prevalences were higher in Koulpissy (Dmf=350mf/mL), Seiga (Dmf=2330mf/mL and Renghin (Dmf=183mf/mL) and relatively low in Saptan (Dmf=50mf/mL). In 2015, *W. bancrofti* was detected only in two villages namely Seiga and Tensobentenga with low microfilariae prevalence compared to 2014 reaching mean infection of 17mf/mL in each site but no more in Saptan and Koulpissy. Additionally to *W. bancrofti*, *Mansonella perstans* was found in 2014 in Saptan with a mean prevalence of 1.64 % with 50 mf/mL.

Mosquito abundance and species composition

A total of 29,415 *Culicidae* were sampled by the two collection methods with 9,272 in August 2014 and 20,143 in September 2015 throughout the six sites both indoors (11,924 mosquitoes) and outdoors (10,243 mosquitoes). In addition, *An. gambiae* s.l. was the predominant species found in both round of surveys (87.25%) followed by *An. nili* (5.72%) and *An. funestus* s.l. (1.24%) in Saptan. *Aedes* (0.82%), *Culex* sp (5.06%) and *Mansonia* (0.005%) were found in relatively low proportions (Table 2).

Anopheles gambiae s.l. species composition.

An. coluzzii was the predominant species in Koulpissy, Seiga and Renghin whatever the year. However, *An. gambiae* represented more than 80 % of the complex species in Tangonko and Tensobentenga in August 2014 but not in September 2015 where *An. coluzzii* were found predominant in these localities (Figure 2-4). *An. arabiensis* was retrieved

August 2014						
Site	Number persons examined	Nb of MDA	LF positive cases	Total number of Microfilaria (in 60µl)	Microfilarial density (Dmf)	Prevalence of Wb infection (%)
Seiga	140	12	1	14	233mf/mL	0.71
Koulpissy	106	12	3	21	350 mf/mL	2.83
Renghin	81	12	1	11	183mf/mL	1.23
Tangonko	93	12	0	0	0	0
Tensobtenga	110	12	0	0	0	0
Saptan	122	17	1	3	50mf/mL	1.64
Total	652	-	6	49	92mf/ML	0.92
September 2015						
Seiga	296	13	1	1	17mf/mL	0.34
Koulpissy	282	13	0	0	0	0
Renghin	226	13	0	0	0	0
Tangonko	87	13	0	0	0	0
Tensobtenga	233	13	1	1	17mf/mL	0.43
Saptan	209	19	0	0	0	0
Total	1333	-	2	2	6mf/mL	0.15

Table 1: Prevalence of *Wuchereria bancrofti* in human populations from the study sites (LF: lymphatic filariasis).

in low frequencies in all sites except in Tensobtenga in 2014 but not occurring in 2015 collections. *An. gambiae* was the main species of the complex in Saptan whatever the year.

Prevalence of *Wuchereria bancrofti* in mosquito populations

The infection of *W. bancrofti* within *An. gambiae* s.l. populations were revealed by conventional PCR in two samples sets from Koulpissy in 2014 with an infection rate of 4.5% (1.17-11.4) and Tensobtenga in 2015 reaching an infection rate of 1.04% [0.03-5.2] (Table 3). The species PCR of each positive pool revealed that all positive pools were identified as *An. coluzzii* as well as in Koulpissy and Tensobtenga. Furthermore, the pools of Saptan composed by *An. gambiae* s.l., *An. funestus* s.l. and *An. nili* collected both in 2014 and 2015 were tested first using LAMP technique and secondly with conventional PCR. The results obtained with LAMP-PCR technique showed that both *An. funestus* s.l. and *An. nili* were infected by *W. bancrofti* respectively in 2014 and 2015 with related infection rates of 0.5% (0.05-2.9) and 0.06% [0.002-0.3] respectively (Table 4). However, these results were checked by conventional PCR that confirmed only one pool of *An. nili* as effectively positive to *W. bancrofti* reaching an infection rate of 0.8% (0.3-1.4). The other pools of *An. funestus* failed to be confirmed by conventional PCR. No *An. gambiae* s.l. pool was tested positive to *W. bancrofti* neither by LAMP none by conventional PCRs in Saptan.

