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Malaria and Lymphatic Filariasis Co-Transmission in Endemic Health Districts in Burkina Faso

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Abstract

Introduction: Lymphatic filariasis (LF) and malaria are two vector-borne diseases which parasites can simultaneously infect human or mosquito. In Burkina Faso, studies mainly focused on the control of these diseases independently. Hence, there is a lack of information on their co-transmission of to both human and vector. The present study aimed at providing baseline data from endemic areas in Burkina Faso towards a successful integrated management of both diseases. **Methods:** The study was carried out in six sites distributed in the East, Center-East and South-West regions of Burkina Faso. Data were collected in August 2014 and September 2015. The infection rates in human and vector populations, vector diversity, trophic and resting behavior were investigated. To determine the disease prevalence nocturnal finger-prick blood sample and microscopic observations were performed. Vectors collected by human landing catches and pyrethrum spray collections. Biochemical and molecular analyses were performed to identify *Anopheles gambiae sensu lato* sibling species, and to determine vector infection rate and their blood meal origins. **Results:** Results indicate residual transmission of LF and malaria in human and vector populations. A low co-infection rate (<1%) with *Wuchereria bancrofti* and *Plasmodium falciparum* was noted in both human and mosquito. *Anopheles gambiae s.l.*, *An. funestus s.l.* and *An. nili* were by order the main potential vectors encountered. It was in majority parous females and exhibited endophagic and exophagic behavior. Parasite's co-infec-

tion was found with *An. coluzzii* and *An. nili* only. **Conclusion:** The present study has provided basic information on the (co-)transmission of both diseases in the study areas. These results will be useful for further investigations towards the development and implementation of a better integrated strategy to control these diseases.

Keywords

Wuchereria bancrofti, *Plasmodium falciparum*, Mosquitoes, Co-Infection

1. Background

Vector borne diseases, lymphatic filariasis (LF) and malaria, constitute high burdens of public health. Indeed, according to the World Health Organization (WHO), 228 million of morbidity and 405,000 deaths due to malaria were estimated in 2018 [1]. In addition, 51 million people are suffered from LF worldwide in 2017 [2]. Malaria and LF are transmitted by the same mosquito species and then can be co-transmitted to both mosquito and human in West Africa [3]. In this part of continent, the parasites responsible for malaria and LF are mainly *Plasmodium falciparum* and *Wuchereria bancrofti* [4] respectively and their major vectors are *Anopheles gambiae s.l.* and *An. funestus s.l.* [3] [4] [5] [6]. Significant advances in the monitoring, control, and elimination of LF and malaria have been recorded along years [7] [8]. In Burkina Faso, the control of LF using Mass Drug Administration (MDA) with ivermectin + albendazol is ongoing since almost two decades. At the time of the current study, most endemic communities would have stopped transmission and started transmission assessment survey (TAS) or post-MDA surveillance [9]. However, in some health districts particularly in the Center-East, East and South-West regions of the country the prevalence of microfilariae is still above 1%, which is far from the elimination level [9] [10]. To fight against malaria, a national control program is undertaken since 1991. The aim of this program is to reduce morbidity and mortality related to malaria by cases management, chemoprevention and vector control. Currently, the country has made significant efforts in preventing malaria by vector control through long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) of insecticides [11] [12]. Despite these efforts, malaria remains endemic and is responsible of many cases of morbidity and mortality in the country [13].

Parasites of LF and malaria are transmitted mainly by *An. gambiae s.l.* and *An. funestus s.l.* in the country [5]. According to the WHO Office for Africa, integrated vector management is the best approach to improve the efficacy, cost-effectiveness, ecological soundness, and sustainability of both diseases control [14] [15]. Indeed, in areas where malaria and LF are transmitted by the same vectors, interventions against malaria such as the use of LLINs and indoor residual sprays had significant impact, which may has been even greater against LF than malaria [16] [17] [18]. However, the change in mosquito biting and resting

behavior [19] [20], in addition to insecticide resistance [21] [22] could jeopardize the success of vector control operations. Therefore, to set up towards the designation and implementation of an integrated, simultaneous attack against LF and malaria would be to understand both parasites transmission. Moreover, although number of studies that have investigated the impact of LLINs on malaria [23] and that of MDA on LF [9] [10], little information is available on the co-transmission of these diseases to both human and vector populations. To develop an effective integrated management strategy of these diseases, it is important to know their co-transmission patterns. The present study has investigated the vector behavior and the co-transmission of *W. bancrofti* and *P. falciparum* to both mosquito and human populations in areas of Burkina Faso where malaria is endemic and LF transmission persist.

2. Methods

Study sites

The study was carried out in six sites, distributed in Fada, Koupéla, Ouargaye and Diébougou health districts (**Figure 1**). These sites were selected on the basis of prevalence data of LF and malaria from surveillance activities carried out by the National Neglected Tropical Disease Control Program (NNTDCP), National Malaria Control Program (NMCP) and previous studies [9] [10] (**Table 1**). The study sites are: Seiga, Koulpissy (in Fada); Renghin (in Koupéla); Tangonko, Tensobtenga (in Ouargaye) and Saptan (in Diébougou). The number of inhabitants of each village in 2016 was in Koulpissy 1747; Seiga 1425; Tangonko 505; Tensobtenga 1627; Renghin (Baskouré) 479 and Saptan 346.

