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Anopheline occurrence and the risk of urban malaria in the city of Ouagadougou, Burkina Faso

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Abstract
Urban populations are growing rapidly, particularly in West Africa and this has a major implication for the risk and control of malaria. In the city of Ouagadougou, knowledges about malaria transmission and its vector ecology is not sufficient for appropriate vector control measures. Three districts of the city of Ouagadougou were selected using a geographical approach that took into account the city heterogeneity. This approach was based on the analysis of the level of urbanization leading to urban and peri-urban sites. Timely adult mosquitoes and larva collection were done. Collected mosquitoes were identified and sorted by physiological status and genus. Blood meals sources and *Plasmodium* circumsprozoite Protein were assessed for anopheline mosquitoes. In total, 10158 mosquitoes including 7555 adults and 2603 larvae were caught. The population was composed of 71.9% of *Culex* spp., 22.2% of *Aedes* spp., 5.7% of *Anopheles* spp. and 0.2% of *Mansonia* spp. The majority of *Anopheles* specimens were caught in peri-urban area (66.7% for Yamtenga and 17.5% for Zongo); while only few anophelines were found in urban setting (Dapoya, 15.8%). Puddles and metal dishes were found to be the productive breeding sites for anopheline mosquitoes. The most important part of blood meal source were human (66.7%) with some being mixed blood meal. A large amount of undetermined sources (21.1%) were found in peri-urban area leading to some animals sources not recognized in our antigen set. *Plasmodium* infection rate was 08.05% with the infectious mosquitoes found to be more prevalent in peri-urban setting (91.7%) compared to urban area (8.3%). These data are crucial to assess the risk of malaria transmission in the city of Ouagadougou.

Keywords: Urbanization, anopheline, malaria, blood meal, *Plasmodium*, Burkina Faso

1. Introduction
The urbanization process is a major challenge in many African countries today. Rapid and unplanned population growth is at the root of many environmental problems, including water scarcity, pollution, housing, poor sanitation and poor solid waste management in households. This physiognomy of urban centers should reduce the risk of malaria transmission due to the lack of suitable breeding sites for vector development. However, urban malaria is a reality [1] and would be due among other things to the multiplication of suitable habitats induced by the presence of non-formal and disordered hydro-agricultural installations coexisting permanently with urbanization [2, 3].

Once saved by land degradation phenomenon because of the abundance of arable land, the regions of Burkina Faso are now subject to an environmental crisis. Internal migration of populations of degraded areas to those in favor of agro-pastoral activities contribute to accelerating the degradation of these lands. Human alteration of habitats can play an important part in changing the ecological balance within which mosquitoes breed, develop and transmit diseases [4, 5].

The breeding ecology of the species vary considerably in different localities, thus significantly influencing mosquito-borne disease transmission in such areas [6, 7]. Man-made environmental modifications have been suspected to be at the origin of the diversification and radiation of the major human malaria vector [8], through the creation of new ecological niches in marginal habitats [6]. The spread of new agricultural practices combined with the creation of water reserves has been hypothesized to be the original environmental change prompting ecological niche specialization within *Anopheles gambiae* through divergent selection in these new habitats [9].

Urban human populations are growing rapidly, particularly in West Africa and this has a major implication for the risk and control of malaria [10]. Malaria transmission has been documented in urban settings with measurable entomological inoculation rates (EIR) in some areas [3]. However, in the city of Ouagadougou, knowledges about malaria transmission and its vector ecology in this capital appears to be old and may not be sufficient to implement appropriate vector control measures. Indeed, early studies done in the city of Ouagadougou found that malaria vectors distribution is linked to rainy season, gardening and urban agriculture[1, 2]. Then, malaria transmission appear to be heterogeneous with the highest prevalence found near the urban dams and the peripheral areas [11]. It has also been showed by these studies that one of the major sources of malaria cases would be migration between rural and urban areas [2, 12]. This immigration could created some precarious conditions in peripheral setting with poor housing system and poor sanitation leading to a high morbidity and mortality in these areas. A decade after the latest study, with the growing anthropogenic influence on the natural environment, such as changes in land use and deterioration of ecosystems with the plastic hazard, urban malaria couple with emerging infectious diseases like dengue remain the main causes of hospitalization in Ouagadougou [13]. So, understanding the ecology of malaria vectors this city and quantifying transmission is essential for the adequacy of the control strategies proposed by the malaria control programs.

