


Research Article

First Observations of *Plasmodium malariae* on the Long-Term Malaria Vector Control Program on Villages of the Balombo Region of Angola

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Abstract

At the request of the National Malaria Control Program (NMCP), a long-term vector control program was implemented in 2007 in eight villages around the Balombo town (Angola) to compare the efficacy of four methods of indoor vector control. These methods included (1) Long-Lasting deltamethrin Insecticide treated Nets (LLIN PermaNet[®] 2.0 model or P.2.0); (2) association of P.2.0 and deltamethrin Insecticide Treated Plastic Sheetting (delta-ITPS)-Zero Fly[®] model; (3) delta-ITPS alone; and (4) 2 rounds of lambda-delta-cyhalothrin Inside Residual Spraying (IRS) followed by installation of delta-ITPS. Cross-sectional parasitological surveys (CSS) were done every two months. *Plasmodium* species determination, parasitemia and gametocytes presence, and evolution in time were analyzed. A total of 190 CSS was done between 2007 and 2011, *Plasmodium* spp. were observed in 5,431 of the 21,804 TBF done (24.9%). *Plasmodium malariae* alone was observed in 22 TBF (0.4%) and mixed infections *P. falciparum* and *P. malariae* in 44 TBF (0.8%). Our study confirms the presence of *P. malariae* in Angola, which must be known due to its special clinical impact, quartan fever, kidney failure, chronicity, symptomless carriers, persistence for several years with long term recrudescence and reported cases of resistance to classical ACTs. The prevalence of *P. malariae* decreased after implementation of vector control methods. The burden of *P. malariae* needs to be studied to reach the goal of malaria elimination by 2030.

Keywords: Cross-sectional malaria surveys, vector control, *Plasmodium malariae*, mixed infections.

Introduction

A great amount of work has been devoted to *Plasmodium malariae* since its first description by Laveran as *Oscillaria malariae* in 1881, then by Grassi and Feletti in 1890 under the name *Haemamoeba malariae* [1-10], until recent studies included genome sequencing [11- 19]. *P. malariae* is a particular species, especially at the clinical and parasitological levels, with a 72-hr erythrocyte cycle inducing a quartan fever with a slow development (15 days in both liver and *Anopheles* mosquito) [20, 21]. The parasite density is always low compared to *Plasmodium falciparum* and it produces clinical relapses, considered now as recrudescence, due to the asexual forms that persist in the host's blood [20]. Considered benign, this *Plasmodium* species was used in the 40's in experimental infections for the treatment of neurosyphilis [10]. Though, symptoms include nephropathies [22-26], long asymptomatic persistence of several years before access to recrudescence [27] that may induce transfusional malaria [28]. Cases of glomerulonephropathy associated

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with *P. malariae* infections were also reported with a disease that may persist and evolve despite the apparent elimination of parasites and with detectable chronic renal disease observed even 3-5 years after the so-called primary infection [29].

This species presents genetic polymorphism [30-34], it can be transmitted by palearctic or tropical vectors [35, 36], and due to its possibility to infect human and simian subjects [37, 38], it was considered as an anthrozoönotic species. This plasmodial species has a scattered world-wide distribution in malarious regions of Africa [39, 40], South America [41], Asia and Pacific [42] with a variable frequency in time and space according to the technique of investigations and biological analysis. With the recent acceleration of malaria control operations, the frequency of *P. falciparum* is decreasing [17, 43, 44] and a special interest has to be given to other plasmodial species, including *P. malariae*, which could persist for many years [45], or even the whole life of infected persons [19]. The genome of *P. malariae* has recently been decrypted [18, 46], which explains a case of resistance with a clinical recrudescence after treatment with the combination of artemether-lumefantrine (a full course of 6 doses over 3 days, equivalent to a total dosage of 6.2 mg/kg of artemether and 37.4 mg/kg of lumefantrine). The explanation given was that the primary infection would have been polyclonal, but the recrudescence was due to a resistant clone [46]. In 1937, the death of a 27-years old man in India was reported following what appeared to be the third malaria crisis access, with no particularly high fever, but parasitological examination revealed a high density of *P. malariae*. Despite treatment with quinine (injection and oral), resulting in the disappearance of microscopically observed *Plasmodium*, the subject died possibly from heart problems [47]. Overall, *P. malariae* is