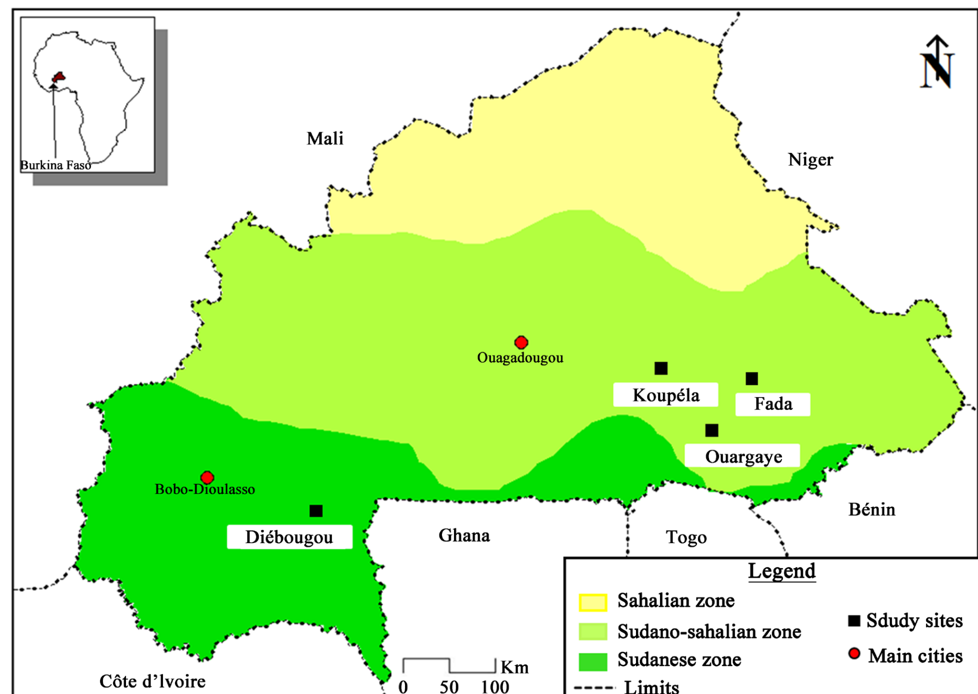

Figure 1. Map of Burkina Faso showing the locations of study sites.

Table 1. Prevalence of *W. bancrofti* and *P. falciparum* infection, number of mass drug administration (MDA) rounds, therapeutic coverage rate and LLINs coverage rate in the study sites.

Site	Year 2014				
	Prevalence of Wb infection (%)	Prevalence of Pf infection (%)	Nb rounds of MDA	Coverage rate for LF treatment	Coverage rate for LLINs (%)
Seiga	1.7	89.4	12	79.6	77.9
Koulpissy	3.2	89.4	12	79.6	77.9
Renghin	0	81.8	12	80.3	96
Tangonko	0.59	89.8	12	81.4	96
Tensobtenga	1.08	89.8	12	81.4	96
Saptan	3.9	90.4	17	83.8	84.4
	Year 2015				
Seiga	3.27	86.3	13	86.3	-
Koulpissy	3.27	86.3	13	86.3	-
Renghin	0	82.4	13	82.4	-
Tangonko	3.48	84.3	13	84.3	-
Tensobtenga	3.48	84.3	13	84.3	-
Saptan	6	94.7	19	94.7	-

Wb: *Wuchereria bancrofti*; Pf: *Plasmodium falciparum*; Nb: Number; LF: Lymphatic filariasis; LLINs: Long Lasting Insecticidal Nets.

The health districts of Fada, Koupéla and Ouargaye are in the Sudano-sahelian zone with two seasons, a dry season, and a rainy season. The dry season extends from November to April. The rainy season extends from May to October with an annual rainfall of 600 to 800 mm and the maximum peak in August-September. This zone is characterized by water systems sparse and savannah vegetation type. Diébougou health district is in the Sudanese zone, with an annual rainfall of 1000 to 1200 mm which extends from May to October. The dry season extends from November to Mars. This zone is characterized by a dense hydrographic network and wooded type savannah vegetation dotted with clear forest and gallery.

Nightly thick blood smears collections for parasitological analysis

The parasitological samples were collected in August 2014 and September 2015 from individuals of between 05 and 65 years of age. Collections were performed in all the sites between 12 pm to 02 am on two day every month. Parents' consent was obtained before any blood collection from their children. Three drops of blood corresponding to 20 µl each or about 60 µl were collected into a clean glass object. The thick blood films were air-dried for one night at room temperature and were fixed in ethanol, then stained the next day with 10% Giemsa (Sigma) in phosphate buffer (pH 7.0) for 30 minutes. Afterwards, they were soaked, rinsed, dried, and then wrapped with toilet paper and transported to the laboratory. Every slide was examined by two independent readers under microscope at x100 magnification to determine for the presence or not of micro-

filariae and trophozoites. The species of microfilariae and trophozoites were identified. All the positive cases were notified to the health center for treatment as recommended by NNTDCP and NMCP.

Mosquito collections

Mosquitoes were collected in August 2014 and September 2015 by two sampling methods, the human landing catch (HLC) and the Pyrethrum Spray Catch (PSC). Human landing catches (HLC) was used to estimate the human biting rates. In each village, five houses were randomly chosen and they were distributed approximately 100 m apart. In such a way to have a representative geographical distribution and during two consecutive nights in both periods. In a house assigned to HLC, two local volunteers, one indoors and the other outdoors, collected mosquitoes landing on exposed legs and feet from 20:00 to 06:00. Collectors (all adult males) were regularly rotated to reduce collector-mediated bias in the results and supervision was provided to ensure collectors stayed awake thus reducing any potential for biting. The mosquitoes collected in each house were stored by collection origin (indoor and outdoor) and in hourly tranches. To estimate mosquito abundance in each village, indoor resting mosquitoes were collected in the both period by PSC between 06 am to 09 am. In each site a total of 10 houses were randomly selected per collection period. The collected mosquitoes were morphologically identified according to the morphological identification key describe by Gillies and Coetzee [24]. The repletion status of *An. gambiae s.l.* and *An. funestus s.l.* females collected from the PSC was recorded. All vectors collected and analyzed in the field were kept in Eppendorf tubes containing silica gel for further analysis in the laboratory.