2. Materials and Methods

2.1 Study area

The study was done in Ouagadougou (12° 21′ 58″ N and 1° 31′ 05″ W) the capital city of Burkina Faso. Located in the center of the country, the city harbored approximately 2 637 303 inhabitants in 2016 [14].

The city is subject to a tropical savannah climate, including two mains seasons: the dry season and the rainy season. The annual rainfall is 750 to 900 mm. The rainy season is between June and October, and the dry season consists of two parts: a cold and dry season is between November and January, and the hot and dry season is between February and May. Ouagadougou is ongoing in urbanization with variety of ecological situations.

The study was conducted in three districts: Dapoya, Yamtenga, Zongo. These districts were selected using a geographical approach that took into account the city heterogeneity. This approach was based on the analysis on the level of urbanization. Indeed, Dapoya is an old urbanized and densely populated district, characterized by close-together houses with numerous households. Yamtenga and Zongo are peripheral connecting to the town eastern and western part respectively. Yamtenga is characterized by condensed precarious houses built with local materials. However in Zongo houses are disperse.

2.2 Sampling design

Each site was visited three times according to the seasons; representing a visit to hot dry season, a visit during the rainy season and another in cold dry season. During these visits, two consecutive days of adult mosquitoes collection were done indoor and outdoor. Larvae sampling, were performed in breeding sites composed of different types of water bodies (ponds, puddles, footprints, used tires, dishes, etc.) found in the concessions.

2.3 Mosquito adult collections

In each study site, both traditional and modern houses were sampled for mosquito collection. During two consecutive nights, CDC light traps were used to catch adult's mosquitoes in each study site between 8:00 pm and 6:00 am. Indoor resting females were also caught by an aspirator catch in the CDC-sampling houses around 5:00 before the CDC installation and in the morning (6:00 to 7:00 am) after the CDC sampling. Outdoor resting mosquitoes were also sampled by aspirator by visiting some microhabitants outside and near the sampled houses. All mosquitoes were transported in laboratory and knocked-down in freezer and conserved at -20 °C for identification.

2.4 Larva sampling and rearing

In each study area, all water collections were numbered, categorised and prospected for the presence of mosquitoes larvae. Mosquito larvae were found in a great variety of habitats. So, different sampling techniques were needed to ascertain the presence or absence of immature mosquitoes and collect them. For example, surveys in the jars, used tires and other containers that may contain mosquito larvae were performed. Approximately 500 ml of water containing larvae were taken by breeding sites prospected and brought back to the laboratory for screening and breeding of mosquito larvae. For dams the dipper method were used for sampling. Different habitat types were surveyed to enroll a various mosquito. On the sampling site, predators were eliminated prior to conditioning and transportation of larvae to the laboratory. Larvae were reared in forage water in insectary until adult and killed in freezer for identification.

2.5 Laboratory processing of mosquitoes

Collected adult mosquitoes including both CDC, aspirator catches and insectary-emerged adults were identified using standard morphological identification keys [15]. The anophelines blood meal sources were analyzed by ELISA using antibodies for human and most frequently found animals in the study sites [16]. The infection rate was also analyzed via the circumsporozoite (CS) proteins of *Plasmodium* detection [17].

2.6 Data analysis

The districts and concessions were characterized according to the type of house, the use of insecticide and impregnated mosquito net, the presence of animals, the number of children and inhabitants in the concession. Moreover, for the analysis, mosquitoes frequencies were calculated by concession, district, collection type, and stage of development. Proportions were compared by the Chi-Square test of Pearson or Fischer exact test. The significance rate was set at 0.05.

3. Results

3.1 Houses and breeding sites characterization

Houses characteristics in the surveys sites are summarized in Table 1. A total of 167 houses were sampled during the study. In Zongo and Yamtenga districts, more mixes houses were sampled (75.9%; 68.3%) and fewer modern houses (24.1%; 18.3%) respectively. However for Dapoya houses were mainly modern (59.2%). Particularly for Yamtenga, some traditional houses were found (13.3%). ITN ownership and

insecticide use and the presence of fire were quite the same in the three districts. However, Domestic animal were commonly found in Dapoya visited concessions (71.4%, p=0.005) compared to Zongo (43.1%) and Yamtenga (45.5%). When looking at the population inside houses, we notice that the mean number of person per house is higher at Dapoya (8.40 ±4.6, p-value = 0.003) than Zongo (6.2 ±2.9) and Yamtenga (5.9±2.9).