still a neglected human malaria parasite, not well understood that still contributes up to 10% of malaria infections in sub-Saharan Africa [34]. The objective of the study was to evaluate the prevalence of both *P. malariae* and *P. falciparum* in a mesoendemic malaria region of Angola where our study has been ongoing since 2007 [48-50]. In addition, it seemed important to pay a particular attention to these *Plasmodium* species in the framework of the study aiming at comparing the efficacy of four vector control methods, including long-lasting deltamethrin-impregnated mosquito nets (LLIN), deltamethrin insecticide-treated plastic sheeting (ITPS), association LLIN and ITPS, and lambda-cyhalothrin indoor residual spraying (IRS), in the Balombo region of Angola and to monitor its possible evolution with the implementation of different vector control operations.

Methodology

Study area

The Balombo study area in the Benguela Province of Angola was already described [48]. This study was made between February 2007 and December 2011, with sampling collections made every 2 months, in eight villages around the town of Balombo (12°21'S, 14°46'E) located at an altitude of 1,220 meters in a mountainous area of ancient volcanoes with a thermal spring at the west entrance to the city (Fig. 1). The vegetation is a tropical savannah with shrub prairie, the original forest has been severely degraded for crop development and domestic use. There is a well-marked dry and cold season in June to August and abundant rains from November to April. Temperatures vary between a minimum of 10°-15°C in June-August and a maximum of 30-35°C in March and April.



Figure 1: Map of collections sites in the 8 villages around the Balombo town, Benguela Province, Angola

Parasitological surveys

Parasitological surveys were carried out as part of a multidisciplinary evaluation of a vector control operation aiming at comparing four vector control methods in paired villages. The complete protocol of vector control method implementation is detailed in Brosseau et al. [48] and it included the following steps: (1) PermaNet 2.0 Long lasting deltamethrin-impregnated mosquito nets (LLIN) provided in Cahata and Caala; (2) association of LLIN with Zero Fly®, deltamethrin insecticide-treated plastic sheeting (ITPS) that were pinned on the hut walls in Capango and Canjala; (3) deltamethrin-treated ITPS Wall Lining (WL) model alone and also pinned on the walls in Chissequele and Barragem; and (4) conventional lambda-delta-cyhalothrin indoor Residual Spraying (IRS) with two rounds/year (every six months), followed by the installation of ITPS in Libata and Candiero in order to increase the long lasting efficacy of the treatment. Cross-sectional parasitological surveys were conducted on a regular basis every two months focusing on random samples of the population under 15 years of age. At the time of the field investigations, part of the population present in the village usually came to the team to have a blood test and, for obvious ethical reasons, a thick blood film (TBF) was prepared from each one of these volunteers and these TBF were coloured in the field with Giemsa. Then, microscopic observations were done at the medical department of the Angolese Sonamet Company located in Lobito, which supported the study through their Malaria Control Program (MCP).

To compare the efficacy of each vector control method, a subsample of children ≤ 15 years old was extracted from the original list to standardize the samples to be compared, but in the present study dealing with *P. malariae* the whole samples were considered with no age limits. Then, the number of blood smears done per village varied between 1,856 TBF in Capango to 3,387 TBF in Caala during the five years of the study (Table 1) and were constituted of people from all age groups, from 2 to 75 years old. Young children were accompanied by their volunteer mother. During the surveys a supplementary drop of blood was also obtained on filter paper for further immunological analysis at IRD in Montpellier, France for estimating the *Anopheles* biting pressure on the population before and after implementation of the four vector control methods [48]. Ethical approval was obtained from the National Malaria Control Program of the Ministry of Health of Angola, the Ethical authority in charge of approving studies on malaria research in Angola. In addition, written consent signed by the head of each household was obtained for all individuals enrolled in the study by Malaria Control Program (MCP) of the Sonamet Company. Microscopic parasitological examinations were done by the same team throughout the study with a regular double-checking by V. Foumane, parasitologist at OCEAC (Organisation de

coordination pour la lutte contre les endémies en Afrique Centrale), Yaoundé, Cameroon, of 10% random sample slides. Results of these analyses were provided to the village health worker for further action to be taken by the National Malaria Control Program (NMCP) of Angola.