Discussion

This study was one of the first which attempted to validate a molecular diagnostic tool to investigate infection status of *Wuchereria bancrofti* especially within vector populations in Burkina. The results were consistent both in human and vector populations being in reduced trend from one year to another as well as by the intensity of the infection in human populations as revealed by the microfilariae density and by

the infection rate within vector populations. These results were confirmed two years after in the same areas by Kima et al. [5] who had also notified many cases of *W. bancrofti* in human populations confirming thus the hotspot status of this transmission belt [5]. Compared to Ghana, a neighboring country experiencing the elimination of LF, the infection of *W. bancrofti* in human population was rare or absent after 11 to 16 MDAs that lead to conduct post TAS evaluation studies [8]. Here in Burkina Faso many investigations are still needed before decision making to stop the MDA. Nevertheless, when comparing the prevalence in human populations from 2014 to 2015, it can be noted an important reduction of *W. bancrofti* either in of microfilariae or in the number of villages with positive cases. This situation can be explained as a progress resulted by integrated vector control measures implemented with the high coverage of LLINs in nationwide but also by the continuing MDA realized in these sites [20]. Analyzing the prevalence of *W. bancrofti* in vectors populations, it can be noted that the main and potential vectors of LF in Burkina Faso were assumed to be anopheline mosquitoes as early reported by very old studies [21]. Globally the biting dynamic in these sites was dominated by *An. gambiae* s.l. but mostly identified as *An. coluzzii* and *An. gambiae*, the third species of the complex, *An. arabiensis* being very rare in the collections. It is fair to assume that in our studies the results were considered as preliminary as we did not dissect any mosquitoes for microscopic identification of microfilaria (L3) nor testing PSC collected mosquitoes by PCR for *W. bancrofti* infection. So no detail can be documented like those realized in Ghana [22]. Nevertheless the molecular detection indicated that belonging the *An. gambiae* complex represented only by two species, all the pools tested positive were composed by *An. coluzzii* that suggested that in the Centre-East and East parts, this species should be the most involved in *W. bancrofti* transmission. Furthermore, the vector bionomics in the West and specifically in the South-

August 2014											
Sites	Collection site	<i>Anophelinae</i>					<i>Culicinae</i>			Total	
		<i>An. gambiae s.l.</i>	<i>An. funestus s.l.</i>	<i>An. nili</i>	<i>An. pharoensis</i>	<i>Anopheles sp.</i>	<i>Aedes s.p.</i>	<i>Culex sp.</i>	<i>Mansonia s.p.</i>		
Seiga	Indoor	634	1	0	1	0	7	1	1	645	1155
	Outdoor	494	0	0	0	0	15	1	0	510	
Koulpissy	Indoor	639	0	0	0	0	5	0	7	651	1325
	Outdoor	658	0	0	0	0	12	0	4	674	
Renghin	Indoor	496	0	0	0	0	1	30	0	527	959
	Outdoor	369	4	0	3	1	13	38	4	432	
Tensobtenga	Indoor	633	0	0	0	0	1	0	1	635	1352
	Outdoor	699	0	0	0	0	7	8	3	717	
Tangonko	Indoor	517	0	0	0	0	2	66	0	585	1198
	Outdoor	504	0	0	0	0	7	102	0	613	
Saptan	Indoor	198	206	120	0	4	19	2	48	597	1038
	Outdoor	143	74	127	0	9	45	0	43	441	
September 2015											
Seiga	Indoor	2500	0	0	0	0	9	2	0	2511	4832
	Outdoor	2292	0	0	0	0	26	3	0	2321	
Koulpissy	Indoor	1286	0	0	0	0	7	0	0	1293	2310
	Outdoor	997	0	0	0	0	15	0	5	1017	
Renghin	Indoor	2058	0	0	0	0	7	13	0	2078	3546
	Outdoor	1441	0	0	4	0	11	12	0	1468	
Tensobtenga	Indoor	1021	0	0	0	0	3	5	2	1031	1602
	Outdoor	549	0	0	1	0	4	5	12	571	
Tangonko	Indoor	445	0	0	1	1	4	92	0	543	1005
	Outdoor	382	1	0	0	2	9	65	3	462	
Saptan	Indoor	214	22	570	1	8	3	7	3	828	1845
	Outdoor	105	8	851	0	16	1	26	10	1017	

Table 2: Total vector collected by Hunan Landing Catches (HLC) method.

