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Anopheles rufipes remains a Potential Malaria Vector after the First Detection of Infected Specimens in 1960 in Burkina Faso

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Abstract

Malaria transmission is assured in Africa mainly by the species of the complex *Anopheles gambiae* followed by *Anopheles funestus*. But as the malaria elimination is becoming more and more realistic, it is crucial to consider all vectors involved in *Plamodium* transmission even though in local scale. In this prospect we performed this study to confirm if *Anopheles rufipes* remains potentially able to transmit *Plasmodium* since former findings reported this species to be potential malaria vector in Burkina Faso toward 1960. Our data recorded one female of *An. rufi*pes infected by a *Plasmodium* parasite at the oocyst stage suggesting that this mosquito species still remains a potential human malaria vector. However, future field and laboratory studies are needed to confirm *An. rufipes* vector competence and capacity.

Keywords: *Anopheles rufipes*; Infection; Oocyst, *Plasmodium falciparum*; Vector

Introduction

The parasite *Plasmodium falciparum* is responsible for the majority of deaths due to malaria worldwide, particularly amongst children less than five years of age and pregnant women living in sub-Saharan Africa [1]. This parasite is transmitted through infected bites of female mosquitoes belonging to the genus *Anopheles*. Only few Anopheles species are involved in malaria parasite transmission in Africa. In Burkina Faso and other West African countries, *Anopheles gambiae* s.s. Giles (now confirmed as a complex of two species, namely *Anopheles coluzzii* and *Anopheles gambiae)* [2], *Anopheles arabiensis* Patton and *Anopheles funestus* Giles have been associated with the highest entomological inoculation rates for several decades [3-6]. In the past, *Anopheles nili* was also a major malaria vector species in the south-western region of the country, although its population has decreased drastically over the two latest decades due to environmental modification by human activity [3]. The potential epidemiological role of other species such as *Anopheles rufipes* was also demonstrated in the north and south-west localities of Burkina Faso by Holstein [7]. Despite the fact that specimens of *An. rufipes* were found infested by *Plasmodium* oocysts in the field several decades ago, further investigations including field surveillance of malaria transmission are needed to better assess the potential role and implication of this species in local malaria transmission system. In 2008, during entomological surveys, *An. rufipes* mosquitoes were frequently found, in large numbers, at the end of the rainy season (October-November) in homes of the village of Soumousso. Based on this observation, we included *An. rufipes* in the panel of major malaria vectors (*An. gambiae sl* and *An. funestus)* from which routine infection rates were estimated. The overall goal of this paper is to re-evaluate whether, a couple of decades after the previous data, if *An. rufipes* remains competent for *Plasmodium* infection.

Materials and Methods

Sampling site

Soumousso (11°00'46"N, 4°02'45"W) is a typical Guinean savannah village located approximately 55 km East of Bobo-Dioulasso, the second largest town of Burkina Faso. There are two distinct seasons over the year. The rainy season occurs from May to October, with an annual average of rainfall of 1000-1200 mm. Anopheles breeding sites consist mainly of rain-filled puddles and a semi-permanent swamp. Four main malaria vectors are found in this village, including both molecular forms M and S (respectively *An. coluzzii* and *An. gambiae*, see above) of *An. gambiae*, *An. funestus*, and *An. nili*. *An. arabiensis* is occasionally reported at low frequency (<5% of *An. gambiae s.l.* samples). *An. rufipes* is also reported towards the end of the rainy season but as it has been considered a non-vector species, its bionomic and dynamics have not been considered until now.

Mosquito collection and infection detection

During October and November 2008, resting wild blood-fed female mosquitoes (including *An. gambiae sl, An. funestus and An. rufipes*) were caught using a mouth aspirator in the living room of human dwellings early in the morning [8]. They were kept under in sectary conditions for 5 days in IRSS lab, until all surviving mosquitoes were dissected in 2% mercurochrome and their midguts examined for *Plasmodium* oocyst infection. Mosquitoes were morphologically identified [9,10]. The carcasses of all dissected mosquitoes were then used for CSP ELISA tests according to the protocol of Burkot et al.[11], modified by Wirtz et al. [12].