Determination of age gradient of mosquitoes

To estimate vectors parity rate in each site, the ovaries of 150 females from *Anopheles* genus caught by HLC indoors and outdoors methods and random selected were monthly dissected. The parity rate was determined by observing the coiling of ovarian tracheoles [25]. Examination of dissected ovaries allowed to separate parous females (which had already laid eggs at least once) of nulliparous females (which had not yet laid eggs).

Mosquito blood meal source detection

Blood-fed females of *Anopheles* genus from PSC were used to determine host preference for blood meal intake. A random selection of 50 specimens per site and per period were analyzed by a direct enzyme-linked immune-sorbent assay (ELISA) [26] using anti-host (IgG) conjugated against human, bovine, pig, goat or sheep and donkey blood. Vector anthropophilic rate was calculated as the proportion of mosquitoes fed on human based on the total number of analyzed females.

Species composition

A sub-sample of 360 unfed females of *An. gambiae s.l.* were processed by Polymerase Chain Reaction (PCR) for molecular identification at the species level by using legs and wings. Their heads and thoraces were used to determine the

infection status. Genomic DNA of mosquitoes was extracted with 2% cetyl trimethyl ammonium bromide (2% CTAB). Then, Sine 200X 6.1 locus protocols described by Santolamazza *et al.*, [27] were used to identify the members of *An. gambiae* complex.

Detection of *Plasmodium falciparum* and *Wuchereria bancrofti* infections in the vectors

For *W. bancrofti* and *P. falciparum* detection, only *An. gambiae s.l.*, *An. funestus s.l.* and *An. nili* species heads and thoraces were tested. Two PCR methods were used to determine the vectors infection with both parasites: the conventional PCR and the Loop-mediated isothermal amplification PCR (LAMP), using DNA from heads and thoraces of mosquitoes grouped by pool. The conventional PCR permitted to analyze the vectors collected in all sites. To determine *W. bancrofti* and *P. falciparum* infection by conventional PCR, DNA amplification was carried out following the procedure described by Farid *et al.*, [28] and Morassin *et al.*, [29] respectively. During analyze, the primers for the LAMP PCR have been received and used for the *W. bancrofti* gene detection 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 procedure described by Takagi *et al.*, [30]. 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 (Seiga, Koulpissy, Renghin, Tensobtenga and Tangonko).

Data analysis

Statistical analyses were performed using R software. The R commander package, version 4Ri386, was used to perform the chi-square (χ^2) test with a probability threshold *p-value* = 5%. The chi-square was used to compare the percentages of entomological parameters between species and collection period of the same site. The prevalence of *W. bancrofti* and *P. falciparum* infection in human was determined as the number of positive cases divided by total number tested. The chi-square test was used to compare both infections prevalence in human for each study site by collection period. The "Pool Screen® 2.0" software, using the algorithm of Katholi *et al.,* [31] was used to calculate the parasite infection rates in vectors with 95% confidential interval.

Ethical Approval

Ethical approval was obtained from Institutional Ethics Committee of the Institut de Recherche en Sciences de la Santé and registered as N˚A08/2014/CEIRES. Written informed consent describing the potential risks and benefits of the study was obtained from all study participants before commencing the study and re-confirmed on each experimental night. Volunteers were screened for malaria and lymphatic filariasis parasites during recruitment. Those who were found malaria or lymphatic filariasis positive were offered treatment free of charge according to WHO recommendations. All Volunteers recruited received drug as a prophylactic measure during the study period to prevent disease.

3. Results

Prevalence of microfilariae and trophozoites in human populations

A total of 1985 thick blood smears were analyzed, of which 652 were from August 2014 and 1333 were from September 2015 for all study sites. The results on LF prevalence are summarized in Table 2. The highest prevalence (upper 1%) was recorded in Koulpissy, Renghin and Saptan in August 2014. In September 2015, the prevalence of LF significantly decreased compared to August 2014. Infection was only found in human populations in two sites namely Seiga and Tensobtenga (Table 2).

With regards the *P. falciparum* infection (Table 2), it was high in all sites with prevalence between 56.36% and 81.43% in August 2014. In September 2015, prevalence decreased in all sites, ranging between 24.89% and 54.07%. Significant difference was observed in the prevalence of malaria in human populations between the two periods of collection ($p\text{-}value = 2^{-6}$).

For all human populations analyzed for LF and malaria co-infection, prevalence was 0.16% in August 2014 and 0.08% in September 2015 (Table 2). Only one co-infection case was found in each period of collection, precisely in Koulpissy (August 2014) and in Seiga (September 2015).

Table 2. Summary of lymphatic filariasis and malaria prevalence in human populations

Site	Number persons examined	LF cases positives	Malaria cases positives	Co-infection cases positives	Prevalence of *W. bancrofti* infection (%)	Prevalence of *P. falciparum* infection (%)	Prevalence of both parasites co-infection (%)
August 2014							
Seiga	140	1	114	0	0.71	81.43	0
Koulpissy	106	3	74	1	2.83	69.81	0.94
Renghin	81	1	68	0	1.23	83.95	0
Tangonko	93	0	60	0	0	64.52	0
Tensobtenga	110	0	62	0	0	56.36	0
Saptan	122	1	85	0	1.64	69.67	0
Total	652	6	463	1	0.92	71.01	0.16
September 2015							
Seiga	296	1	75	1	0.34	25.34	0.34
Koulpissy	282	0	82	0	0	29.08	0
Renghin	226	0	76	0	0	33.63	0
Tangonko	87	0	24	0	0	27.59	0
Tensobtenga	233	1	58	0	0.43	24.89	0
Saptan	209	0	113	0	0	54.07	0
Total	1333	2	428	1	0.15	32.11	0.08

LF: Lymphatic filariasis.