<i>Anopheles gambiae s.l.</i>					
Localities	Date of collection	Nb pools tested	Nb positive pool	Nb mosquitoes tested	Infection rates
Siega	Aug 2014	13	0	100	0
	Sept 2015	10	0	100	0
Koulpissy	Aug 2014	15	4	100	4.5 (1.17-11.4)
	Sept 2015	10	0	100	0
Renghin	Aug 2014	14	0	100	0
	Sept 2015	10	0	100	0
Tensobtenga	Aug 2014	13	0	100	0
	Sept 2015	10	1	100	1.04 (0.03-5.2)
Tangoko	Aug 2014	13	0	100	0
	Sept 2015	10	0	100	0

Table 3: Infections rates of *Wuchereria bancrofti* in *An. gambiae s.l.* populations from 5 study sites assessed by conventional PCR.

Type of PCR	2014				2015			
	Nb specimens	Nb pools tested	Nb positive pool	Infection rates	Nb specimens	Nb pools tested	Nb positive pool	Infection rates
<i>An. funestus</i>								
LAMP PCR	385	6	1	0.5 (0.05-2.9)	33	2	0	-
Conventional PCR	385	9	0	-	33	2	0	-
<i>An. nili</i>								
LAMP PCR	256	8	1	0.012 (0.1-1.2)	1423	65	10	0.71 (0.3-1.4)
Conventional PCR	256	8	0	-	1423	65	1	0.06 (0.002-0.3)
<i>An. gambiae s.l.</i>								
LAMP PCR	186	6	0	-	206	9	0	-
Conventional PCR	186	6	0	-	206	9	0	-

Table 4: Infection rates of *Wuchereria bancrofti* in *An. gambiae s.l.*, *An. funestus* and *An. nili* by populations compared between LAMP and conventional PCRs at Saptan in the South West region.