Statistical analysis

Data were analyzed and graphs constructed with GraphPad Prism6® software (San Diego, CA, USA). Distribution of parasitemia were analyzed with the non-parametric Mann-Whitney statistical test.

Results

Prevalence of *Plasmodium malariae*

From February 2007 to December 2011, 190 field surveys were done and 21,804 thick blood smears were prepared from which 5,431 were positive for *Plasmodium* (24.9%), 5,365 with *P. falciparum* (98.8%), 22 (0.4%) with *P. malariae*, and 44 (0.8%) with a mix of *P. falciparum* and *P. malariae* (Table 1).

Parasitemia of *P. malariae* alone or coinfections *P. malariae* and *P. falciparum*

P. malariae parasitemia, alone or associated with *P. falciparum*, were significantly not different with p-value = 0.1127. For single or mixed infections, the geometric means were of 466 and 787, and the medians of 450 and 746 respectively (Table 2).

Influence of vector control methods

The presence of *P. malariae*, alone or associated with *P. falciparum*, according to the vector control method is

Table 1: Parasitological surveys done from 2007 to 2011 in 8 villages of the Balombo region

Village	No surveys	No TBF done	No TBF +	No <i>P.f.</i>	No <i>P.m.</i>	No <i>P.f.</i> + <i>P.m.</i>
	Caala	25	3,387	1,004	1,000	1
Cahata	25	2,873	833	819	7	7
Capango	24	1,856	314	312	1	1
Canjala	24	2,952	931	915	4	12
Chisséquéélé	23	2,551	463	460	2	1
Barragem	23	2,525	548	534	3	11
Candiero	23	2,761	524	523	0	1
Libata	23	2,899	814	802	4	8
Total	190	21,804	5,431	5,365	22	44
		100%	24.9%	98.8%	0.4%	0.8%

No, number; TBF, thick blood film; TBF+, positive thick blood film; *P.f.*, *P. falciparum*; *P.m.*, *P. malariae*

reported in Table 3. Similar percentages of infections were found when considering *P. malariae* and mixed *P. malariae* + *P. falciparum* with values ranging from 1% in villages with LLIN alone and IRS, 1.4% in villages with LLIN and Zero Fly, and 1.7% in villages with ITPS, while *P. falciparum* percentages vary between 98.3% and 99%. Besides, the number of infections with *P. malariae* alone or mixed with *P. falciparum* decreased drastically, by a factor of 4.5 after vector control operations with values of 54 out of 66 infections (82%) before to 12 (18%) after vector control implementation (Table 4). However, the parasitemia of *P. malariae* infections alone or in coinfection with *P. falciparum* did not change with vector control implementation as shown by p-values of 0.0778 for *P. malariae* infections alone (median = 480 before and 233 after VC) and p-value= 0.923 for coinfections (median = 804 before and 853 after VC) (Fig. 2).

Table 2: Parasitemia of *P. malariae* alone or associated to *P. falciparum*

Indicator/ infections	<i>P. malariae</i>	<i>P. malariae</i> + <i>P. falciparum</i>
Arithmetic mean (parasites/μl)	1,065 ±1,840	1,955 ±4,008
Geometric mean	466	787
Median (sample size)	450 (n=22)	746 (n=44)
Minimum value (parasites/μl)	32	133
Maximum value (parasites/μl)	8,301	24,000

Table 3: Presence of *Plasmodium malariae* according to vector control method implemented (TBF= thick blood film; LLIN= Long lasting insecticide treated net; ITPS= insecticide treated plastic sheeting; ZF= Zero Fly model of ITPS; IRS= Inside residual spraying)

Method of vector control	No surveys	No TBF done	No TBF +	No <i>P.f.</i>	No <i>P.m.</i>	No of mixed <i>P.f.</i> + <i>P.m.</i>
LLIN alone	50	6,260	1,837	1,819	8	10
		100%	29.3%	99.0%	0.4%	0.5%
LLIN+ZF	48	4,808	1,245	1,227	5	13
		100%	25.9%	98.6%	0.4%	1.0%
ITPS alone	46	5,076	1,011	994	5	12
		100%	19.9%	98.3%	0.5%	1.2%
IRS then ITPS	46	5,660	1,338	1,325	4	9
		100%	23.6%	99.0%	0.3%	0.7%