Vector abundance and *Anopheles* species composition

The *Culicidae* fauna collected by the two collection methods in all health districts was composed of different species (Table 3 and Table 4). A total of 29,183 mosquitoes were collected with 9098 in August 2014 and 20085 in September 2015 throughout the four health districts. The number of vectors was significantly different between the periods of collection (*p-value* = 0.000016). However, the number of mosquitoes caught by HLC indoor (11,924 mosquitoes) was not significantly higher than that recorded outdoor (10,243 mosquitoes) (*p-value*

Table 3. Mosquito species composition collected by Hunam Landing Catches (HLC) method.

Sites	Collection site	August 2014								Total	
		Anophelinae					*Culicinae*				
		An. gambiae s.l.	*An. funestus s.l.*	*An. nili*	*An. pharoensis*	*Other Anopheles*	*Aedes s.p.*	*Culex s.p.*	*Mansonia s.p.*		
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 4. Mosquito species composition and physiological status of *An. gambiae s.l.* and *An. funestus s.l.* collected by pyrethrum spray catches (PSC) method in the study sites.

Sites	*Anophelinae*										*Culicinae*		Total
	An. gambiae s.l.				*An. funestus s.l.*				*An. nili*	*Other Anopheles*	*Culex s.p.*	*Aedes s.p.*	
	Unfed	Fed	Half-gravid	Gravid	Unfed	Fed	Half-gravid	Gravid					
August 2014													
Seiga	7	159	0	49	0	0	0	0	0	0	14	1	230
Koulpissy	48	617	5	159	0	0	0	0	0	0	40	1	870
Renghin	21	157	5	103	0	0	0	0	0	0	49	1	336
Tensobtenga	12	132	3	28	0	0	0	0	0	0	40	0	215
Tangonko	12	206	0	104	0	1	0	0	0	2	156	0	481
Saptan	6	58	1	4	2	32	3	7	0	0	0	0	113
Total	106	1329	14	447	2	33	3	7	0	2	299	3	2245
September 2015													
Seiga	218	816	49	260	0	0	0	0	0	5	441	2	1791
Koulpissy	107	687	79	170	0	0	0	0	0	0	10	0	1053
Renghin	233	845	71	233	0	0	0	0	0	0	76	0	1458
Tensobtenga	36	249	24	44	0	0	0	0	0	0	43	0	396
Tangonko	12	64	5	29	0	0	0	0	0	0	126	0	236
Saptan	3	55	3	1	0	0	0	1	2	0	3	1	69
Total	609	2716	231	737	0	0	0	1	2	5	699	3	5003

= 0.062). *Anopheles gambiae s.l.* was predominant in both collection periods (87.25%) in all sites followed by *An. nili* (5.72%) and *An. funestus s.l.* (1.24%) in Saptan. *Aedes s.p.* (0.82%), *Culex s.p.* (5.06%) and *Mansonia s.p.* (0.005%) were found in relatively low proportions (Table 3 and Table 4).

***Anopheles gambiae sensu lato* sibling species distribution**

Overall, 360 *An. gambiae s.l.* (n = 30 per site) were analyzed to identify the sibling species encountered in the study sites. In August 2014, *An. coluzzii* was found predominant in Koulpissy (86.7%) and Renghin (76.7%). However, *An. gambiae* represented more than 60% of the complex species in Seiga, Tangonko, Tensobtenga and Saptan. In September 2015, *An. coluzzii* was the most encountered in all the sites except in Saptan and Tangonko where *An. gambiae* was predominant with more than 50% (Figure 2). *An. arabiensis* was observed in low frequencies in all the sites regardless the collection period.

Vector biting and resting behaviors

The variations in the feeding behaviors of *An. gambiae s.l.*, *An. funestus s.l.*, and *An. nili* in both collection periods are shown in Figure 3. Overall, in all sites, *An. gambiae s.l.* exhibited endophagic behavior in August 2014 as well as in September 2015 (*p-value* = 0.05). Indeed, 50% to 70% of this species were collected indoor regardless the site and the period. In Saptan, *An. funestus s.l.* was

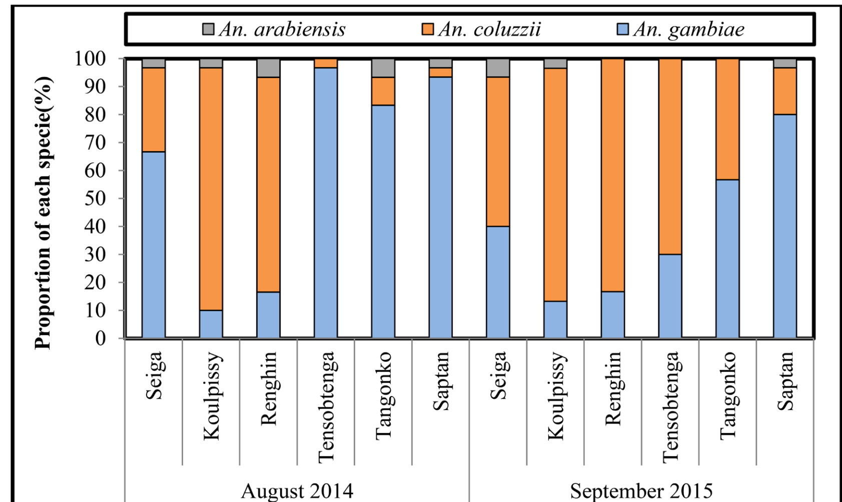

Figure 2. *Anopheles gambiae s.l.* sibling species composition following the study sites.

