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IMMUNOLOGY AND HOST-PARASITE INTERACTIONS - ORIGINAL PAPER

# Surveillance of *Plasmodium malariae* infection among inhabitants of rural areas in Ouidah–Kpomasse–Tori Bossito health district, Benin

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## Abstract

Among the *Plasmodium* species that infect humans, *P. falciparum* has been largely studied in malaria endemic areas. However, *P. malariae* infection is less documented among the human population. This study aimed to monitor the prevalence and distribution of *P. malariae* in Southern Benin. A cross-sectional survey was conducted in rural localities in the Ouidah–Kpomasse–Tori Bossito (OKT) health district in Southern Benin from June to October 2019. Socio-demographic data were collected using a questionnaire, while malaria infection data were obtained on the one hand by microscopy diagnosis and, on the other, by nested polymerase chain reaction (PCR). Based on microscopy, the prevalence of *P. malariae* mono-infection and coinfection of *P. falciparum*, *P. malariae* was respectively 2.3% and 1.2% in the OKT health district. This prevalence was higher ($P < 0.01$) than that reported by Damien *et al*. (2010) 10 years ago in the same study area with 0.7% and 0.3% of *P. malariae* and *P. falciparum/P. malariae*, respectively. Based on PCR analysis, *P. malariae* prevalence was 14.1%, including 5.2% of mono-infection and 8.9% of mixed infection with *P. falciparum*. Sub-microscopic *Plasmodium* infections were high (30.6%) and more pronounced in older participants (>20 years). The present study revealed that *P. malariae* increased in the OKT health district with a high prevalence of submicroscopic infection. Since our results provide valuable evidence of increasing *P. malariae* infection, the National Malaria Control Programs (NMCPs) must consider *P. malariae* when designing future measures for effective control and malaria treatment.

**Keywords** Prevalence · *Plasmodium* · *P. malariae* · Rural inhabitants · Ouidah–Kpomasse–Tori Bossito

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## Introduction

Malaria infections still remain a major cause of morbidity and mortality in populations living in Sub-Saharan Africa. Nearly 229 million malaria cases were recorded globally, leading to 409,000 deaths in 2019 and 94% of the deaths occurred in Africa (WHO, 2020). In Benin, the prevalence of the disease has been estimated at 39% in children under 5 years (INSAE, 2018).

Despite the scale-up of control interventions such as long-lasting insecticide-treated nets (LLINs), indoor residual spraying (IRS), and antimalarial drugs, malaria continues to be a serious public health problem in many African countries (Winskill et al. 2020). In the Global Technical Strategy for Malaria 2016–2030 (WHO-GTS), the World Health Organization (WHO) sets the goal to eliminate malaria in some countries (based on data from 2015) by 2030 ( WHO, 2016). To reach this objective, taking into account all malaria parasites for prevention, diagnosis, and treatment would be an important and promising strategy (WHO, 2015).

To date, five parasites from the genus *Plasmodium* such as *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi* are involved in the transmission of malaria (Bejon et al. 2014), and this transmission is ensured within both host populations (human and vector) by *Plasmodium* spp. gametocyte carriers (Schneider et al. 2007). Among *Plasmodium* spp., *P. falciparum* is the most common species found in Africa (WHO, 2019a, 2019b, 2019c) and the most pathogenic (Brazier et al. 2017). In West Africa, *P. malariae* is mostly found in sympatry with *P. falciparum* (Collins and Jeffery 2007). Compared to *P. falciparum*, *P. malariae* are much less prevalent ( Mueller et al., 2007 and typically asymptomatic (Collins and Jeffery 2007).

Previous studies have shown that untreated *P. malariae* infections lead to nephrotic syndrome and are associated with severe anemia (Ehrich and Eke, 2007; Langford et al., 2015). Despite these symptoms that *P. malariae* could cause to human health, *P. falciparum* has been the focus of all the surveillance over the years (Gnémé et al. 2013).

Malaria surveillance is mandatory because it can generate data on malaria infection, allowing NMCPs to set priorities by developing and implementing appropriate strategies for disease control (Bridges et al. 2012). However, surveillance of malaria infection would not bring significant progress without paying attention to all malaria parasites species, especially non-falciparum in those countries where they coexist with *P. falciparum*.

Some studies have shown the increase of *P. malariae* prevalence over the years. It has been reported that the prevalence of *P. malariae* has increased from 0.9% in 2007 to 13.2% in 2010 in Burkina Faso (Gnémé et al. 2013) and the Colombian Amazon Region (43.8% in 2016) (Camargo-Ayala et al. 2016). More recently, a study in Tanzania reported persistent detection of *P. malariae* over a 22-year period and a 2-fold increase in its prevalence from 2010 to 2016 (Yman et al., 2019)

Ten years ago, a previous study conducted in the Ouidah–Kpomassè–Tori Bossito health district had reported *P. malariae* infection among rural inhabitants (Damien et al. 2010). The increasing prevalence of *P. malariae* observed in the above countries could also be the case in the Ouidah–Kpomassè–Tori Bossito health district since no study has been made to monitor *P. malariae* infection in this area yet. Surveillance of *P. malariae* infection is then needed within the population to unveil the burden of this non-falciparum parasite. In the present study, we sought to assess the current distribution and prevalence of *P. malariae* in the OKT health district and then compare this prevalence to that found in this area 10 years ago to inform NMCP for adjusting current malaria control strategies.

## Materials and methods

### Study sites description

The cross-sectional survey was carried out in rural localities of Ouidah and Kpomasse municipalities located in the OKT health district in the South of Benin (Fig. 1) from June 2019 to August 2019 and from September 2019 to October 2019, which correspond to the malaria transmission periods. Ouidah and Kpomasse municipalities are located respectively at 42 km and 57 km from Cotonou. The climate is subequatorial and has two rainy seasons (a long rainy season from April to July and a short one from October to November) and two dry seasons (a long dry season from December to March and a short one in August and September).

### Sampling procedure

Six villages were randomly selected in each Ouidah–Kpomasse–Torri Bossito health district based on the previous data on *P. malariae* infection in the human population in this area (Damien et al. 2010) (Fig. 1).

Before the sample collection, sensitization sessions were held with local authorities and the population to explain the study's objectives and requested the authorities' support for the study implementation. All the volunteers who signed the informed consent and willing to participate in the study were included. A structured questionnaire was administered to record the participant's socio-demographic and environmental data (age, gender, temperature, bednet use, type of housing, breeding animals).

