Experimental study: the relationship between *Plasmodium falciparum* gametocyte carriage and mosquitoes infectiousness in two sympatric ethnic groups in Burkina Faso

Aims: The lower susceptibility of the Fulani to malaria compared to Mossi was previously described in Burkina Faso in West Africa. The mature gametocyte stage of *Plasmodium falciparum* is known to be the only stage capable of infecting the mosquito though this process is disrupted by the action of immunity and other factors as well. Our study aims to assess the ability of two sympatric ethnic groups known to have different susceptibility to *Plasmodium falciparum* malaria, to infect mosquitoes through an experimental membrane feeding assay.
Methodology: Study participants were gametocyte carriers aged from 2 to 12 years recruited in the village of Barkoundouba where Fulani and Mossi are living in sympatric. A venous blood was obtained from each participant for direct membrane feeding assay of insectary reared mosquitoes. Blood fed mosquitoes were stored for 7 days with sugar water as the only food source, then dissected for the microscopic detection for oocysts.
Results: A total of 1050 mosquitoes were used for the experimental infections. Eight day after feeding, a total of 897 mosquitoes were dissected, 275 from the Fulani and 622 from the Mossi group. With an average of 43 stomachs examined by experimentation, the mosquito infestation rate was 10.5 % in Fulani and 13.2% in Mossi group (p=0.569). The fed mosquito rate was 95 % and 95.6 % in Fulani and Mossi ethnic group respectively (p=0.241). The rate of survival mosquitoes after the feeding was 96.5 % and 87.5 % in Fulani and Mossi ethnic group respectively (p=0.088). The proportion of dissected mosquitoes was 100 % and 99.2 % in Fulani and Mossi ethnic group respectively (p=0.138) leading to an average oocystic load of 249 in Fulani and 21 in Mossi group. The success rate of DMFA in both groups combined was 57.14%. Indeed, this rate was 33.33% and 66.67% in Fulani and Mossi group respectively.
Conclusion: Our study showed that there is no significant difference found between the two ethnic group with the fed, survival, dissected and the infested mosquitoes rate. However, the average of oocystic load was higher in Fulani than the Mossi group despite the low infection in Fulani group. There is a need to explore the mechanism underlying such difference between the two ethnic groups.
Keywords: *P. falciparum* gametocytes, mosquitoes, infectivity, ethnicity, DMFA

1. INTRODUCTION

The transmission of malaria parasites begins with the presence of mature, sexual stage gametocytes in the peripheral blood of an infected individual. Once ingested by a blood feeding female Anopheles mosquito, gametocytes within a mosquito develop into sporozoites which are transmitted via the saliva of a feeding mosquito to the human bloodstream. However, this classical parasite development in the mosquito can be negatively affected at each of these steps. Then, as a consequence, not all gametocytes that are ingested by mosquitoes result in sporozoites in the salivary glands, and the transmission of malaria embodies more than just the epidemiology of gametocytes [1]. As previously described, the level of gametocytes in the blood, often referred to as gametocytemia, constituted an important factor highly associated with the probability of human-to-mosquito transmission [2, 3]. Therefore, despite of the considerable progress made in the last decade in reducing the burden of malaria by wide-scale deployment of insecticide-treated nets and efficacious artemisinin combination therapy (ACT) as first-line antimalarial treatment [4], a specific focus on malaria reducing interventions is needed [5]. The *Plasmodium* gametocytes, being the parasite blood stages responsible for human to mosquito parasite transmission [6]. The control of malaria transmission remains one of the focal point of such intervention to further move towards malaria elimination. In that context, a better understanding of the gametocyte infectiousness with regard to human immunity that could influence the success of mosquito infection will help in a good implementation of malaria reduction intervention strategies.

Different previous studies in Burkina Faso, established the role of human genetic background on *P. falciparum* parasite carrying, anti-malaria humoral immune responses, or malaria morbidity in two sympatric ethnic group, Fulani and Mossi [7, 8]. But still today little is known on their respective genetic background effect on the gametocyte infectivity to mosquito.