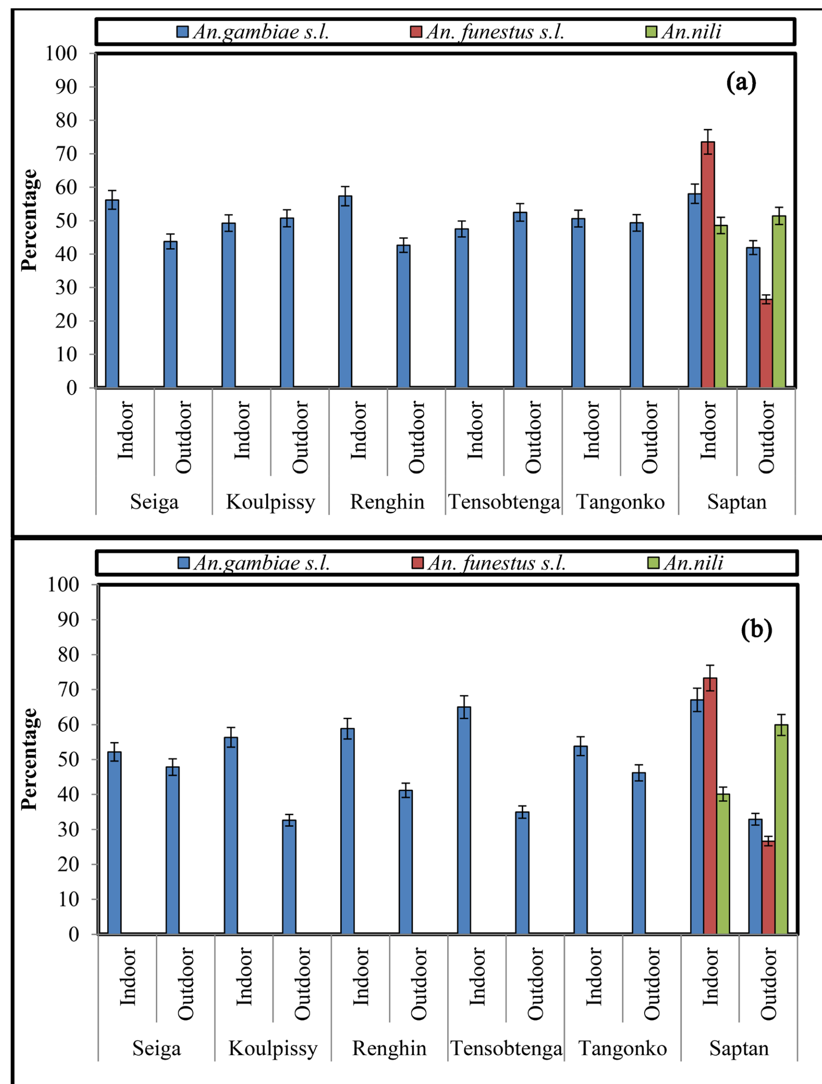

Figure 3. Feeding behaviors of *Anopheles* species in the study sites, (a) August 2014 and (b) September 2015.

also found endophagic regardless the site and the period. *Anopheles nili,* showed exophagic as well as endophagic behaviors in Saptan in August 2014 (**Figure 3(a)**) but exhibited an exophagic trend in September 2015 (**Figure 3(b)**).

The residual fauna collected by PSC showed that *An. gambiae s.l.* were relatively the main vector in all sites except in Saptan where few females of *An. funestus s.l.* were collected in August 2014 (**Figure 4**). These vectors exhibited an endophily behavior with high number of specimens resting indoor. No *An. nili* was collected in both collection periods. Therefore, this vector is potentially exophilic. More mosquitoes were collected in September 2015 than in August 2014 regardless the site (p-*value* < 0.05).

Parity rate of *Anopheles gambiae sensu lato*

Figure 5 shows the parity rates in all sites in both collection periods. In Seiga, Koulpissy, Renghin, Tensobtenga and Tangonko, females caught indoor as well as outdoor in August 2014 were majorly parous (which had already laid eggs at least once) (**Figure 5(a)**). In these sites, the mean parity rate was 68.44% (CI: 56.4 - 86.52) for *An. gambiae s.l.* However, in Saptan, more parous *An. gambiae s.l.* females were caught indoor (parity rate 80%; CI: 75.22-84.30) than outdoor (46.67%; CI: 40.2 - 52.30) (**Figure 5(a)**). In September 2015, the vectors sampled in Seiga, Tensobtenga and Tangonko were in majority parous in indoors as well as outdoors (**Figure 5(b)**). During this same collection period, the parity rates of vectors collected in Koulpissy and Renghin have decreased compared to August 2014. However, in Koulpissy the parity rate was higher indoor than outdoor. In Saptan, females collected indoor as well as outdoor were majorly parous (**Figure 5(b)**), but the parity rate was higher outdoor than indoor. The parity rates were not different between indoor and outdoor in all sites by collection period (p-*value* = 0.1027). However, a significant different was noted in parity rates

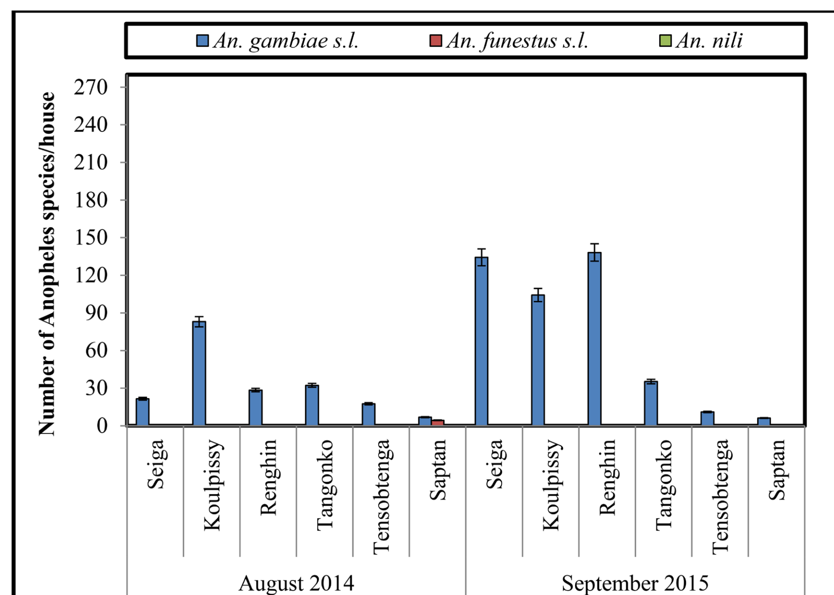

Figure 4. Mean number of mosquitoes collected by Pyrethrum Spray Catch (PSC) throughout the study sites.