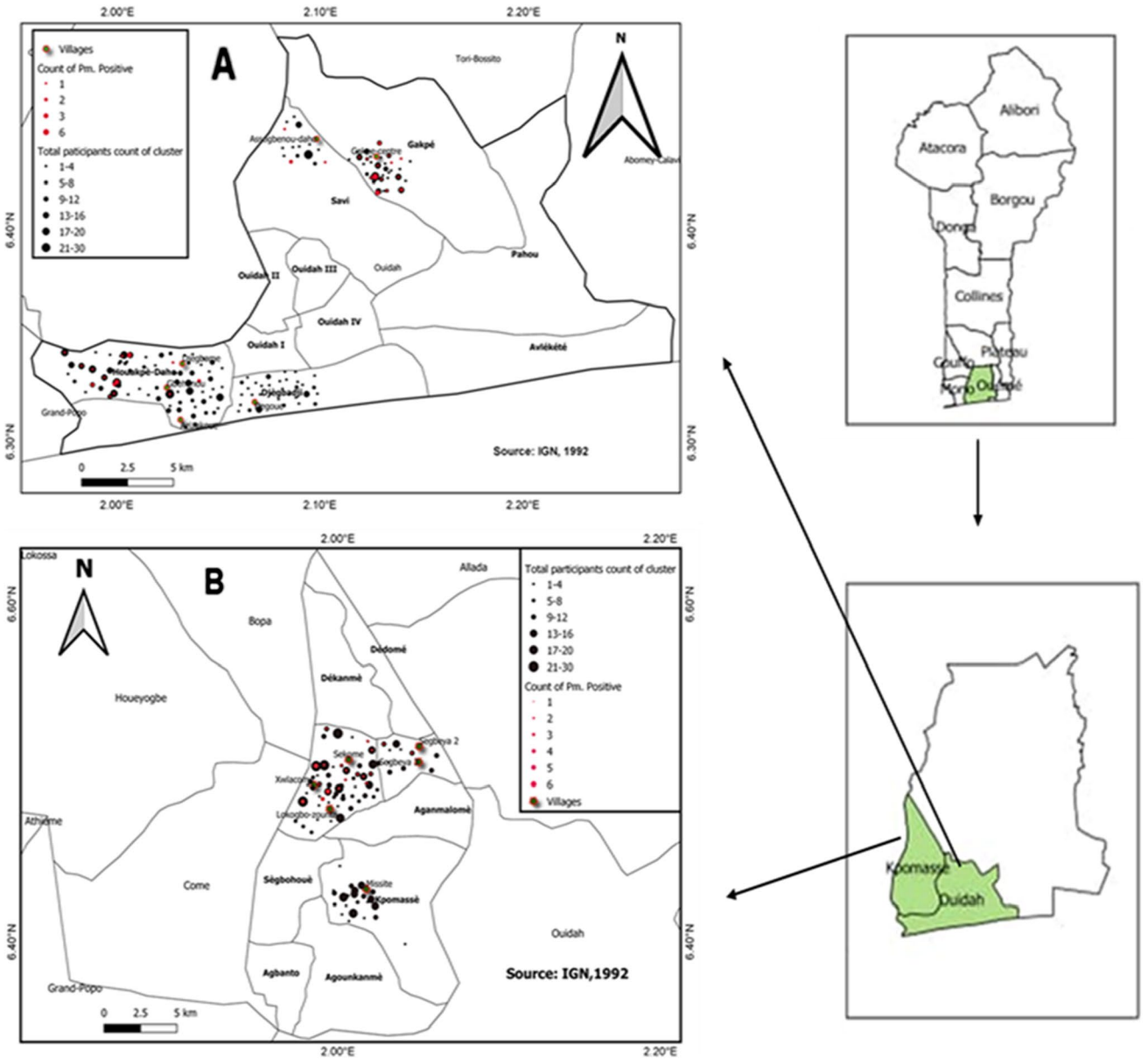


**Fig. 1** Map showing rural localities surveyed in Ouidah (**A**) and Kpomassè (**B**) municipalities, the geographical distribution of *P. malariae* cases (mono and coinfection) and participants recruited. For each cluster, the size of the black dot represents the number of participants per household, and the size of the red dot represents the number of positive *P. malariae* by microscopy

Approximately 60 µl of capillary blood was collected from each participant, 20 µl was used to prepare a thick and thin smear on a glass slide for detection and quantification of the asexual stages and/or gametocytes of *Plasmodium* spp. using microscopy, 40 µl was used to prepare two biological replicates of blood spots on filter paper to perform molecular analysis. The filter paper and the slide were dried and then brought to the laboratory of the Fondation pour la Recherche Scientifique (FORS) located at Cotonou and the laboratory of Vector-Borne Infectious Diseases located at the Institut Régional de Santé Publique/University of Abomey-Calavi (IRSP/UAC), Benin. *Plasmodium* positive cases by microscopy among recruited participants were treated by the medical staff with Artemisinin-based combination therapy (ACT), according to WHO and national malaria control policy (WHO,2004.; Zinsou and Cherifath, 2017).

## Plasmodium infections detection using blood slide microscopy

The prepared slides were fixed with methanol, let to dry, and stained with Giemsa diluted at 20% before examining

under the microscope for detection and quantification (parasite densities) of the type of *Plasmodium* species (asexual and sexual forms) following WHO recommendations (OMS, 2014). The parasite density was evaluated on the leukocyte count (white blood cells). The number of parasites per µl of blood in a thick smear was established in relation to the number of leukocytes counted. At least 200 leukocytes were counted if the number of parasites counted on the slide was greater than or equal to 100. However, if the number of parasites counted was less than 100, it was necessary to count up to 500 leukocytes. In some cases, the parasitaemia was so high that hundreds of parasites were counted per reading field. Therefore, it was appropriate to count up to 100 leukocytes. A slide was defined as negative if no parasites were found after examining at least 100 fields (OMS, 2014).

The number of parasites per µl of blood in a thick smear was established in relation to the number of leukocytes counted and assuming that there were 8000 leukocytes per µl of blood. The parasite density was determined following the formula: Parasite density = number of parasites × 8000/number of leukocytes read.

Sexual and asexual parasite stage densities were reported separately for all *Plasmodium* species.

All the positive cases of *P. malariae* and coinfection *P. falciparum*/*P. malariae* have been validated by a minimum of two microscopists, and slides with conflicting results were re-read until a consensus was reached. Quality control was frequently made for 10% randomly selected slides by a senior biologist.

### Plasmodium species (P. falciparum, P. malariae) identification by PCR

An overall of 1449 samples from 8 villages was randomly selected for *P. malariae* and *P. falciparum* amplification among the total blood spots samples collected. Parasite DNA was extracted using the Chelex extraction method (Plowe et al., 1995).