This present experimental study was carried out to evaluate the infectiousness by feeding laboratory-bred mosquitoes and to compare this infectiousness of *P. falciparum* gametocytes between the Fulani and Mossi, two sympatric ethnic group living in Burkina Faso.

2. MATERIALS AND METHOD

2. 1. Study area and populations

The study was carried out in Burkina Faso in Barkoundounba village, which is organized in two quarter where two sympatric ethnic groups are living separately: Barkoundouba Peulh where live only the Fulani and Barkoundouba Mossi with only the Mossi ethnic group. The two quarter are 3 km apart and situated at 35 km at northeast of Ouagadougou, the capital city of Burkina Faso (Fig 1). In the study area, malaria transmission is hyper-endemic during the rainy season, and lasts from June to October. The annual entomological inoculation rate ranged from 10 to 500 infective bites per individual. *P. falciparum* is responsible for over 90% of malaria infections [9].

A total of 461 asymptomatic children aged between 2 and 12 years of age were enrolled in this study during a cross sectional surveys. The study population included 255 Fulani and 206 Mossi.

2. 2. Study methods

2. 2.1. Participants recruitment strategy

A cross-sectional survey was conducted from September to November 2017. Every day during the study period, about thirty (30) blood smears were made from the study participant at the local dispensary for detection of asymptomatic and gametocyte-bearing subjects. Potentials participants of the study were examined by physicians for clinical signs, axillary body temperature measurement before subjected to blood collection from finger pricks for malaria diagnosis. In case of fever (temperature ≥ 37.5°C), a presumptive malaria treatment was given according to the national malaria treatment guidelines. Only participants with asymptomatic malaria infection were enrolled in the study if they were carrying gametocytes of *P. falciparum*.

Eligible subjects were invited the next day to the CNRFP insectarium for venous blood collection for carrying out mosquitoes' membrane feeding assay. Body temperature, vital signs, history of medications during the last two weeks of the patient were assessed by the study clinician prior to the blood draw.

2. 2. 2. Detection of *Plasmodium falciparum* gametocyte carriers by microscopy

The thick and thin blood smear collected were air dried and stained with 6 % Giemsa for 35 minutes and examined at the CNRFP laboratory based in Ouagadougou. Briefly, one hundred high powers field per thick smear were examined for malaria parasites assessment by two skilled microscopy specialists independently and the mean parasite density was considered as final results. A third reading was requested in case of discrepancy between the two readers (qualitatively or when the difference between the two first readers exceeded 30 %). In this case, the mean of the two closest parasite densities was used as final result. Trophozoïtes and gametocytes densities were assessed by counting against respectively 200 leukocytes and 1000 leukocytes of blood and converted into parasite density per microliter by assuming a standard count of 8000 leukocytes/µl blood. A slide was considered as negative if no asexual parasite stages were found after examination of 100 power fields.

2. 2. 3. Membrane feeding assays

In membrane feeding assays, venous blood was taken in heparin tube using syringes that were pre-warmed up to 37°C in an incubator. For whole blood membrane feeding experiments, blood samples were offered to mosquitoes immediately without sample modulation. For *Anopheles gambiae*, 2-7 day old mosquitoes were commonly used.

In the current study, the starved mosquitoes were allowed to feed for 15-20 min via an artificial membrane (Parafilm) attached to a water-jacketed glass feeder to maintain the temperature at approximately 37°C. After feeding, unfed mosquitoes were removed. Blood-fed mosquitoes were kept at a temperature range from 26 to 28°C with permanent access to a 10 % sucrose solution without further blood meals. Mosquito midguts were dissected 7-8 days later in 0.4 % mercurochrome in phosphate buffered saline (PBS) or distilled water. The number of oocysts in the mosquito midgut was recorded to determine the prevalence as well as the intensity of infection [10].