Table 4: Prevalence and number of cases in parentheses of *P. malariae* alone (*P.m.*) or associated to *P. falciparum* (*P.m.* + *P.f.*) in surveys done before and after vector control (VC) implementation

Vector control methods	Before VC		After VC	
	<i>P.m.</i>	<i>P.m.</i> + <i>P.f.</i>	<i>P.m.</i>	<i>P.m.</i> + <i>P.f.</i>
LLIN alone	57% (8)	43% (6)	0	100% (4)
LLIN + ZF	14% (2)	86% (12)	75% (3)	25% (1)
ITPS alone	27% (4)	73% (11)	50% (1)	50% (1)
IRS then ITPS	36% (4)	63% (7)	0	100% (2)
Sub-Total	82% (18/22)	82% (36/44)	18% (4/22)	18% (8/44)
Total	82% (54/66)		18% (12/66)	

LLIN= Long lasting insecticide treated net; ITPS= insecticide treated plastic sheeting; ZF= Zero Fly model of ITPS; IRS= Inside residual spraying.

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Influence of the season

The analysis of *P. malariae* infections observed during the dry and rainy season surveys shows a similar overall number with respectively 30 and 36 infections, with twice less *P. malariae* alone infections in the rainy (32%) than the dry season (68%) (Table 5).

Influence of gender

The 66 infections of *P. malariae* alone or associated with *P. falciparum* included 28 men and 38 women. The parasite load of *P. malariae* were similar in men and women (p-value= 0.14; respective median 480 and 299) and the parasitemia of mixed infections *P. malariae* + *P. falciparum* were also similar in men and women (p-value= 0.93; respective median = 804 and 1,040) (Fig. 3).

Influence of age

Our sample was not prepared to be representative of the village populations since it was intended to mainly evaluate the impact of vector control operations on *P. falciparum* infections in children (≤ 15 years). However, for *P. malariae* evaluation, 2 age groups were considered such as <5 years old, considered as at-risk group, and > 5 years old. The results showed a similar number of infections in both groups with 11 carriers <5 years old and 11 carriers > 5 years old for infections with *P. malariae* alone, and 20 carriers <5 years old and 24 carriers > 5 years old for mixed infections *P. malariae* + *P. falciparum*.