Nested PCR targeting the 18S rRNA gene region of plasmodia was performed using a previously described protocol (Georges et al. 1993) with slight modification. Nested PCR was performed within two reactions: The first round of DNA amplification was performed using rPLU5 and rPLU6 primers (Supplementary Table 1) to amplify the *Plasmodium* genus. The PCR mixture had a total volume of 15 µl, containing 5 µl DNA template, 1X PCR buffer, 166 nM dNTPs, 0.7mM MgCl2, and 1 U of OneTaq DNA. In the secondary reaction (nested 2), the species-specific primers rFAL1/rFAL2 (133.33 nM), rMAL1/rMAL2 (333.33 nM) were used in separate 15 µl reactions for the identification of *P. falciparum* and *P. malariae* species, respectively. The template for the secondary reaction was 1 µl of the primary reaction product. Details of the primers used are provided in Table 1.

The primary and nested PCR reaction cycling conditions included an initial denaturation at 95℃ for 5 min, followed by 35 cycles, a second denaturation at 94℃ for 30 s, primer annealing step at 55℃ (for primary) or 58℃ (for nested) for 1 min, sequence extension at 68℃ for 1 min, and a final extension at 68℃ for 5 min. PCR products were run for 40 min on a 2% agarose gel stained with a 0.5 µg/ml ethidium bromide solution and visualized under an ultraviolet transilluminator. The expected size of the PCR products was 205 bp and 140 bp for *P. falciparum* and *P. malariae*, respectively.

### Data analysis

Data were recorded into Microsoft Excel and exported into R version 3.5.3 and GraphPad Prism 8.0.2 software (San Diego, California USA). The normality of data distribution was checked using the Shapiro–Wilk test (Shapiro and Wilk, 1965).

The main variable was the prevalence of *Plasmodium* spp. (both asexual and sexual form). It was calculated as the proportion (in percentage) by dividing the number of individuals identified as positive for *Plasmodium* spp. parasites by the total number of participants. Then, we compared our microscopy results with that of Damien et al. (2010) to assess the trend of *P. malaria* prevalence 10 years later.

The correlations between socio-demographic, environmental factors, and the *P. falciparum* and *P. malariae* infections were explored.

The patient was considered to have fever was when an axillary body temperature is equal to or above 37.5℃. Age was stratified into six groups: <1, 1–4, 5–9, 10–14, 15–19, and ≥20 years, according to WHO guidelines (WHO, 2019a, 2019b, 2019c). Differences in participant characteristics and prevalence among age groups were assessed using Fisher's exact and $\chi^2$ according to the case. For all statistical tests, a 5% level of significance was used.

## Results

### Socio-demographic characteristics of the study population

A total of 2289 participants were recruited, encompassing 1393 from six villages in Ouidah and 896 from six villages in Kpomasse. The study population participants aged from 0 to 105 years old (mean age 26.5; SD ± 23.9). The percentage of males and females in the study population was 41.3% (945/2289) vs. 58.7% (1344/2289), respectively. Moreover, gender distribution varies significantly across villages (*X*-squared = 23.325, df = 11, *p*-value = 0.0159). Only 61 of 2289 participants (2.6%) had a fever (≥37.5℃) at the time of the survey and did not present any other symptoms of

**Table 1** Demographic profile of the 2289 participants distributed by district

| Characteristics | Ouidah (1393) | Kpomasse (896) |
|---|---|---|
| **Age** | 26.7 SD ± 24.2 | 26.2 SD ± 23.6 |
| | *N* (%) | *N* (%) |
| **Gender** | | |
| Female | 785 (56.4) | 559 (62.4) |
| Male | 608 (43.7) | 337 (37.6) |
| **Age group** | | |
| <1 | 9 (0.6) | 0 |
| 1–4 | 196 (14.1) | 123 (13.7) |
| 5–9 | 247 (17.7) | 186 (20.8) |
| 10–14 | 179 (12.8) | 115 (12.8) |
| 15–19 | 108 (7.8) | 66 (7.4) |
| >20 | 654 (46.9) | 406 (45.3) |
| **Animal breeding** | | |
| Yes | 994 (71.4) | 431 (48.1) |
| No | 399 (28.6) | 465 (51.9) |
| **Fever** | | |
| Yes | 45 (3.2) | 16 (1.8) |
| No | 1348 (96.8) | 880 (98.2) |
| **Protection barriers** | | |
| **Bednet use** | | |
| Yes | 1276 (91.6) | 735 (82.0) |
| No | 117 (8.4) | 161 (18.0) |
| **Concept of how to protect themself against malaria** | | |
| Drug use (CTA) | 84 (6.0) | 23 (2.6) |
| Traditional herbal infusion use | 285 (20.5) | 142 (15.8) |
| Environmental health | 20 (1.4) | 16 (1.8) |
| Insecticide use | 17 (1.2) | 7 (0.8) |
| Bednet use | 262 (18.8) | 213 (23.8) |
| Traditional herbal infusion use/bednet | 35 (2.5) | 30 (3.3) |
| No response | 690 (49.5) | 465 (51.9) |
| **Environmental risk factors** | | |
| **Presence of objects likely to be mosquito breeding sites near the housing** | | |
| Yes | 318 (22.8) | 45 (5) |
| No | 1075 (77.2) | 851 (95) |
| **Type of housing** | | |
| Modern | 834 (59.9) | 277 (30.9) |
| Old-fashioned | 559 (40.1) | 619 (69.1) |

The table shows the general and socio-demographic characteristics of the participant in the two study areas: Ouidah (1393 participants) and Kpomasse (896 participants)

*N*, number; %, percentage; *SD*, standard deviation

malaria. The socio-demographic characteristics of participants from Ouidah and Kpomasse were shown in Table 1.

## Prevalence of Plasmodium spp. infection using blood slide microscopy

Overall, 76.3% (1745/2289) participants were non-infected, and 23.7% (544/2289) were infected by *Plasmodium* spp. Among participants infected, 22.4% (512/2289) were identified as single species infections, and 1.4% (32/2289) were identified as co-infections (Fig. 2). *P. falciparum* was the most prevalent *Plasmodium* parasite and displayed a prevalence of 19.8% (454/2289), followed by *P. malariae* and *P. ovale* with a prevalence of 2.3% (53/2289) and 0.2% (5/2289), respectively. Moreover, the co-infection of *P. falciparum/P. malariae*, *P. falciparum/P. ovale*, and *P. malariae/P. ovale* displayed a prevalence of 1.2% (28/2289), 0.1% (3/2289), and 0.04% (1/2289) respectively (Table 2).