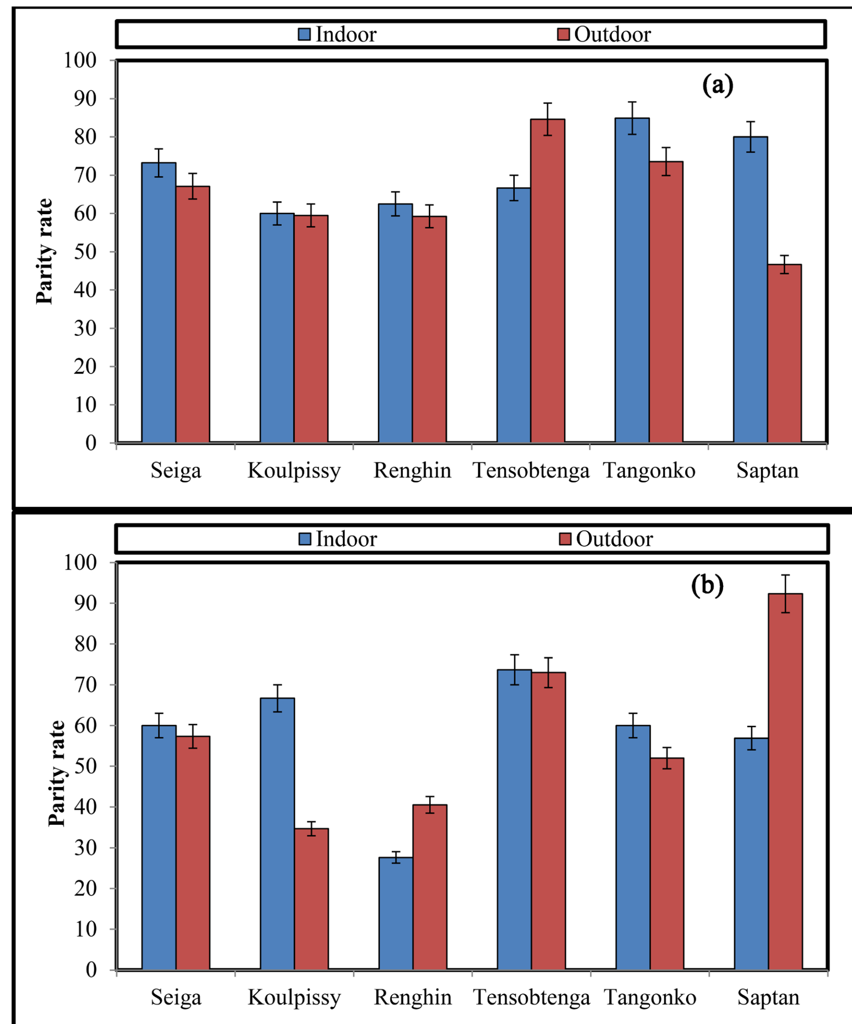

Figure 5. Parity rates of *Anopheles gambiae s.l.* collected in the study sites, August 2014 (a) and September 2015 (b).

between collection period (*p-value* < 0.05).

Blood meal source of *Anopheles gambiae* sensu lato collected by Pyrethrum Spray Catch

In all the sites, *An. gambiae s.l.* vectors showed anthropophagic behavior except in Koulpissy and in Tangonko where mixed human and animal blood meal was found in high proportion up to 80% in August 2014 (Figure 6). In September 2015, low proportion and or no mixed blood meal was found in *An. gambiae s.l.*

Prevalence of *Wuchereria bancrofti* and *Plasmodium falciparum* infection in *Anopheles* populations

Table 5 shows the prevalence of *W. bancrofti* and *P. falciparum* in *An. gambiae s.l.* populations in Seiga, Koulpissy, Renghin, Tensobtenga and Tangonko in both collection periods. *Wuchereria bancrofti* infection was found only in Koulpissy (Fada health district) in August 2014 with an infection rate of 4.5% (CI: [1.17 - 11.4]) and in Tensobtenga (Ouargaye health district) in September 2015

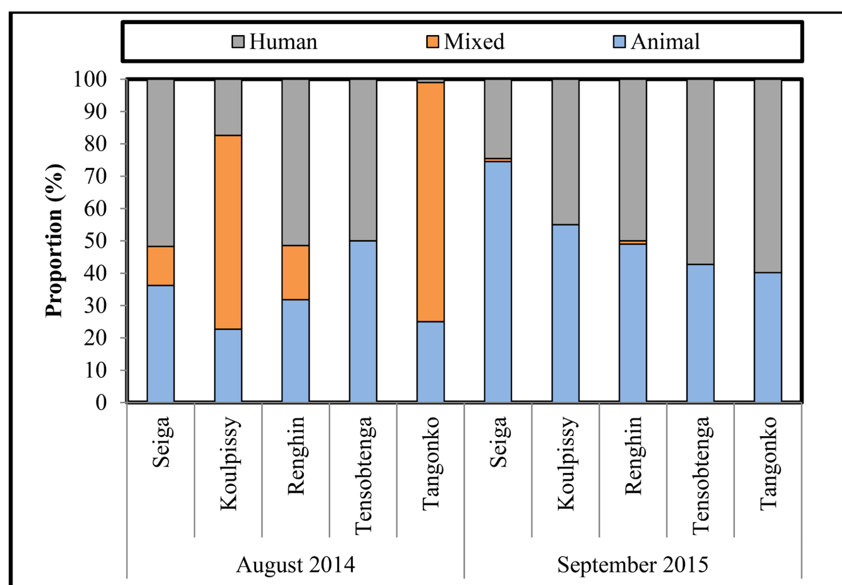

Figure 6. *Anopheles gambiae s.l.* blood meal source of in the study sites.