2. 2. 4. Experimental infections of mosquitoes

A total of 1050 mosquitoes (50 mosquitoes per subject) were used for the experimental infection using the blood of the 21 subject's gametocytes carriers given a total of, 300 mosquitoes in Fulani group and 750 mosquitoes in the Mossi group.

Indeed, adult mosquitoes of 2-3 days old were conditioned in cups (50 mosquitoes / cup / participant) and fasted for 12 hours before the test. Each cup was placed under a cell to allow blood sampling. After gorging, ungorged mosquitoes or partially gorged were removed with a mouth vacuum cleaner. Gorged individuals were transferred into new cups and raised in optimal conditions for 7 days and dissected on the 8th day for oocysts assessment in the stomach.

2. 2. 5. Oocysts detection

The mosquitoes were dissected blindly in relation to the ethnic group by experienced microscopists. This dissection was carried out under a binocular magnifying glass, using Dumont n°5 forceps. The mosquito is held by a clamp at the chest and the abdomen is stretched with a second clamp placed at the 7th abdominal segment, the exoskeleton tearing itself free then the intestine. This is delicately detached at both ends and placed into 50 µl of sterile PBS. The carcass was also kept separately for future molecular analysis.

The intestines were deposited in a drop of 0.4% mercurochrome solution and observed under a microscope (X20) to quantify the number of oocysts. When no mosquitoes in a cup (participant) were infected, the carrier was considered non-infesting.

The presence or absence of oocyst (s) makes it possible to assess the individual's ability to infect the vector in relation to his parasitological and serological status.**2. 3. Data analysis**

Statistical analyses were done using Stata software version 13.1. The differences between the proportions were assessed by the Chi-square or Fisher test. They were considered significant for $p < 0.05$. Multifactorial analysis by logistic regression was also performed on the success of infections and by multiple linear regression on the percentage of mosquitoes infested, with gametocyte density, presence or density of asexual erythrocyte stages and age of gametocyte carriers as independent variables and success of infections or percentage of mosquitoes infected as related variables.

3. RESULTS

Our accepted population for infectivity tests was 21 participants with an average age of 7.9 years, including 14 males and 7 females subjects. All these participants slept under mosquito nets and had no history of antimalarial treatment in the two weeks prior to the enrolment.

3.1. *Plasmodium falciparum* parasites prevalence and densities

A total of 461 blood smears (255 and 206 from Fulani and Mossi group respectively) were examined using microscopy technique. *Plasmodium falciparum* trophozoites were detected in 83 (32.55 %) and 103 (50 %) blood smear from Fulani and Mossi respectively. However, only thirty-three (33) were *P. falciparum* gametocyte carriers: nine (9) from Fulani groups and twenty-four (24) from the Mossi ethnic group. From those 33 gametocyte carriers, only 21 (6 Fulani and 15 Mossi) were subjected to the membrane mosquitoes feeding at the insectarium according to the study criteria (Table 1).

Table 1: *P. falciparum* parasites prevalence and densities

	***P. falciparum* Prevalence (%)**		***Geometric mean parasite density***	
	Asexual	**sexual**	**Asexual**	**Sexual**
Fulani	32.55	3.53	1645 [1152-2349]	32 [14-71]
Mossi	50	11.65	1321 [939-1859]	29 [18-47]
p-value	< 0.001	0.001	0.004	NS

NS= No Significant p-value

The overall plasmodial and gametocyte index was estimated at 40.35% and 7.16 % respectively in the study population during the investigation period.

3. 2. Feeding experimental results

A total of 897 fed mosquitoes were dissected eight days later: 275 and 622 from experiments in Fulani and Mossi gametocyte carriers respectively. This represents an average of 43 mosquitoes per gametocyte donor (6 Fulani and 15 Mossi). In Fulani and Mossi, 6 and 15 DMFA were done respectively.