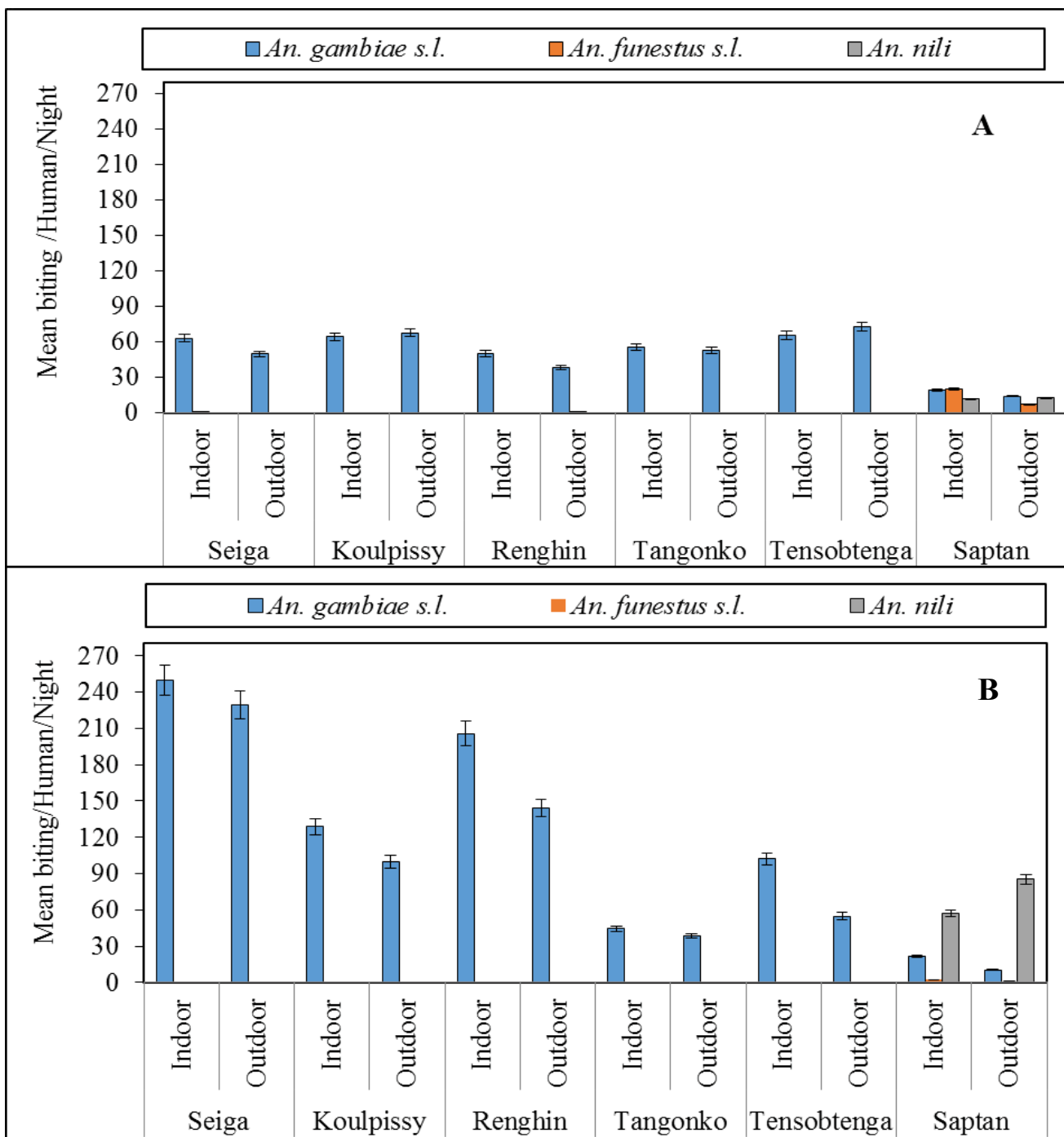


Figure 2: Mean bites per person per night from indoor HLC in the study sites. (A) August 2014 and (B) September 2015 throughout study sites.

West region of Burkina Faso revealed that *An. funestus s.l.* and *An. nili* were very dynamic and even playing locally an important role in malaria transmission. It was naturally that we should assume that *An. funestus s.l.* was tested positive to *W. bancrofti* infection that was also validated in Ghana and in old reports documenting filariasis transmission pattern in the West region of the country [8,21,23]. It was surprising that these results were not validated at the same time by the two techniques such as LAMP-PCR and conventional PCR as showed by our results. These results could be explained by the fact that the LAMP-PCR should be less specific than the conventional PCR regarding to *W. bancrofti*. Nevertheless, as this species is abundant and playing a key role in malaria

transmission it is fair to consider that it should be a potential vector of *W. bancrofti* in South-West health region. Then surprisingly *An. nili* the third vector after the *An. gambiae s.l.* and *An. funestus s.l.* was tested positive to *W. bancrofti* for around 10 pools with LAMP-PCR and only one positive pool with the conventional PCR. Nevertheless, this result confirmed that *An. nili* was implicated in *W. bancrofti* as well as malaria transmission in this area biting indoors and outdoors being more exophilic (PMI report) So its implication in *W. bancrofti* transmission remained to be explored as it was the first time this species was found positive. Then knowing that in the field *An. nili* females were found in relatively high abundance and then no longer infected by *W. bancrofti*, it