Results and Discussion

Out of a total of 729 mosquitoes dissected in October and November 2008, *An. gambiae s.l* was predominant (58.44%), followed by the significant presence of *An. funestus* (29.63%) and *An. rufipes* (11.93%). The dissection revealed a highly variable distribution of the number of oocysts (from 1 to 100) in the infected females. In particular, the single oocyst-positive *An. rufipes* harbored five oocysts. Estimation of the prevalence of the infection was focused

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on a qualitative assessment. The results showed that 4.69 % (20/426) of *An. gambiae s.l.* females dissected were positive for the presence of oocyst compared to *An. funestus* and *An. rufipes*, respectively, with 1.85 % (4/216) and 1.15% (1/87). No *An. rufipes* or *An. funestus* mosquitoes were found positive for sporozoite detection by ELISA-CSP, while some of them were oocyst-positive. Several non-mutual exclusive hypotheses may explain this pattern. Firstly, some studies have revealed the existence of a barrier to infection between mosquito midgut and salivary glands. In particular, some mosquito immune pathways appear to specifically target the sporozoites and prevent their migration [13-15]. It is therefore possible that while *An. funestus/ rufipes* are permissive hosts to *Plasmodium* oocyst stages, they are not competent for the sporozoites stages. Second, it is possible that, in Soumousso, populations of *An. rufipes* and *An. funestus* do not live long enough to transmit the plasmodium sporozoites compared to *An. gambiae*. This may be due to several factors including intrinsic genetic differences impacting adult lifespan, or a relatively higher sensitivity to pyrethrinoids used for the impregnation of long-lasting insecticide nets compared to *An. gambiae* [3]. Finally, when uninfected *An. rufipes* and *An. funestus* may have the same adult longevity as *An. gambiae* but display decreased longevity when infected possibly because of a lower tolerance to malaria infection.

In addition to the undeniable positivity found in the single female at oocyst stage, recent preliminary findings gathered in our lab using experimental *Plasmodium falciparum* infections, have shown that *An. rufipes* can be infected with sporozoïtes (Mouline, unpublished results). Thus, based on the assumption that any positive mosquito at oocyst stage should carry sporozoite stages later, we have estimated the overall *Plasmodium* prevalence per female, by combining positive mosquitoes with oocyst and/or sporozoites. Thus, the infection rates reached 27.93% for *An. gambiae sl*, 1.85% for *An. funestus* and 1.15% for *An. rufipes* (Table 1). The infection rate was significantly higher for *An. gambiae* than *An. funestus* ($P<0.0001$, df =1). In previous studies, the susceptibility of *An. rufipes* to *Plasmodium* infection was estimated among a total of 9 females examined [7]. The same authors also reported few specimens of *An. rufipes* with sporozoites in the salivary glands in Oursy, a locality of the northern region of Burkina Faso (formerly known as Haute-Volta). Our study demonstrates that *An. rufipes* mosquitoes can continue to serve as a potential malaria parasite vector species in specific areas of Burkina Faso. However, its real contribution to malaria epidemiology in Burkina Faso should be better investigated. Current mosquito control strategies are targeting malaria vectors belonging to the *An. gambiae* complex of species. Such studies that underline the potential role of other vector species such as *An. funestus*, *An. nili* and *An. rufipes* in malaria transmission intensity could serve to improve malaria control programs in Burkina Faso.

	Oocyst stage		**Sporozoite stage**		**Prevalence of infection**
	Positive	Negative	Positive	Negative	
An. gambiae (N=426)	**04.69%**	**95.31%**	**25.35%**	**74.65%**	**27.93%**
	(20/426)	(406/426)	(108/426)	(318/426)	(119/426)
An. funestus (N=216)	**01.85%**	**98.15%**	**00.00%**	**100.00%**	**01.85%**
	(4/216)	(212/216)	(0/216)	(216/216)	(4/216)
An. rufipes (N=87)	**01.15%**	**98.15%**	**00.00%**	**100.00%**	**01.15%**
	(1/87)	(86/87)	(0/87)	(87/87)	(1/87)

N: Total number of mosquitoes for each species; n: number of mosquitoes for te

Table 1: Summary of mosquito infectivity in the field for different species [% (n/N)].

Conclusion

The detection of *Plasmodium* oocysts in *An. rufipes* in the field suggests that this species is potentially capable to serve as a malaria vector mosquito. Due to their significance among the local vector population from specific locations in Burkina Faso, a routine entomological surveillance is required to better elucidate its vector capacity.

Conflict of Interest

Authors declare no conflict of interest.

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