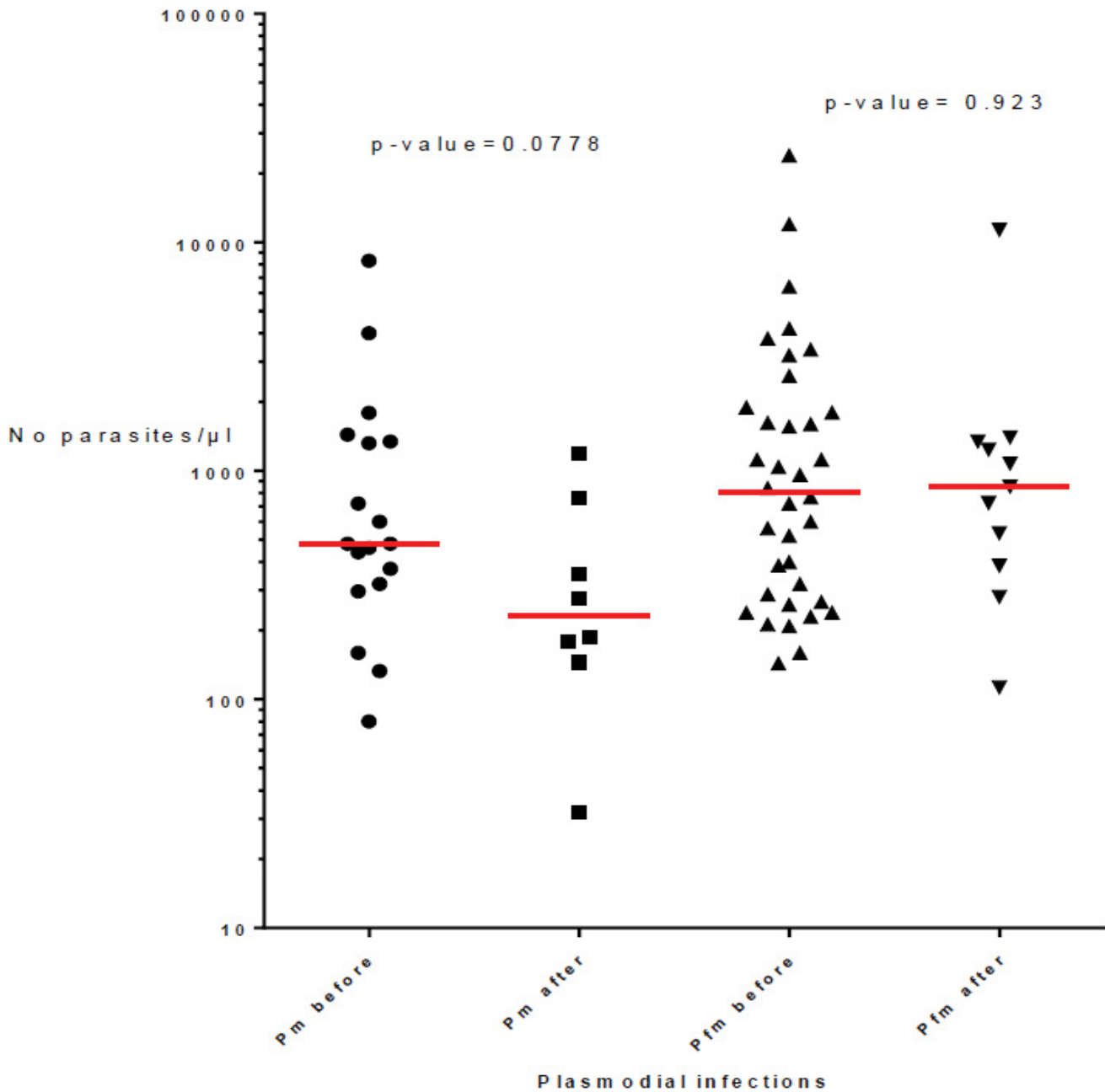


Figure 2: Evolution of the parasitemia of *P. malariae* (Pm) alone or associated with *P. falciparum* (Pfm) with median (red line) before and after vector control implementation

Table 5: Number of positive slides and prevalence of *P. malariae* (*P.m.*) alone or associated to *P. falciparum* (*P.f.+P.m.*) according to season

Plasmodium species	Rainy season		Dry season	
	<i>P.m.</i>	<i>P.f. + P.m.</i>	<i>P.m.</i>	<i>P.f. + P.m.</i>
No positive slides	7/22	23/44	15/22	21/44
Prevalence	32%	52%	68%	48%
Total	30/66 (45%)		36/66 (55%)	