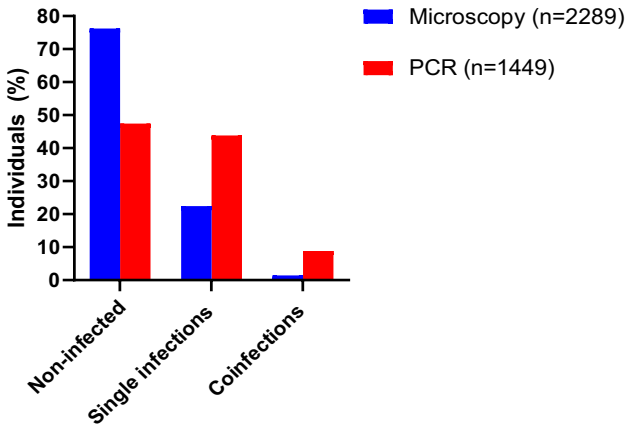


**Fig. 2** Overall individuals non-infected, single-infected, and coinfected by *Plasmodium* spp. using microscopy and PCR

*P. malariae* and coinfection *P. falciparum/P. malariae* were found in seven villages and six villages out of the 12 villages, respectively (Supplementary Fig. 1).

There was no variability in *P. malariae* prevalence within the age groups ($X$-squared = 7.3584, df = 5, $p$-value = 0.1953), and the prevalence ranged from 1.6 to 4.6% (Fig. 3). In contrast, there was a significant difference in the prevalence of *P. falciparum* among the different age groups ($X$-squared = 114.53, df = 5, $p$-value < 0.0001). Furthermore, the highest load of both *P. falciparum* and *P. malariae* parasitemia was observed in the age group 1–4 years, and the smallest parasitemia of *P. falciparum* was observed in the age group >20 years (Fig. 3).

A total of 5.59% of participants (128/2289) were carrying *Plasmodium* spp. gametocyte including 13.28% (17/128) and 82.81% (106/128) accounted for *P. malariae* and *P. falciparum*, respectively. Furthermore, *P. malariae* gametocytes carriage was higher in participants aged 1 to 9 years, while *P. falciparum* gametocytes carriage was higher in participants aged 15–19 years (Fig. 4).

Comparing our study microscopy result with Damien and Collaborator's work (Damien et al. 2010), there was no significant difference in *Plasmodium* spp. infections detected in our study 23.7% (544/2289) with *Plasmodium* spp. infections 23% (654/2838) shown in Damien et al. (2010) study ($p$-value = 0.5). However, a comparison of *P. malariae* infection (2.3 vs. 0.7) and co-infection *P. falciparum/P. malariae* (1.2 vs. 0.3) between both studies revealed a significant difference ($P < 0.01$) (Table 2).

## Prevalence of Plasmodium spp. infection detected by PCR

Overall, out of 1449 samples subjected to PCR analysis, 47.4% (687/1449) individuals were non-infected and 762 individuals, 52.6% (762/1449), displayed *Plasmodium* spp infection including 43.7% (634/1449), 8.8% (128/1449) as mono and co-infection, respectively (Fig. 2). *P. falciparum* mono-infection was detected in 38.5% (558/1449) of participants, followed by mixed infections of *P. falciparum/P. malariae* 8.8% (128/1449) and *P. malariae* mono-infection 5.2% (76/1449) (Table 3).

The prevalence of *Plasmodium* spp. infections in different geographical settings and age groups are shown in Figs. 5 and 6, respectively. *P. falciparum*, *P. malariae*, and mixed infection *P. falciparum/P. malariae* were detected in all the eight villages except Degoue, where the mixed infection was not detected, and *P. falciparum* was the dominant species followed by mixed infection.

Sub-microscopic *Plasmodium* infections were high (30.6%) and more pronounced in older participants >20 years (Fig. 7).

Furthermore, there was a correlation between the following characteristic: age and fever and the occurrence of *P.*

**Table 2** Prevalence of *Plasmodium* infection detected by microscopy in our study and Damien et al.'s study carried out in 2010

| ***Plasmodium* spp. infections** | **Microscopy ($n_1 = 2838$), Damien et al. (2010) study data** | | **Microscopy ($n_2 = 2289$). present study data** | | ***p*-value** |
|---|---|---|---|---|---|
| | *N* | Prevalence (%) | *N* | Prevalence (%) | |
| **All *Plasmodium* positive** | 654 | 23 | 544 | 23.8 | 0.5 |
| *P. falciparum* | 593 | 20.9 | 454 | 19.8 | 0.4 |
| *P. malariae* | 21 | 0.7 | 53 | 2.3 | <0.001 |
| *P. falciparum* + *P. malariae* | 9 | 0.3 | 28 | 1.2 | 0.001 |
| *P. ovale* | 14 | 0.4 | 5 | 0.2 | 0.1 |
| *P. falciparum* + *P. ovale* | 15 | 0.5 | 3 | 0.1 | 0.01 |
| *P. malariae* + *P. ovale* | 1 | 0.03 | 1 | 0.04 | 0.8 |
| *P. falciparum* + *P. malariae* + *P. ovale* | 1 | 0.03 | 0 | 0 | |

*N*, number of participants carrying *Plasmodium* infection; $n_1$, total number of participants recruited in Damien study in 2010; $n_2$, total number of participants recruited in our study

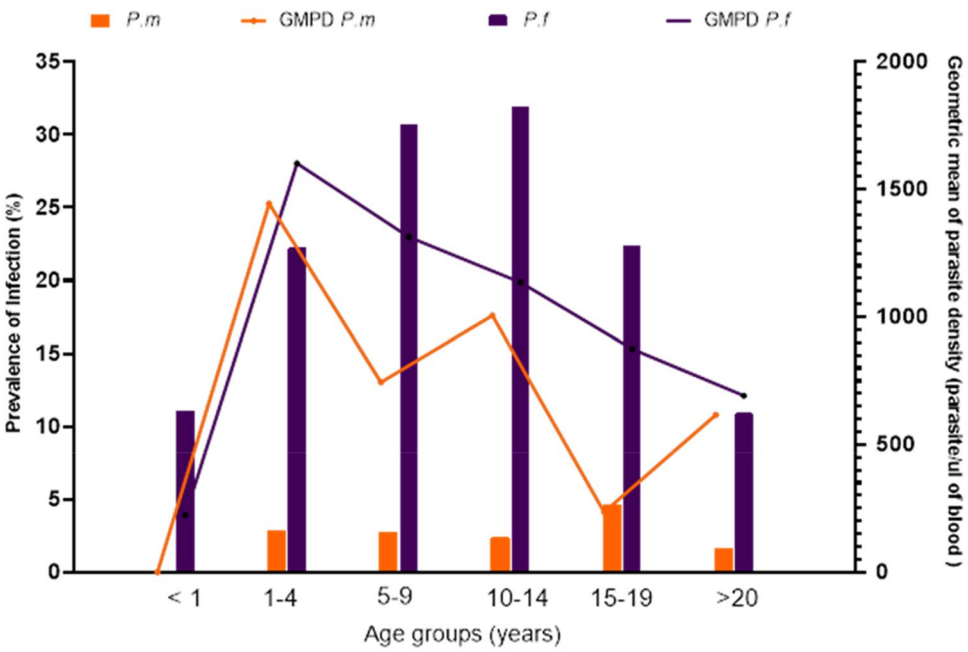


**Fig. 3** Age-specific prevalence of *P. malariae*, *P. falciparum*, and the geometric mean of parasite density (GMPD) within the study population based on microscopy