Table 5. Infections rates of *Wucherira bancrofti* and *Plasmodium falciparum* of *An. gambiae s.l.* populations from Seiga, Koulpissy, Renghin, Tensobtenga and Tangonko assessed by conventional PCR.

Site	*Anopheles gambiae* s.l.						
	Date of collection	Nb pools tested	Nb mosquitoes tested	Nb positive pool *W. bancrofti*	*W. bancrofti* infection rates % [CI]	Nb positive pool *P. falciparum*	*P. falciparum* infection rates % [CI]
Seiga	Aug 2014	13	100	0	0	2	2.1 [0.2 - 7.3]
	Sept-2015	10	100	0	0	0	0
Koulpissy	Aug 2014	15	100	4	4.5 [1.17 - 11.4]	4	4.5 [1.17 - 11.4]
	Sept-2015	10	100	0	0	2	2 [2.2 - 7.54]
Renghin	Aug 2014	14	100	0	0	2	2.1 [0.2 - 7.3]
	Sept-2015	10	100	0	0	1	1.04 [0.03 - 5.2]
Tensobtenga	Aug 2014	13	100	0	0	5	6.5 [1.9 - 15]
	Sept-2015	10	100	1	1.04 [0.03 - 5.2]	1	1.04 [0.03 - 5.2]
Tangonko	Aug 2014	13	100	0	0	1	1 [0.03 - 5.2]
	Sept-2015	10	100	0	0	1	1.04 [0.03 - 5.2]

Nb: Number.

with infection rate of 1.04% (CI: [0.03 - 5.2]). *Plasmodium falciparum* infection was found in all the sites in both collection periods, except in Seiga in September 2015. The highest infection rates of *P. falciparum* were observed in Tensobtenga (6.5%, CI: [1.9 - 15]) and Koulpissy (4.5%, CI: [1.17 - 11.4]) in August 2014. The prevalence of *P. falciparum* decreased in September 2015 compared to August 2014.

In addition, the pools of Saptan constituted by *An. gambiale s.l.*, *An. funestus s.l.* and *An. nili* sampled both in August 2014 and in September 2015 were tested

first using LAMP technique and secondly with conventional PCR for *W. bancrofti* detection. The results of LAMP technique showed that both *An. funestus s.l.* and *An. nili* were infected by *W. bancrofti* respectively in August 2014 and September 2015 with related infection rates of 0.5% [0.05 - 2.9] and 0.06% [0.002 - 0.3] respectively (Table 6). Therefore, these results were checked by conventional PCR that confirmed only *An. nili* as effectively positive to *W. bancrofti* reaching an infection rate of 0.8% [0.3 - 1.4] in September 2015. The pool positive of *An. funestus s.l.* failed to be confirmed by conventional PCR. No *An. gambiae s.l.* pool was tested positive to *W. bancrofti* neither by LAMP PCR none by conventional PCRs in Saptan. However, in Saptan, the infection status of *P. falciparum* was confirmed only by conventional PCR within *An. gambiale s.l.*, *An. funestus s.l.* and *An. nili* populations. The results showed that *An. gambiae s.l.* was higher infected with infection rates of 0.9% (CI: [0.02 - 4]) and 2% (CI: [0.38 - 5.7]) in August 2014 and September 2015 respectively (Table 6). Only one pool of *An. nili* confirmed by conventional PCR as effectively positive to *P. falciparum* reaching an infection rate of 0.8% (CI: [0.3 - 1.4]) in September 2015. No *An. funestus s.l.* pool was tested positive to *P. falciparum* by conventional PCRs in Saptan.

A co-infection of *W. bancrofti* / *P. falciparum* was found within *An. gambiae s.l.* populations in Koulpissy (Fada health district) in August 2014 with an infection rate of 2.25% (CI: [0.58 - 5.7]) and in Tensobtenga with an infection rate of 1.04% (CI: [0.03 - 5.2]) in September 2015 (Table 7). In Saptan, only *An. nili* was found to be co-infected with an infection rate of 0.8% (CI: [0.3 - 1.4]).

Anopheles gambiae s.l. sibling species identification revealed that all positive

Table 6. Infections rates of *Wuchereria* bancrofti and *Plasmodium falciparum* of *An. gambiae s.l.*, *An. funestus s.l.* and *An. nili* by populations compared between LAMP and conventional PCR at Saptan in the Diébougou health district.

Type of PCR	August 2014						September 2015					
	Nb specimens	Nb pools tested	*W. bancrofti* Nb positive pools	*W. bancrofti* infection rates %[CI]	*P. falciparum* Nb positive pools	*P. falciparum* infection rates %[CI]	Nb specimens	Nb pools tested	*W. bancrofti* Nb positive pools	*W. bancrofti* infection rates %[CI]	*P. falciparum* Nb positive pools	*P. falciparum* infection rates %[CI]
An. funestus												
LAMP PCR	385	6	1	0.5 [0.05 - 2.9]	-	-	33	2	0	0	-	-
Conventional PCR	385	9	0	0	0	0	33	2	0	0	0	0
An. nili												
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	0	0	0	1423	65	1	0.06 [0.002 - 0.3]	1	0.06 [0.002 - 0.3]
An. gambiae s.l.												
LAMP PCR	186	6	0	0	-	-	206	9	0	0	-	-
Conventional PCR	186	6	0	0	1	0.9 [0.02 - 4]	206	9	0	0	3	2 [0.38 - 5.7]

Table 7. Co-infections rates of *Wucherira bancrofti* and *Plasmodium falciparum* of *An. gambiae s.l.* populations from the five study sites assessed by conventional PCR.