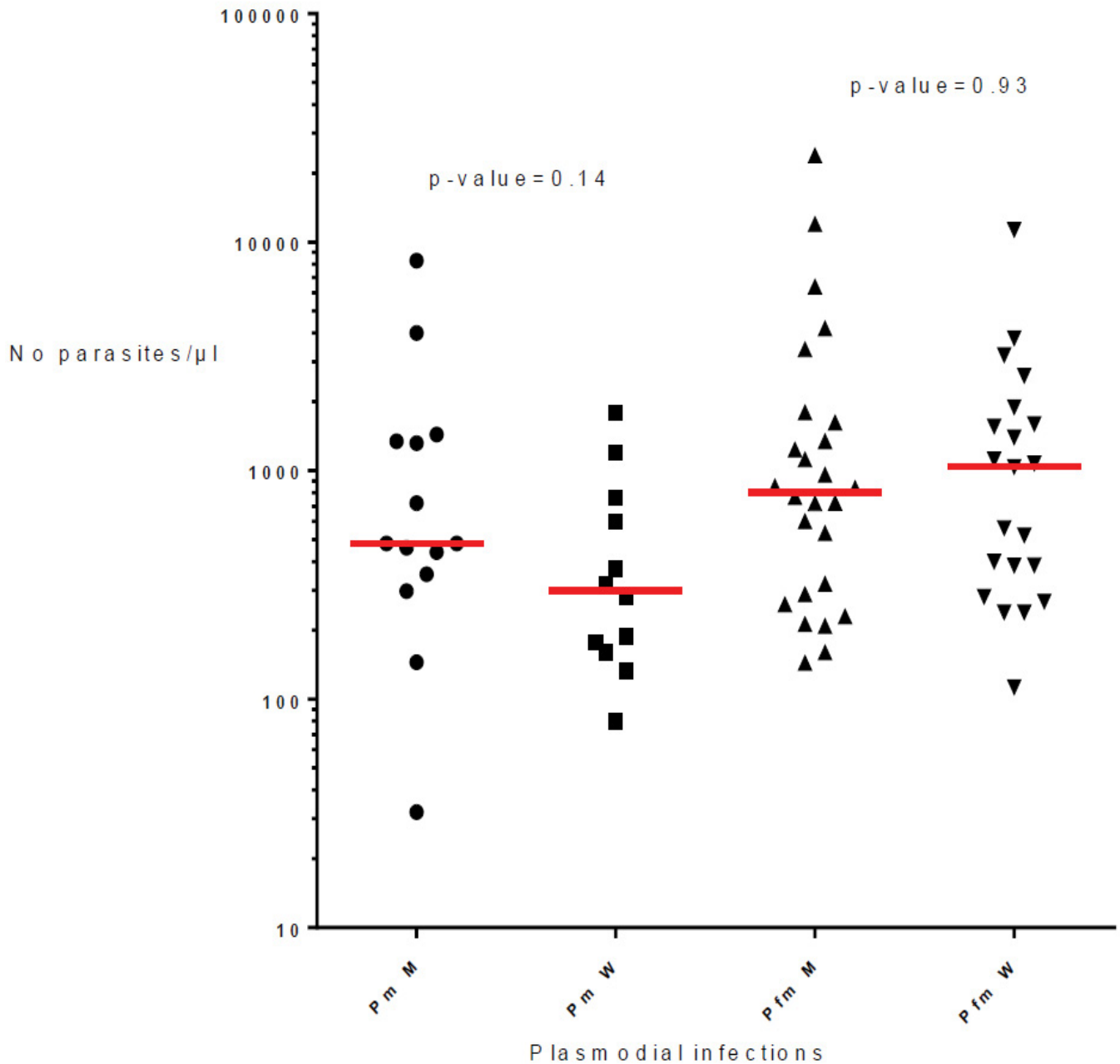


Figure 3: Parasitemia due to *P. malariae* alone (Pm) or associated with *P. falciparum* (Pfm) with median (red line) according to the gender. M, men; W, women

Discussion

According to the last WHO malaria report [44], Angola is the fifth country with the highest number of malaria cases and deaths in the world, accounting respectively for 3.4% and 3.2% of the global cases and deaths in 2022, ranking 5th and 7th among the African countries. For the past 10 years, malaria figures in Angola have been steadily increasing with an incidence that jumped from 3.5 million confirmed cases in 2011 to 9.2 million in 2022, and a mortality that

nearly doubled from 6,909 to 12,474 deaths respectively out of a total population of 35.6 million, all living in malaria-risk regions. In the Balombo region of Angola (Benguela Province), malaria is mesoendemic and all reported cases are due to *P. falciparum* with no report of the occurrence of *P. malariae* or *P. ovale*, due to their rarity, although records have shown the presence of these *Plasmodium* species in other parts of Angola [51- 55]. For instance in northern Angola, on a total of 3,316 blood samples collected, 541 (16.3%) were *Plasmodium* infected, out of them 477 (88.2%) were

due to *P. falciparum* alone and 35 (6.5%) were coinfections *P. falciparum* and *P. malariae* [53]. In this study by Fancony et al. [53], *P. malariae* infections were reported by molecular methods and not observed with usual optical microscopy such as our study.

The five years longitudinal surveys in the eight villages around Balombo (Angola) confirmed the presence of *P. malariae* in the Balombo region of west-central Angola and its very low prevalence, with 22 cases out of 21,804 thick blood smears observed (0.1%), and this could explain why this species is rarely reported in single sectional surveys, deserving continuous training of microscopic technicians [56]. For example, during continuous training operations for microscopists from health centers in Bengo, Benguela and Luanda Provinces in Angola, there was an increase in the sensitivity and specificity of examinations after training, while noting false positive *P. vivax* case in Bengo and *P. malariae* infection on a slide considered as negative [57]. Using combined techniques such as microscopy and PCR assays, the prevalence of *P. malariae* mono-infections at 1% was found in the Democratic Republic of Congo, country neighbouring Angola [58], while it was at 2.5% in Cameroon and coinfections *P. falciparum*/*P. malariae* at 17% using the same two techniques and RDTs [59].