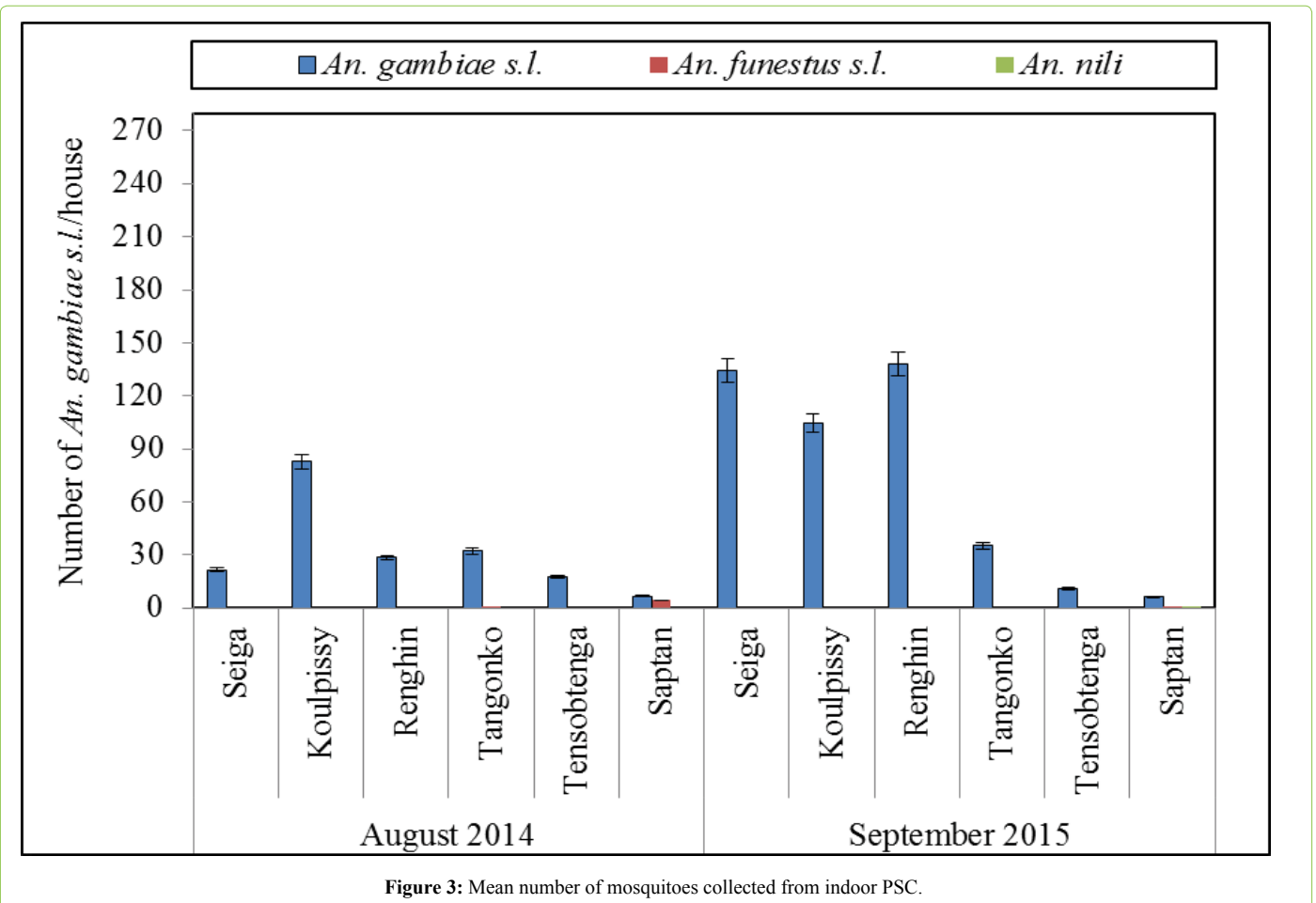


Figure 3: Mean number of mosquitoes collected from indoor PSC.

is not clear if it was only some specimens infected or if this taxon developed competitiveness to transmit LF parasites. Further investigations were recommended in this hotspot area where *An. nili* is very prolific to better clarify the role of this species in LF transmission. As this species is biting more specifically in some villages outdoor, it can sustain outdoor residual transmission of *W. bancrofti* where conventional control tool like LLINs cannot be deployed.

In other hands, the accuracy of the LAMP technique which had been validated in many studies as the xenomonitoring tool remained to be recalibrated in our context as the results were early step data and need to be extended to a large sampling size and regions [24,25]. Doing so we should perform the two techniques rigorously in the same series of samples to calculate how strongest the margin of error between the two is. Of course, if xenomonitoring is essential tool for many countries aiming to perform TAS validation studies before the LF elimination beyond the horizon 2030, its validity and accuracy need to be exempt of critics [26].

Nevertheless, and with regard to the prevalence of LF and the infection rates within human in these sites we can state that our results were consistent, following the same trends when one may consider vector or human populations. It is evident that the incidence of LF in these areas is still persisting. Integrated and innovative actions are needed for its elimination by 2030 [26]. These actions can be focused on integrated vector control as luckily LF and malaria share

the same vectors in Burkina Faso. In addition it is crucial to implement community-based actions leading to make drugs available for anyone without any discrimination and social inequity during the MDA. But to achieve the success socio-anthropological surveys may be implemented in association with entomological investigations to better understand the bottleneck that block the elimination of such long time neglected disease.

Conclusion

The study has documented the persistence of *W. bancrofti* infection in human and vector populations after more than 11 rounds of mass drug administration in Burkina Faso. Entomological assessment showed that *An. gambiae*, *An. coluzzii*, *An. funestus* and *An. nili* were the *Anopheles* species largely dominating in all sites during the collection periods. *An. coluzzii* was detected positive to *W. bancrofti* being the potential vector of this parasite in the Centre and East region whereas in the South West, surprisingly *An. nili* was confirmed by the two molecular techniques positive to *W. bancrofti* and so considered as potential vector of LF for the first time in Burkina Faso. However, as all these data remain preliminary, further additional surveys and investigations are needed to better calibrate the two molecular techniques. Nevertheless, it raises evidence that the use of molecular diagnostic tool is crucial to better define endpoints for LF elimination.