The mosquito infection rate was 10.5 % among Fulani and 13.2% among Mossi group (p=0.569). The fed mosquito rate was 95 % and 95.6 % among Fulani and Mossi respectively (p=0.241). The rate of survival mosquitoes after the feeding was statistically comparable (p=0.088) between the two ethnic group Fulani (96.5 %) and Mossi (87.4 %). The rate of dissected mosquitoes was 100 % and 99.2 % among Fulani and Mossi respectively (p=0.138). There is no significant difference found with the fed, survival, dissected and the infested mosquitoes rate. However, the oocyst average of oocystic load was higher in Fulani (249) than the Mossi (21) group despite the low infection in Fulani group (Table 2, fig 1).

The gametocytes of 9 carriers (42.86 %) did not infect mosquitoes. Among them, 4 were the Fulani ethnic group (geometric mean gametocyte density was 33 GF/µl) and 5 in the Mossi ethnic group (geometric mean gametocyte density was 43 GF/µl). On the other hand, 12 (57.14 %) gametocytes carriers were infective for anopheles (10 in Mossi and 2 in Fulani). For these positive infections, the percentage of infected mosquitoes was 19.30 % and the average number of oocysts per infected stomach was 14.09. The geometric mean of gametocyte was 126 and 61 GF/µl among Fulani and Mossi respectively. The successful rate of feeding was 33.33 % (2/6) in Fulani and 66.66 % (10/15) in Mossi group.

Table2: Mosquitoes feeding results in the two ethnic groups

	Fed mosquitoes		Survival mosquitoes		Dissected mosquitoes		Infected mosquitoes		Oocyst density
	%	N	%	N	%	N	%	N	
Fulani	95	285	96.5	275	100	275	10.5	29	30
Mossi	95.6	717	87.5	627	99.2	622	13.2	82	8
p-value	**0.241**		**0.088**		**0.138**		**0.569**		**< 0.0001**