In Angola, a cross-sectional survey conducted in 733 Chinese and Southeast Asian migrants showed that most infections were due to *P. falciparum*, although infection and/or exposure to *P. vivax* and *P. malariae* was also detected [54]. Based on these very few reports, more attention should be given to the detection of non-falciparum infections in order to know what and where plasmodial species are circulating in Angola, and more widely in the south of the Saharan region for a more precise diagnosis of patients in order to provide them an appropriate malaria prophylaxis [60]. In addition, it is important to know where this plasmodial species is circulating for the diagnosis of patients being examined in non-endemic areas, to avoid kidney failure or any other complications due to *P. malariae*. *Plasmodium* infections with non-falciparum species, also referred to Non-Falciparum Malaria (NFM), are responsible for 25% of cases imported into Europe [55] and a retrospective analysis of the records from January 2006 to August 2016, recorded in a hospital in Portugal, revealed 19 cases of NFM among the 225 malaria cases including 12 cases from Angola. Out of these 19 NFM cases, 7 (37%) were due to *P. malariae*. The most frequent symptom was fever and the biological analysis showed thrombocytopenia. Treatments provided to these 19 NFM cases were quinine-doxycycline (11 patients), chloroquine (six patients) or artemether-lumefantrine (two patients) with cure in all cases. In Henan Province of China, three patients, including two returning from Angola and one from Equatorial Guinea, had symptoms such as irregular fever, headache, chills. Two cases had elevated total bilirubin and

splenomegaly and were confirmed as *P. malariae* infection by microscopic examination. Both were cured with artemisinin-based combination therapy (ACT) [52], while the usual drug can have crucial side effect of G6PD deficiency and must be given only with strict medical care in main hospital. In Jiangsu Province of China, an observational study of imported malaria cases was carried out for the period of 2011-2014 [51]. Out of 1,268 imported malaria cases, *P. falciparum* cases accounted for 83.4% (n = 1,058), and it was noticed that the proportions of *P. ovale* and *P. malariae* increased during these four year-period. Therefore, this increase in rare *Plasmodium* species originating from south-Saharan Africa and Southeast Asia needs to be better monitored at all levels of health providers focusing on diagnosis and treatment of malaria. In addition, due to long latency periods and misdiagnosis of *P. malariae* and *P. ovale*, there is an increased risk of re-introduction of malaria in China, declared malaria-free by WHO in 2021 [51, 61, 62]. A recent meta-analysis of the occurrence of *P. malariae* and *P. ovale* in the world revealed that 51% of these species were reported from the African region and each species was accounting for 3.16% and 1.69% respectively on this continent [17]. This prevalence of *P. malariae* is 10 times higher than the one found in this study (0.3% including mixed infections), which may be due to the fact that this species is often misidentified as *P. falciparum* or totally missed under microscopic examination due to the low parasite density compared to *P. falciparum* [63]. Then, the use of optical microscopy to search for *Plasmodium* parasites must be, in future studied, accompanied by PCR assays to confirm, and eventually increase the number of positive samples. This is particularly important as *P. malariae* shows a lower parasitemia compared to *P. falciparum*, therefore more difficult to detect under a microscope [21, 64].

To improve the diagnosis of certain fevers, anaemia [65] and nephropathies, it is important to be aware of the presence of *P. malariae* and thus avoiding misdiagnosis [66] in endemic and non-endemic areas [64, 67, 68] with the critical issues of mistreatment [60, 69-74]. The persistence of *P. malariae* even after ACT treatment is of great concern [75, 76], as well as its well-known long chronicity [3] with an amazing persistence for several years, such as a *P. malariae* case reported 32 years after the initial infection of stay in malaria-endemic areas [77]. It was reported that infections have been transmitted by blood transfusion twelve years, twenty years, and thirty years after infection of the donors [3]. In addition, it is important to highlight the fact *P. malariae* is a parasite of both man and African apes (chimpanzee and gorilla) [38, 78] and with current and foreseen environmental changes and forest destruction, the risk of human infections could greatly increase as it was recently seen with *P. knowlesi* in Indonesia and even more Malaysia [79-89].