Site	*Anopheles gambiae s.l.*				
	Date of collection	Nb pools tested	Nb mosquitoes tested	Nb positive pool *W. bancrofti*/*P. falciparum*	Co-infection rates %[CI]
Seiga	August 2014	13	100	0	0
	September 2015	10	100	0	0
Koulpissy	August 2014	15	100	2	2.25 [0.58 - 5.7]
	September 2015	10	100	0	0
Renghin	August 2014	14	100	0	0
	September 2015	10	100	0	0
Tensobtenga	August 2014	13	100	0	0
	September 2015	10	100	1	1.04 [0.03 - 5.2]
Tangonko	August 2014	13	100	0	0
	September 2015	10	100	0	0

samples for *W. bancrofti* infection and *W. bancrofti*/*P. falciparum* co-infection were *An. coluzzii*. However, the positive samples for *P. falciparum* were mainly composed of *An. gambiae* followed by *An. coluzzii* in all sites in both collection periods.

4. Discussion

Integrated management of LF and malaria in the hotspot health districts in Burkina Faso is poorly documented. To control LF and malaria, vector management integrated is currently implementing. However, to set up a better control strategy, it is important to obtain local information on the prevalence of these diseases, the vector diversity and behavior. The main objective of the present study was to collect baseline data towards developing and implementing an effective strategy integrating vector management to tackle both diseases simultaneously. It has investigated the (co-)transmission of malaria and LF and their vector behavior in endemic areas in Burkina Faso.

Malaria was found in almost all the sites while LF was recorded only two sites whiten the study periods in human populations. Malaria prevalence was higher than LF. A low co-infection rate with *W. bancrofti* and *P. falciparum* was noted. Our results are consistent with those of Kima *et al.*, [9] and Gonçalves *et al.*, [32] who reported *W. bancrofti* and *P. falciparum* infections in human populations in the same health districts. The *Plasmodium falciparum* infection rate decreased in September 2015 in all sites compared to August 2014. This may partly due according to the MDA with ivermectin/albendazol [33] [34] and the use of LLINs which reduces the human-vector contact [35].

Anopheles gambiae complex was the main vector collected by HLC and PSC in both collection periods in all health districts. This complex was mainly com-

posed of *An. coluzzii* followed by *An. gambiae* and *An. arabiensis.* In Diébougou health district precisely in Saptan, *An. funestus s.l.* and *An. nili* was collected in high proportion in addition to *An. gambiae s.l.* These observations are consistent with previous studied which have reported the presence of the same vectors in the study areas [3] [21]. In addition, our results show that *An. gambiae s.l.*, *An. funestus s.l.* and *An. nili* are sympatric in Saptan as reported by Soma *et al.*, [36] in Diébougou health district.

Vector densities were higher in September 2015 compared to August 2014 and their distributions differ between sites. This variability may be explained by climate variations since our study sites are in different climatic regions. Indeed, it is known environmental conditions including rainfalls, vegetation, elevation, and anthropism affect the vector distribution [5] [37] [38]. The presence of three vector species (*An. gambiae s.l.*, *An. funestus s.l.* and *An. nili*) in Diébougou health district (Saptan) in contrast to the other sites reveals favorable conditions for the development of these *Anopheles* species.

According to Manguin *et al.*, [4] and Ashton *et al.*, [35], *W. bancrofti* and *P. falciparum* transmission in a vector population depends on the ability of mosquitoes to ingest and support the development of parasites. Thus, the higher infection rates for *P. falciparum* than those of *W. bancrofti* observed in *Anopheles* species can be explained by the latent period of *W. bancrofti* in the vector which is usually long in relation to the vector life expectancy [4]. In contrast, the extrinsic cycle of malaria parasites lasts 9 - 10 days but can sometimes last for only five days [39]. Consequently, more filarial-infected mosquitoes than malaria-infected ones are likely to die before the parasites mature to the infective stage. A support for this is seen in the previous work in an endemic area along the Kenyan Coast where 17 mosquitoes harboured both *P. falciparum* sporozoites and immature stages of *W. bancrofti* while only two had sporozoites and infective larvae [40]. *Anopheles coluzzii*, *An. gambiae* and *An. nili* which were found to carry both parasite genes are considered as the potential vectors in the different study sites. Thus, persistence of LF and the malaria endemicity in the study sites may be correlated with the presence of *An. gambiae*, *An. coluzzii* and *An. nili* found in high proportion with old age vectors that are in majority parous females.

The *Anopheles* species sampled in our study were active during nighttime and fed indoors as well as outdoors. Also, most vectors were exclusively anthropophilic or zoophilic. These different behaviors of *An. gambiae s.l.*, *An. funestus s.l.* and *An. nili* in *W. bancrofti* and *P. falciparum* transmission have been observed and described by several authors [5] [37] [41] [42] in West Africa. Since many mosquitoes were found biting and resting inside the houses, the indoor residual spraying and the use of LLINs could be effective to control the diseases in these study sites. However, suitable insecticide must be employed, knowing the development of resistance by *An. gambiae s.l.* and *An. funestus s.l.* to several classes of insecticides in these areas documented by several authors [36] [43].

Moreover, the control exophilic and exophagic population requires integrated vector control strategies including the environmental management and genetic approaches.

5. Conclusion

The present study has monitored the prevalence of LF and malaria co-infection in both human and vector populations in endemic areas of Burkina Faso. In addition, the vector feeding, and resting behaviors were investigated. Results indicate residual transmission of *W. bancrofti* and *P. falciparum.* However, low prevalence of co-infection in both mosquito and human populations was recorded. The main vectors found were *An. coluzzii*, *An. gambiae* and *An. nili*. This study has provided baseline information which will be useful to the development and implementation of a better integrated strategy to control both diseases.

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Authors' Contributions

SC, SPS, and RKD planned the experiments. SC, SPS and ASH conducted the experiments. SC, SPS and ASH analyzed the data. SC, SPS wrote the manuscript with inputs from ASH, ASN, IS, BR, LK, CB, RWB, GAO and RKD. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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