Overall 88 containers positive for mosquito larvae were sampled. These breeding sites were mainly mud pots, 31 (35.23%) followed by puddles, 19 (21.59%) plastic drums, 17 (19.32%), metal dish/Box, 12 (13.64) and tires, 9 (10.23%). The frequency of container in each site is show in Table 1. Whereas in Dapoya and Zongo, we found few puddles, this type of breeding site was the mostly sampled in Yamtenga (89.5%). More, artificial container like tires, plastic drums/ buckets or metal box were not productive for mosquitoes larva in Yamtenga. We also noticed that a large part of the sampled containers (60.22%) contain organics materials with most common artificial container being metal dish/Box and plastic drums.

Table 1: Households and breeding sites characteristics in the three districts

		Dapoya	**Zongo**	**Yamtenga**
Houses Characteristics	Total Number of Households	49	58	60
	Type of Households (%)			
	Modern	59.2	24.1	18.3
	Mixes	40.8	75.9	68.3
	Traditional	00.0	0.0	13.3
	ITN ownership (%)	85.7	81.0	80.0
	Insecticide used (%)	69.4	56.9	56.7
	Presence of fire (%)	53.1	39.7	58.3
	Presence of domestics animals (%)	71.4	43.1	45.0
	Number of person/house (±SD)	8.4 ±4.6	62 ±2.9	5.9 ±2.9
Types of breeding sites	Total Number of breeding sites	24	45	19
	Metal dish/Box (%)	16.7	17.8	0.0
	Mud pots (%)	37.5	44.4	10.5
	Puddles (%)	0.0	4.4	89.5
	Tires (%)	20.8	8.9	0.0
	Plastic drums/ buckets (%)	25.0	24.4	0.0

SD=Standard Deviation

3.2 Anopheline Mosquitoes diversity and occurrence

In total, 10158 mosquitoes including 7555 adults and 2603 larvae were caught in the three districts. The population was composed of 71.9% of *Culex* spp., 22.2% of *Aedes* spp., 5.7% of *Anopheles* spp. and 0.2% of *Mansonia* spp.). This large proportion of *Culex* mosquitoes mainly come from adult collection. The specific composition of Mosquitoes population according to the stage of development is given in Table 2. Mostly, we found a large percentage of *Aedes* mosquitoes in larvae collection (80.68%); while only 2.0% of mosquitoes collected at adult stage was in this genus.

Table 2: Occurrence of genus of mosquitoes collected at larvae and adult stage

	Adult N(%)	**Larva N(%)**	**All N(%)**
Aedes	151(2.00)	2100(80.68)	2251(22.16)
Anopheles	393(5.20)	189(7.26)	582(5.73)
Culex	6993(92.56)	311(11.95)	7304(71.90)
Mansonia	18(0.24)	3(0.12)	21(0.21)

The majority of *Anopheles* specimens were caught in the district of Yamtenga (388/582, 66.7% followed by Zongo (102/582, 17.5%) and Dapoya (92/582, 15.8%). More than half of anopheline sample come from indoor collection 51.5% (300/582) (Figure 1A) with 29.0% provide by CDC traps (Figure 1B) and 22.5% corresponding to indoor resting mosquitoes caught by aspirator in the daytime. When looking at larva, anopheline larva were found mostly in puddles, 94.7% (179/189 and metal dish/Box 5.3% (10/189). All anophelines breeding sites contain organics materials. In addition, of the anophelines collected, *Anopheles gambiae s.l* was the most common specie (99.0%, n = 576). We also found *Anopheles funestus* (0.8%, n=05) and 01 *Anopheles pharoensis*. While *Anopheles gambiae s.l.* was found in all seasons, the two others species were collected in the rainy season only. Also, Yamtenga harbored all the three anophelines while in Dapoya, only *Anopheles gambiae s.l.* was found.