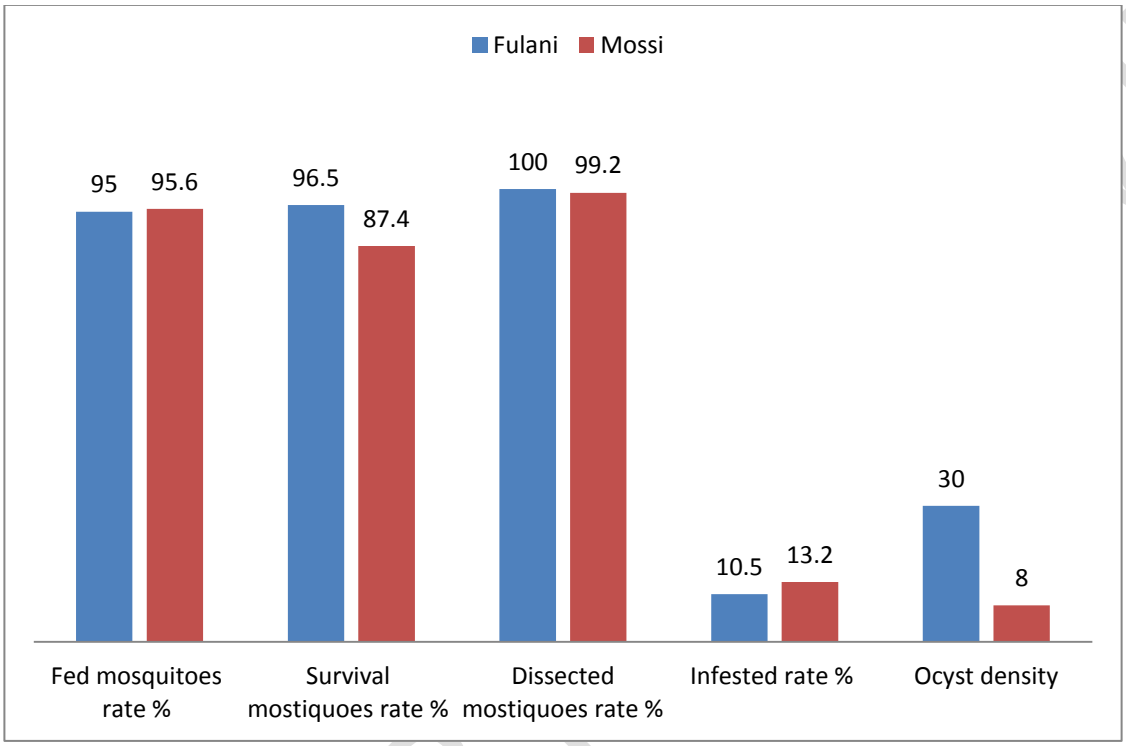

Fig 1: Comparison of the mosquitoes feeding results in the two ethnic groups

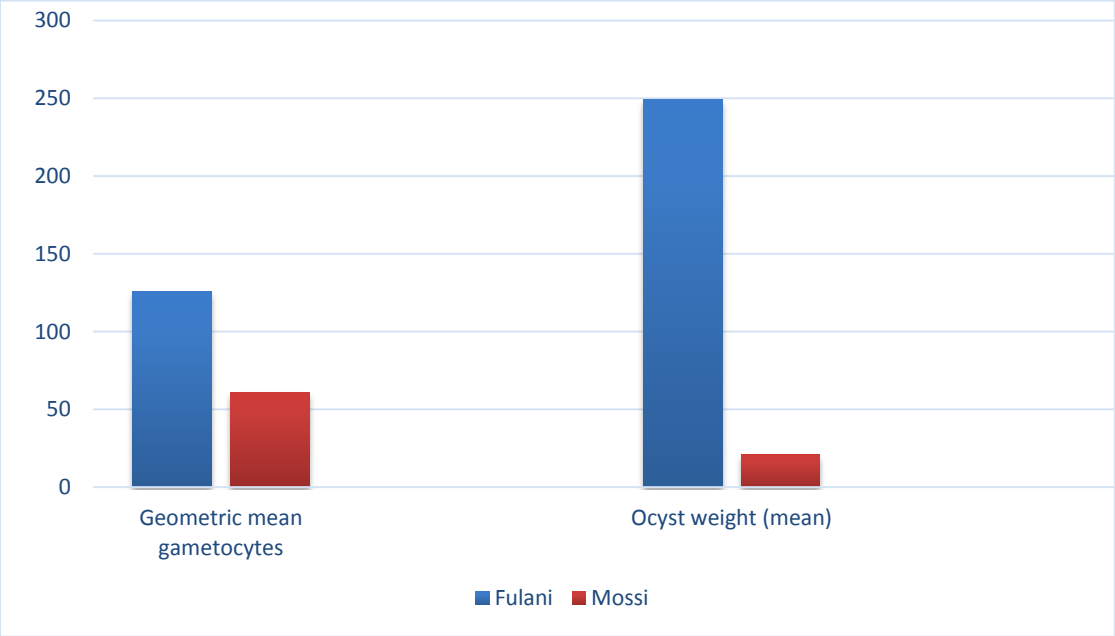

Fig 2: Gametocytes densities and oocyst weight in infected mosquitoes in the two ethnic groups

We observed in our study as presented on the figure 2, a relationship between the gametocyte load and the number of oocysts per infected mosquito. The higher gametocyte density was observed in Fulani group and correlated with the higher number of oocysts compared to the Mossi group. Indeed, there is a positive correlation between gametocyte density and oocystic load according to our results.

4. Discussion

In this study the infectivity of gametocytes has been merely defined as the capability to infect mosquitoes and was not based on the oocyst density. It was previously demonstrated that the number of oocysts developed in individual mosquitoes present a high degree of variability. However, the use of the oocyst density as an appropriate criterion for infectiousness is still debatable since a mosquito bearing one oocyst in his midgut is capable of transmitting the disease [11] following therefore the "all or nothing" law.