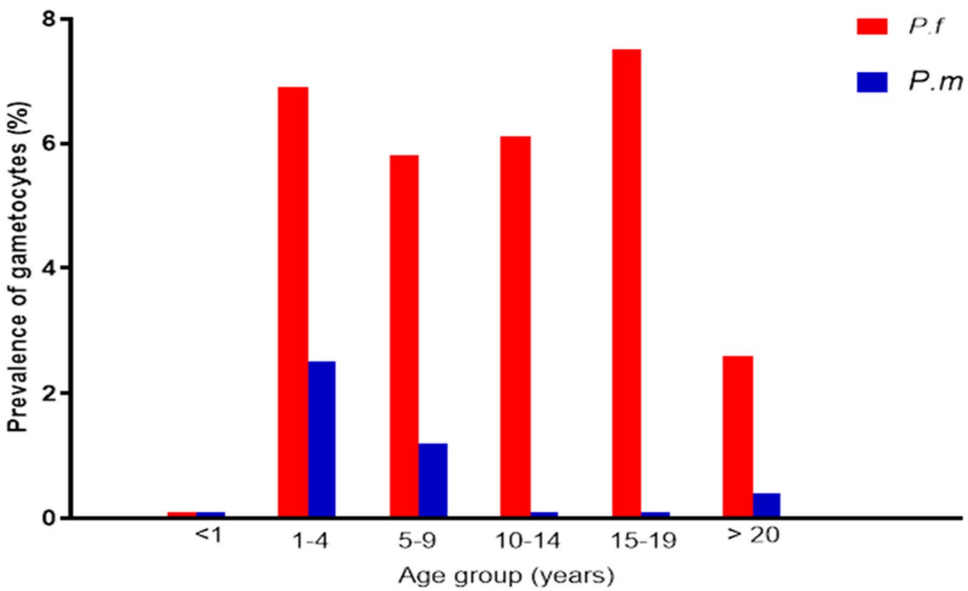


**Fig. 4** Age-specific prevalence of *P. malariae*, *P. falciparum* gametocytes within 2289 participants based on microscopy

**Table 3** Prevalence of *Plasmodium* infection detected by PCR according to the municipalities

| ***Plasmodium spp.* infections** | **Ouidah ($n$= 750)** | | **Kpomasse ($n$ = 699)** | | **Overall ($n$ = 1449)** | |
|---|---|---|---|---|---|---|
| | *N* | Prevalence (%) | *N* | Prevalence (%) | *N* | Prevalence (%) |
| **All *Plasmodium* positive** | 359 | 47.9 | 403 | 57.7 | 762 | 52.6 |
| **Mono-infections** | | | | | | |
| *P. falciparum* | 253 | 33.7 | 305 | 43.6 | 558 | 38.5 |
| *P. malariae* | 42 | 5.6 | 34 | 4.9 | 76 | 5.2 |
| **Co-infection** | | | | | | |
| *P. falciparum/P. malariae* | 64 | 8.5 | 64 | 9.1 | 128 | 8.8 |

*N*, number of participants carrying Plasmodium infection; *n*, total number of participants

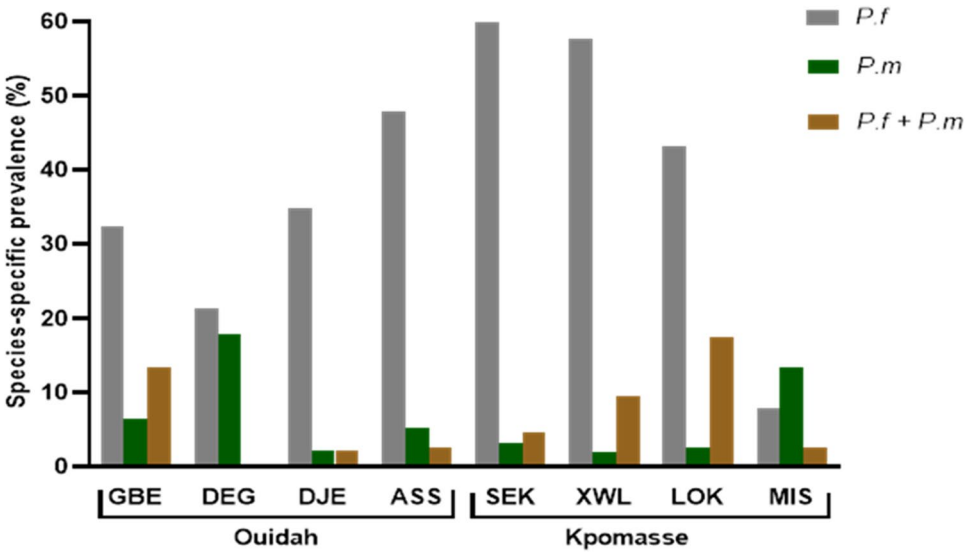


**Fig. 5** Village-specific prevalence of *Plasmodium* spp. detected by PCR (GBE, Gbehonou; DEG, Degoue; DJE, Djegbame; ASS, Assogbenoudaho; SEK, Sekome; XWL, Xlwacome; LOK, Lokogbozounta; MIS, Missite)