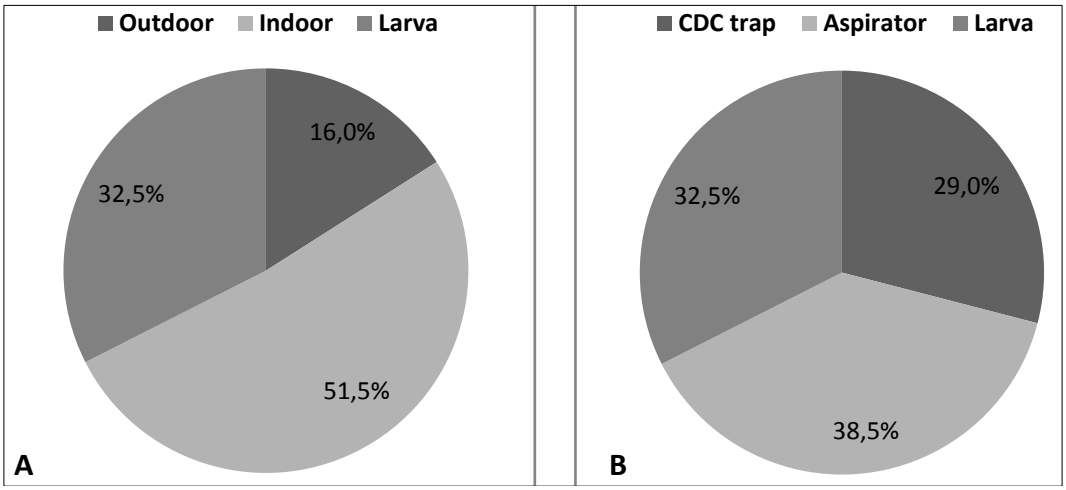

Fig 1: Occurrence of anopheline collected: **A**: according the resting behavior; **B**: According to type of collection

3.3 *Anopheles* blood meal sources and infection rate

The abdominal status of adult *Anopheles* is given in Table 3. An important part of mosquitoes with blood in the gut was observed in all sites, giving 33.7% (n=31), 38.7% (n=86) and 31.6% (n=25) respectively for Dapoya, Yamtenga and Zongo both inside and outside houses. Only few number of gravid mosquito was found in this study.

Table 3: Physiological status of adult anopheline mosquitoes according to the location

	Unfed % (n)	Partialy fed % (n)	Fed % (n)	Gravid % (n)	Males % (n)
Dapoya	41.3 (38)	9.8 (09)	23.9 (22)	1.1 (01)	23.9 (22)
Yamtenga	41.9 (93)	11.7 (26)	27.0 (60)	0.5 (01)	18.9 (42)
Zongo	44.3(35)	17.7 (14)	13.9 (11)	0% (0)	24.1% (19)
Outdoor	20.8 (20)	11.5 (11)	15.6 (15)	0% (0)	52.1 (50)
Indoor	49.1 (146)	12.8 (38)	26.3 (78)	0.7(02)	11.1 (33)

n= number of mosquitoes

When looking at the blood meal sources in the female *Anopheles gambiae s.l* mosquitoes gut (n=132), we found that the most important part of blood meal source were human (66.7%, p=2.147e-15) some being in mixed blood meal. A large proportion were undetermined (21.1%) using the set of antigen (human, Bovin). According the mosquitoes collection site, Yamtenga was found to harbored more undetermined and mixed blood meal sources than the others districts (p=0.03) (Table 4).

For infection via *Plasmodium* Circumsporozoite Protein (CSP) analysis, we found that 08.05% (24/297) were CSP positive. The main part of the positive mosquitoes were sampled at Yamtenga (91.7%) followed by Dapoya (8.3%). No CSP positive anopheline was found at Zongo. The overview of the CSP analysis is given in Table 4.

Table 4: Mosquitoes blood meal sources and infection status in different location

	Dapoya	Yamtenga	Zongo	Total number
Blood meal sources (%)				
Bovin	25.0	50.0	25.0	16
Human	14.9	71.6	13.5	74
Mixte	7.1	92.9	0.0	14
Indetermined	32.1	42.9	25.0	28
***Plasmodium* infection rate (%)**				
CSP negative	22.6	56.2	21.2	274
CSP positive	8.3	91.7	0.0	24

4. Discussion

This study shows that malaria vectors' reproduce and bite in the urban and peri-urban neighborhoods of the city of Ouagadougou. Similarly, the presence of the parasite in mosquitoes demonstrate that malaria transmission is effective in households. When looking at anopheline, the data indicate that three known malaria vectors, *Anopheles gambiae s.l.*, *Anopheles funestus and Anopheles pharoensis* exist in the capital city of Ouagadougou, the central part of Burkina Faso. The former was found on both sites (urban and peri-urban) and during surveys (dry and wet seasons), while the two latter's were only detected in low numbers in the rainy season. This picture of *Anopheles* mosquitoes diversity in Ouagadougou has been previously reported by Fournet *et al*. [2]. These authors found by using CDC trap combined with larva collection these three species and *Anopheles rufipes*. The presence of anopheline vectors during the dry season pointed out the problematic of urban malaria as reported by some authors [18, 19]. This persistence of vector beyond the rainy season confirmed the availability of permanent breeding sites due to anthropogenic activities.