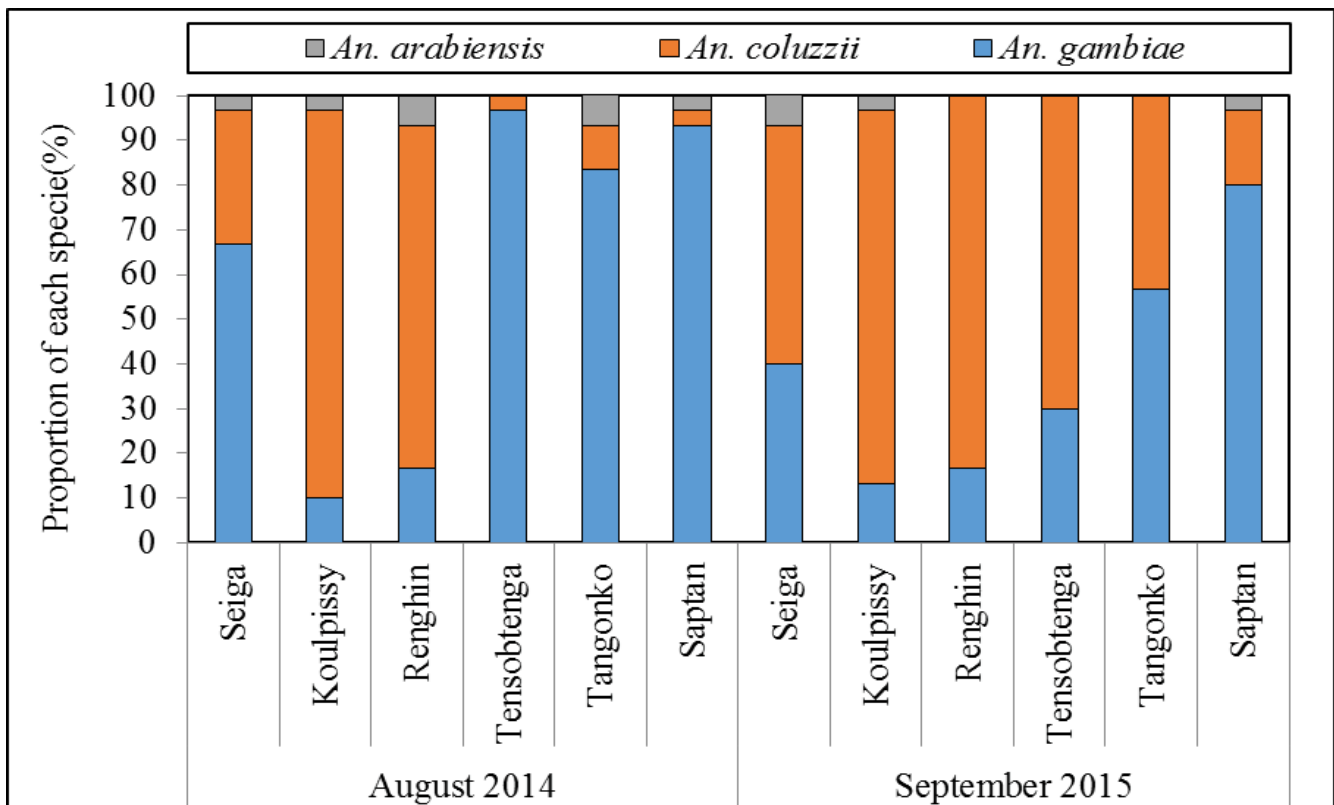


Figure 4: *Anopheles gambiae* s.l. species composition assessed per study sites (n=30 per site).

Declarations

Ethics approval

The works performed in this study did not need consent form which was obtained during the previous surveys.

Consent for publication

Not applicable

Availability of data and materials

All data are included in the manuscript

Competing interests

Authors declared that there is no competing interest

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Authors' contribution

RKD and KB conceived and designed the study. SC and ASH performed the field study, analyzed the data, and drafted the manuscript. RB assured the laboratory analyses. FAS, AD, FF, RWB, IS, and AGO reviewed the first draft of the article. RKD is guarantor of the study. All authors contributed to the drafts and read and approved the final manuscript.

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