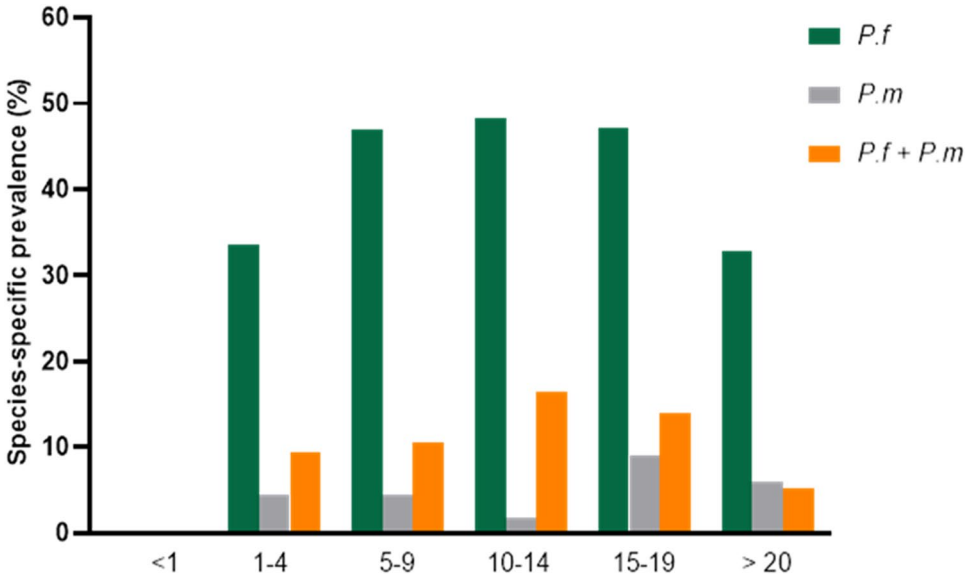


**Fig. 6** Age-specific prevalence of *Plasmodium* spp. detected by PCR

*falciparum* infection (Table 4) ($p < 0.05$). The prevalence of *P. malariae* infection was shown to be correlated with any of the recorded socio-demographic and environmental factors (Table 5) ($p < 0.05$).

## Discussion

In most malaria endemic countries in Africa, *P. falciparum* is the most prevalent (WHO, 2019a, b, c) and has been the focus of malaria research over the years (Woodford et al. 2020). Then, the real epidemiology of other *Plasmodium* species remains poorly understood.

The prevalence of *Plasmodium* parasite infection determined by microscopy and PCR in this study confirms that the *P. falciparum* remains the most prevalent *Plasmodium* spp. in our study area, as previously reported in many studies conducted in Benin and in most of the African malaria endemic areas (Amoah et al., 2019; Damien et al., 2010; Doritchamou et al., 2018; WHO, 2019a, b, c; Gnémé et al., 2013; Peprah et al., 2019).

Here, we observed 2.3% of *P. malariae* infection and 1.2% of *P. falciparum*/*P. malariae* infection, while a previous study carried out 10 years ago showed 0.7% of *P. malariae infections* and 0.3% of *P. falciparum*/*P. malariae* infections, respectively, in the rural inhabitants using microscopy (Damien et al. 2010). These results showed that the prevalence of *P. malariae* and

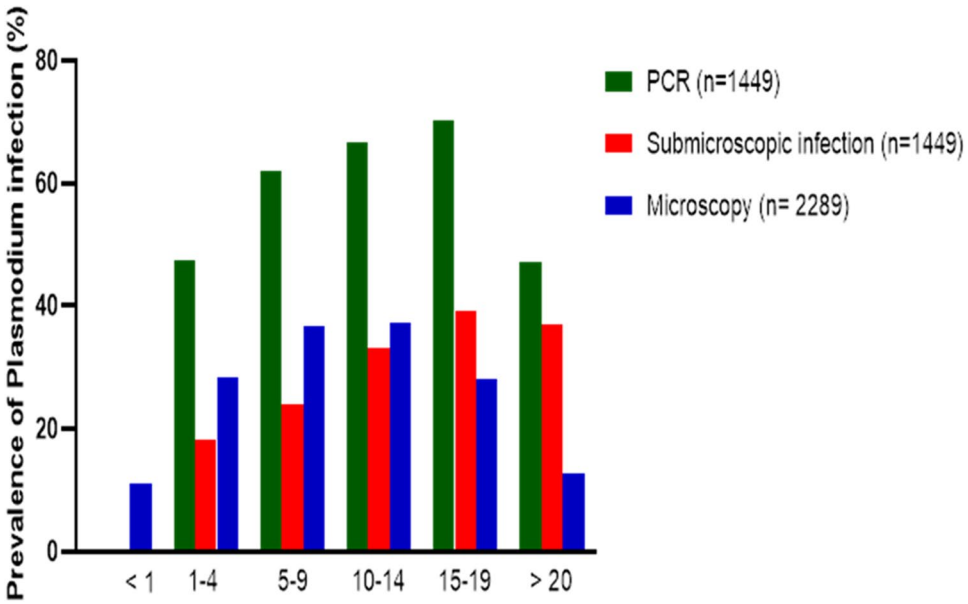


**Fig. 7** Age-specific trend of *Plasmodium* infection in microscopy, PCR, and submicroscopic

*P. falciparum*/*P. malariae* infections are significantly higher, around 3 and 4 times, respectively, after 10 years in the same study area. This finding indicates that *P. malariae* occurrence is increasing in our study area. Some factors such as climate change (humidity and temperature), which could reduce sporogonic cycle duration in competent vectors and thereby increase the vector chance to become infectious (Greenwood 1992), genetic polymorphisms in malaria parasites as demonstrated in the *P. falciparum* infection (Adomako-Ankomah et al. 2017), and positive medication selection (Menard and Dondorp 2017) are well known to contribute to the increase of *Plasmodium* spp prevalence. Based on our data, we speculate that the most likely cause of *P. malariae* increase could be due to the positive selection of this parasite by medication. Indeed, most of the time, the individual living in our study area uses ACT to treat malaria (Zinsou and Cherifath 2017). Since *P. malariae* remains in circulation and is increasing despite the treatment, this may be due to the fact that treatment is not effective against this parasite. A study among Ghanaian school children has shown a persistent detection *of P. malariae* after ACT treatment (Dinko et al. 2013). Conversely, another study reported evidence of a high cure rate of 100% and the proven favorable tolerability profile of ACT on non-falciparum species (Mombo-Ngoma et al. 2012). Hence, it is important to initiate a longitudinal study to assess the response of non-falciparum parasites to commonly used antimalarial drugs, particularly ACT. Many studies have also reported that *P. malariae* (Damien et al., 2010; Gnémé et al., 2013; Yman et al., 2019) and the other non-falciparum species such as *P. ovale* and *P. vivax* infections are increasing in Ghana (Dinko et al., 2013; Browne et al., 2000), Uganda (Betson et al. 2014), Mali (Bernabeu et al. 2012), Senegal (Diallo et al. 2016), and Cameroun (Fru-Cho et al. 2014). More attention should be paid to *P. malariae* and should not be ignored if the goal is malaria elimination, particularly when designing future measures and control interventions. After 10 years, *P. malariae* has increased threefold, so if nothing is done, its prevalence may increase in the population in the coming decade.

In addition, the high proportion (13.3%) of *P. malariae* gametocyte carriers observed during the study period suggests that the parasite transmission will continue occurring in the OKT health district, indicating that specific attention should be paid to *P. malariae*. Many authors demonstrated that gametocytes could maintain the malaria transmission cycle within both host populations (human and vector) (Schneider et al. 2007; Ouédraogo et al. 2010). However, the infectiousness of these gametocytes in circulation determines the success of transmission within both hosts (Bousema and Drakeley 2011). Hence, further entomological studies are also needed to identify the anopheline species that could transmit *P. malariae* in our study site and assess their vectorial capacity to better understand the increased prevalence of *P. malariae*.