Experimental membrane infections of mosquitoes with the blood of naturally infected gametocyte carriers showed that 57.14% of them were capable to infect mosquitoes. This experimental value of mosquito infectivity found in our study was not different from previous values of 58.2% found in Gambia [12] but less than the value of 71% from Awono-ambéné and coll. [13] in Senegal. This difference may be due to the gametocytes density in the respective study populations, high in Senegal (214 gametocytes/µl). No mosquito-infecting meals have resulted in a 100% infection rate. This may be due to the low number of gametocytes actually ingested by anopheles or to the inter-individual variation in the rate of blood digestion by mosquito [14].

The question of why a certain percentage of study participants close to half (42.86%) did not give any infested mosquitoes remains unanswered. The result of the multiple linear regression analysis indicates that gametocyte density is one of the factors determining the success of mosquito infection. The earlier studies found a positive relationship between gametocytemia and sporozoite positive mosquitoes at densities between 50 and 450 gametocytes/µl [15].

Other factors such as the maturity and sex ratio of the gametocytes themselves could also influence the rate of mosquito infection.

Despite the strong positive correlation between gametocyte density and infectiousness, neither the presence nor the density of asexual stages had a significant influence on mosquito infection rates according to our analyses. Indeed, no significant difference was observed for subjects who infected or not mosquitoes regarding the carriage of asexual forms (p=0.251) in our study area. The same observation applies to both the Fulani (p=1.000) and Mossi (p=0.053) groups. In *P. falciparum* malaria, unlike other species, asexual stages do not seem to have any influence on the transmission of the parasite to the mosquito; probably because mature gametocytes appear in the bloodstream at least 10 days after the crisis phase [16, 17] and therefore long time after the peak of cytokine secretion. The results of our study confirmed this affirmation as we observed that the subjects with only asexual parasite were able to infect mosquitoes. However, others studies suggested that high asexual parasite loads seem to be associated with a reduction of gametocyte infectivity, particularly in children with more than 103 parasites/µl of blood [18].

The results of our study showed that the intensity of infection in mosquitoes is dependent on the gametocytemia. Indeed, the higher gametocytes load, the higher the number of oocysts per infected mosquito. Which was in correlation with previous studies assessing mosquitoes infectivity [19], [20], [21], [22]. Our study results showed a mean oocyst density found higher in Fulani and lesser in Mossi.

The relationship between gametocyte density and the infection rate (number of oocysts and proportion of infected mosquitoes) is complex and varies according to a certain thresholds of gametocytemia [3, 23]. Indeed, above a threshold of about 500 gametocytes/microliter, the rate of infection reaches a plateau. *P. falciparum* is also capable of infecting mosquitoes at gametocyte densities below the detection threshold by microscopy, about 15 gametocytes per microlitre [20, 24]. But our study showed that the gametocyte density at 30 gametocytes per microliter wasn't enough to infect mosquitoes in both group.

5. Conclusion

Our study showed that there is no significant difference between the two ethnic groups with regard to the rate of fed, survival, dissected and infested mosquitoes. However, the mean oocystic load was higher in Fulani than in the Mossi group, despite the low infectivity observed in the Fulani group. It is therefore necessary to explore the mechanism underlying this difference between the two ethnic groups.

COMPETING INTERESTS

"Authors have declared that no competing interests exist.".

CONSENT

All authors declare that 'written informed consent was obtained from the patient for publication of this report and accompanying images.

ETHICAL APPROVAL

The study received approval from the Institutional Ethical Committee of the Centre National de Recherche et de Formation sur le Paludisme (deliberation N°2017-000005/MS/SG/CNRFP/CIB) and the Ethical Committee of the Ministry of Health of Burkina Faso (deliberation N°2017-6-074).
Prior to any study-specific procedure, the parents or legally acceptable representative provided written informed consent Participants aged more than 7 years were provided with an additional assent at enrollment.

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