Conclusion

Angola belongs to the Malaria Elimination Eight Regional Initiative (E8), as one of the 8 countries of southern Africa aiming to eliminate malaria by 2030 [90]. The expansion of malaria interventions in Angola in the 20 past years aimed to consolidate malaria control and move towards pre-elimination [91]. However, the case of residual malaria is of great concern and may jeopardize malaria elimination [92-94], especially if all efforts are concentrated on *P. falciparum* only. Non-falciparum species, such as *P. malariae*, are largely forgotten, although this species showed a prevalence of 3.16% in Africa, based on 50 studies, and 17% of coinfections with *P. falciparum* were recently reported from Cameroon [17, 59]. Better knowledge on the prevalence and burden of non-falciparum species is crucial in the aim of achieving malaria elimination in Angola and in the concerned countries of the E8 initiative. To accomplish this task, well-trained microscopists in the identification of non-falciparum species are needed; molecular techniques for the diagnosis of *Plasmodium* species must be popularized; and less expensive diagnostic techniques (such as hypersensitive RTDs) need to be developed.

Résumé

A la demande du Programme National de Lutte contre le Paludisme (PNLP), un programme de lutte antivectorielle a été mis en œuvre dès 2007 dans huit villages autour de Balombo (Angola) pour comparer l'efficacité de quatre méthodes de lutte antivectorielle dans les habitations. Ces méthodes comprenaient (1) des moustiquaires à imprégnation durable (MID) traitée à la deltaméthrine (PermaNet© 2.0 ou P.2.0); (2) l'association de MID P.2.0 et de bâches en plastique traitées avec un insecticide (BPTI), la deltaméthrine (delta-BPTI) - Zero Fly©; (3) delta-BPTI seul; et (4) 2 cycles de pulvérisation de lambda-cyhalothrine à effet rémanent suivis de l'installation de delta-BPTI. Des enquêtes parasitologiques transversales (EPT) ont été effectuées tous les deux mois. L'identification des espèces de *Plasmodium*, la parasitémie, la présence de gamétocytes, et l'évolution dans le temps de ces paramètres ont été analysées. Un total de 190 EPT a été fait entre 2007 et 2011, *Plasmodium* spp. ont été observés dans 5.431 des 21.804 gouttes épaisses (GE) effectuées (24.9%). *Plasmodium malariae* seul a été observé dans 22 GE (0,4%) et des infections mixtes *P. falciparum* et *P. malariae* dans 44 GE (0,8%). Notre étude confirme la présence de *P. malariae* en Angola, qui doit être suivie en raison de son impact clinique, fièvre quarte, insuffisance rénale, chronicité, porteurs asymptomatiques, persistance pendant plusieurs années avec recrudescence à long terme et cas signalés de résistance aux ACT classiques. La prévalence de *P. malariae* a diminué après la mise en œuvre des méthodes de lutte antivectorielle. Le fardeau que représente *P. malariae* doit être étudié afin d'atteindre l'objectif d'élimination du paludisme d'ici 2030.

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Author Contributions

PC designed the study protocol; participated to field surveys, analyzed data and drafted the manuscript. FF supported the study and participated to field surveys. MADS, VF, JCT carried out the field surveys. MADS read all blood slides. VF did initial and ongoing training, participated in laboratory analysis and double-checked part of the blood slides. SM did data analysis and was fully involved in drafting and writing the manuscript. FM participated in data analysis and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data are fully available without restriction.

Declarations

Ethical Approval and consent to participate

This study was conducted in accordance with the Edinburgh revision of the Helsinki Declaration and was approved by the National Malaria Control Program of the Ministry of Health of Angola, the Ethical authority in charge of approving studies on malaria research in Angola. Written consent (signed by the head of each household) was obtained for all individuals enrolled in the study by the SONAMET Company - Malaria Control Program (MCP).

Conflicts of Interest

The authors declare that they have no conflicts of interest in relation to this article.

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