Our result showed the highest prevalence of *P. malariae* infection (14.1%) as mono and co-infection with *P. falciparum* in the OKT health district using PCR. Indeed, most previous studies conducted in this region have not focused on non-falciparum parasites (Savi de Tove et al., 2017) or used molecular assays such as PCR to detect malaria parasites, especially non-falciparum parasites (Damien et al. 2010; Le Port et al. 2011). This high prevalence found indicates that the prevalence of *P. malariae* in mono and co-infection can be better estimated using a molecular tool such as PCR and would be explained by the fact that *P. malariae* is present at low densities (Collins and Jeffery 2007) and is often unrecognized or underestimated by microscopists. In addition,

the national estimate of non-falciparum infection in Benin is 2% (MS, 2015). However, our data showed a very high prevalence of *P. malariae* (7 times). Many authors have shown that the prevalence of *P. malariae* becomes higher than the estimate made by the national programs. In Ghana, the prevalence of *P. malariae* was 12.7%, while the national prevalence is 2–9% (Owusu et al. 2017); in southern Nigeria, *P. malariae* was found at 10% (Oriero et al. 2020), and in Burkina Faso, it has been reported that the prevalence of *P. malariae* increased from 0.9% in 2007 to 13.2% in 2010 (Gnémé et al. 2013). In view of these observations, various authors have called on their national malaria control program to include this parasite in the management of malaria if the goal is to achieve malaria elimination. Given that our study revealed a higher prevalence than that thought by the national malaria control program, we strongly recommend that the Benin National Malaria Control Program take into account the *P. malariae* parasite when implementing strategies against malaria transmission in Benin.

In our study, the prevalence of submicroscopic *Plasmodium* infections was high (30.6%) and was more pronounced in the older participants >20 years. This result suggests that the older participants could harbor undetectable infection under microscopy. This could be explained by the fact that we found lower parasitemia in this age group compared to other age groups and is consistent with previous studies (Idris et al. 2016; Amoah et al. 2019). Indeed, it is well established that the older participants acquired a high level of immunity (anti-parasitic immunity), and that helps them to maintain the parasites at lower densities (Okell et al., 2009). These sub-microscopic infections may be an important source of local transmission since the age group (>20 years) harboring higher sub-microscopic infections are not taken into account in the malaria prevention strategies. A study carried out in Kenya has shown lower parasite density and the highest sub-microscopic infections in participants aged >30 years (Idris et al. 2016). However, these observations (sub-microscopic infections) have to be further explored by implementing longitudinal studies in different geographical settings.

Taking into account that *P. malariae* mono and co-infection could create a lot of damage to human health (Eke, 2007; Langford et al., 2015; Kotepui et al., 2020) and since our results provide evidence that these infections increased after 10 years, we could recommend the following actions to the national malaria program in order to remain in the same vision with the World Health Organization who sets the goal of malaria elimination by 2030 in the Global Technical Strategy for Malaria 2016–2030 (WHO-GTS) ( WHO, 2016). Firstly, there is a need for sensitive and field-applicable methods to identify specifically *P. malariae* in malaria-endemic areas. Secondly, the National Malaria Control Program could extend control strategies to older age groups, as current malaria intervention strategies such as seasonal malaria chemoprevention (SMC) target only children aged 3–59 months, while in our study, the *Plasmodium* spp. infections were higher in older age groups; hence, these groups could serve as reservoirs for future expansion. Thirdly, the Benin National Malaria Control Program should think to initiate some local surveys screening inhabitants in order to evaluate the burden of all the *Plasmodium* species in malaria transmission.

## Conclusion

This study provided insights into malaria infection, indicating that *P. malariae* infection prevalence is increasing and the submicroscopic infection was high and more pronounced in older participants. Since our results provide valuable evidence of increasing *P. malariae* infection, it is important that the NMCP considers this parasite when designing future measures for effective control and malaria treatment. Our study was restricted to the OKT health district in Southern Benin; hence, there is a need to put in place a countrywide epidemiological survey. Moreover, future studies related to malaria vectors should be made to support the above conclusions drawn from the observations.

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**Author contribution** **Romuald Agonhossou**: investigation, methodology, writing – original draft, formal analysis, review and editing. **Romaric Akoton**: investigation, methodology, writing – original draft, review and editing. **Yannelle A. Dossou**: investigation. **Euripide Avokpaho**: investigation. **Jacques D. M. Ntabi**: investigation. **Terence S. Boussougou-Sambe**: investigation. **Nongley N. Francis**: investigation. **Cyrille Ndo**: investigation. **Francine Ntoumi**: review and editing. **Charles S. Wondji**: review and editing. **Ayola A. Adegnika**: review and editing. **Steffen Borrmann**: conceptualization, funding acquisition, methodology, project administration, review and editing. **Saadou Issifou**: conceptualization, funding acquisition, investigation, methodology, project administration, supervision, validation, visualization. **Luc S Djogbénou**: conceptualization, funding acquisition, investigation, methodology, project administration, supervision, validation, visualization, review and editing

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**Data Availability** All data generated or analyzed during this study are included in the article.

## Declarations

**Ethics approval and consent to participate** Ethical clearance (N°115/2018/CER-ISBA/FSS/UAC of 29th October 2018) was given for the study by the Ethical Committee of the Faculty of Sciences and Health, and the authorities approved the study of Ouidah–Kpomasse–Tori Bossito health district. Written informed consent was obtained from the volunteer inhabitants enrolled. The informed consent form and survey were signed/filled out by a parent or tutor for participants aged less than 18 years old. Malaria-positive cases among recruited participants were treated with ACT, according to NMCP guidelines.