Anopheline mosquitoes were more frequent in Yamtenga compared to the others districts in the city. This finding could be link to the difference in houses structure. While Yamtenga harbored more mixes and traditional houses, district like Dapoya presented modern houses. Several studies have showed that a poor quality of houses structure increased anophelines incidence [20-22]. However, it should be noted that this probable association observed between the type of house and the proportion of *Anopheles* is not a causal relationship. Indeed, some authors believe that it is mainly the direct and indirect costs of malaria that would contribute to poverty within a household [23], especially in low-income countries without social security systems. The presence of malaria vectors in urban areas in Africa is relatively well documented and local transmission has been reported in some cities [24]. This situation is linked to inadequate management of larval breeding sites and thus promoting human-vector contact. This result also, confirmed the heterogeneity of malaria transmission as indicated by some authors [2, 11]. These authors showed that malaria transmission became highest from central to peripheral area where landscapes is under modification due to immigration, and urban agriculture.

In the city of Ouagadougou, anophelines breeding sites were mainly, puddles and dishes with some being permanent. This indicated that in urban area, malaria transmission is maintained by natural breeding sites as well as artificial containers. The urbanization process, with the increased of man-made habitats is known to contribute to the large part of these breeding sites. Some study have previously reported this result [2, 3, 7] indicating that eradicating puddles, and destroying breeding sites around urban farm sites can significantly reduce anophelines mosquitoes in the cities [3].

More, the stability of some breeding sites like permanent sites contribute to maintaining and the extent of the population of *Anopheles* mosquitoes in the study areas. It has been reported that underground water could support puddles and created a permanent breeding sites [25] and lead to a sustainable malaria transmission in urban setting. The landscape modification changed the pattern of malaria transmission, while control measures are, so far, focused preferentially towards rural populations. This environmental change must be monitored and controlled by a strategic plan to prevent vector proliferation [2].

In this study, some mosquitoes were collected in small artificial containers, a habitat not commonly associated with *Anopheles* mosquitoes. This result should be taken with caution when we look at increasing of the number of these artificial containers due to a defaulting management system of the solid wastes. Since anophelines are able to survived in polluted water as reported by several authors [3, 26], malaria control should integrated this in any management plan. So, urban vector control strategic should look at how to limit artificial containers like metal and plastic dishes proliferation in urban setting.

These data also mentioned that anophelines in the city of Ouagadougou fed mainly on human sources with some of them being infectious with regard to the prevalence of Circumsporozoite protein found after the ELISA test. The infected anophelines were mainly found in Yamtenga compared to the other districts, suggesting that people living in peripheral areas in Ouagadougou are more exposed to *Anopheles* mosquitoes infection than urban inhabitants [27]. Indeed, in the peripheral areas, animals can go fishing outside the concessions and most often stay away from houses. So, the blood meal source available is mainly human blood. However, in the city center, the animals are kept and raised within the concessions creating another source blood meal, and then reducing the contact mosquitoes and human populations. The infection rate of 08.05% found in this study was higher than the one (06.7%) reported by Fournet *et al.* [2] in their study in 2006. This difference could be due to the fact that in the present study, central and peri-urban mosquitoes were infected. However, in the early study, only peri-rural mosquitoes were sporozoite positive suggesting a link with rural imported malaria. With infective mosquitoes being observed in central areas, the contact human-vector could be more frequent regarding the density of the population in this area.

In contrast, when assuming that malaria may be a zoonotic disease as some studies mentioned [28, 29], and with regard to the prevalence of mixes blood meal and /or undermined ones, further investigation should be done to underline the exact contribution of animals in the malaria reservoirs in urban areas.

5. Conclusion

This study reported *Anopheles gambiae s.l.*, *Anopheles funestus*, *Anopheles pharoensis* in the city of Ouagadougou. These anophelines are highly prevalent in the peri-urban areas with puddles as main breeding sites. They fed mostly on human blood with some of them being sporozoites positives. An important amount of undetermined blood meal sources was found leading to a possibility of animals reservoirs for malaria transmission. These results provided some useful informations on anophelines species, prevalence, breeding sites, blood meal sources and sporozoites rate in the city of Ouagadougou. These data are crucial to assess the risk of malaria transmission in the city of Ouagadougou in order to provide a better management strategic for urban malaria control.

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