**Conflict of interest** The authors declare no competing interests.

## References


Adomako-Ankomah Y, Chenoweth MS, Durfee K et al (2017) High *Plasmodium falciparum* longitudinal prevalence is associated with high multiclonality and reduced clinical malaria risk in a seasonal transmission area of Mali. PLoS One 12:1–15. https://doi.org/10.1371/journal.pone.0170948

Amoah LE, Donu D, Abuaku B et al (2019) Probing the composition of *Plasmodium* species contained in malaria infections in the Eastern region of Ghana. BMC Public Health 19:1–11. https://doi.org/10.1186/s12889-019-7989-1

Bejon P, Williams TN, Nyundo C et al (2014) A micro-epidemiological analysis of febrile malaria in coastal Kenya showing hotspots within hotspots. Elife 2014:1–13. https://doi.org/10.7554/eLife.02130

Bernabeu M, Gomez-Perez GP, Sissoko S et al (2012) *Plasmodium vivax* malaria in Mali: a study from three different regions. Malar J 11:1–6. https://doi.org/10.1186/1475-2875-11-405

Betson M, Sousa-Figueiredo JC, Atuhaire A et al (2014) Detection of persistent *Plasmodium* spp. infections in Ugandan children after artemether-lumefantrine treatment. Parasitology 141:1880–1890. https://doi.org/10.1017/S003118201400033X

Bousema T, Drakeley C (2011) Epidemiology and infectivity of *Plasmodium falciparum* and *Plasmodium vivax* gametocytes in relation to malaria control and elimination. Clin Microbiol Rev 24:377–410. https://doi.org/10.1128/CMR.00051-10

Brazier AJ, Avril M, Bernabeu M, et al (2017) Pathogenicity determinants of the human malaria parasite *Plasmodium falciparum* have ancient origins. mSphere 2:1–13. https://doi.org/10.1128/msphere.00348-16

Bridges DJ, Winters AM, Hamer DH (2012) Malaria elimination: surveillance and response. Pathog Glob Health 106:224–231. https://doi.org/10.1179/2047773212Y.0000000035

Browne EN, Frimpong E, Sievertsen J et al (2000) Malariometric update for the rainforest and savanna of Ashanti region, Ghana. Ann Trop Med Parasitol 94:15–22. https://doi.org/10.1093/infdis/jix686

Camargo-Ayala PA, Cubides JR, Niño CH et al (2016) High *Plasmodium malariae* prevalence in an endemic area of the colombian amazon region. PLoS One 11:1–17. https://doi.org/10.1371/journal.pone.0159968

Collins WE, Jeffery GM (2007) *Plasmodium malariae:* parasite and disease. Clin Microbiol Rev 20:579–592. https://doi.org/10.1128/CMR.00027-07

Damien GB, Djènontin A, Rogier C et al (2010) Malaria infection and disease in an area with pyrethroid-resistant vectors in southern Benin. Malar J 9:1–11

Diallo MA, Badiane AS, Diongue K et al (2016) Non-falciparum malaria in Dakar: a confirmed case of *Plasmodium ovale wallikeri* infection. Malar J 15:1–6. https://doi.org/10.1186/s12936-016-1485-1

Dinko B, Oguike MC, Larbi JA et al (2013) Persistent detection of *Plasmodium falciparum*, *P. malariae*, *P. ovale curtisi* and *P. ovale wallikeri* after ACT treatment of asymptomatic Ghanaian school-children. Int J Parasitol Drugs Drug Resist 3:45–50. https://doi.org/10.1016/j.ijpddr.2013.01.001

Doritchamou JYA, Akuffo RA, Moussiliou A et al (2018) Submicroscopic placental infection by non-falciparum Plasmodium spp. PLoS Negl Trop Dis 12:1–17. https://doi.org/10.1371/journal.pntd.0006279

Ehrich JHH, Eke FU (2007) Malaria-induced renal damage: facts and myths. Pediatr Nephrol 22:626–637. https://doi.org/10.1186/s40168-018-0435-2

Fru-Cho J, Bumah VV, Safeukui I et al (2014) Molecular typing reveals substantial *Plasmodium vivax* infection in asymptomatic adults in a rural area of Cameroon. Malar J 13:1–11. https://doi.org/10.1186/1475-2875-13-170

S Georges S Viriyakbosola X Ping W Jarra 1993 High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. 61 315 320

Gnémé A, Guelbéogo WM, Riehle MM et al (2013) *Plasmodium* species occurrence, temporal distribution and interaction in a child-aged population in rural Burkina Faso. Malar J 12:1–9. https://doi.org/10.1186/1475-2875-12-67

B Greenwood 1992 Malaria — obstacles and opportunities Parasitology Today https://doi.org/10.1016/0169-4758(92)90179-6

Idris ZM, Chan CW, Kongere J et al (2016) High and heterogeneous prevalence of asymptomatic and sub-microscopic malaria infections on islands in Lake Victoria, Kenya. Sci Rep 6:1–13. https://doi.org/10.1038/srep36958

INSAE (2018) Cinquième Enquête Démographique et de Santé au Bénin (EDSB-V)

Kotepui M, Kotepui KU, De Jesus Milanez G, Masangkay FR (2020) *Plasmodium* spp. mixed infection leading to severe malaria: a systematic review and meta-analysis. Sci Rep 10:1–12. https://doi.org/10.1038/s41598-020-68082-3

Langford S, Douglas NM, Lampah DA et al (2015) *Plasmodium malariae* infection associated with a high burden of anemia: a hospital-based surveillance study. PLoS Negl Trop Dis 9:1–16. https://doi.org/10.1371/journal.pntd.0004195

A Port Le L Watier G Cottrell et al 2011 Infections in infants during the first 12 months of life: role of placental malaria and environmental factors PLoS One 6 https://doi.org/10.1371/journal.pone.0027516

Menard D, Dondorp A (2017) Antimalarial drug resistance: a threat to malaria elimination. Cold Spring Harb Perspect Med 7:1–24. https://doi.org/10.1101/cshperspect.a025619

Mombo-Ngoma G, Kleine C, Basra A et al (2012) Prospective evaluation of artemether-lumefantrine for the treatment of non-falciparum and mixed-species malaria in Gabon. Malar J 11:1–6. https://doi.org/10.1186/1475-2875-11-120

Mueller I, Zimmerman PA, Reeder JC (2007) *Plasmodium malariae* and *Plasmodium ovale* – the 'bashful' malaria parasites. Trends Parasitol. 23(6):278–283. https://doi.org/10.1016/j.pt.2007.04.009

Okell LC, Ghani AC, Lyons E, Drakeley CJ (2009) Submicroscopic infection in *Plasmodium falciparum*-endemic populations: a systematic review and meta-analysis. J Infect Dis 200:1509–1517

OMS (2014) Techniques de base pour le diagnostic microscopique du paludisme. Guid du Stag Partie I:

Oriero EC, Olukosi AY, Oduwole OA et al (2020) Seroprevalence and parasite rates of *Plasmodium malariae* in a high malaria transmission setting of southern Nigeria. Am J Trop Med Hyg 103:2208–2216. https://doi.org/10.4269/ajtmh.20-0593

Ouédraogo AL, Bousema T, De Vlas SJ et al (2010) The plasticity of *Plasmodium falciparum* gametocytaemia in relation to age in Burkina Faso. Malar J 9:1–8. https://doi.org/10.1186/1475-2875-9-281

Owusu EDA, Brown CA, Grobusch MP, Mens P (2017) Prevalence of *Plasmodium falciparum* and non-*P. falciparum* infections in a highland district in Ghana, and the influence of HIV and sickle cell disease. Malar J 16:10–17. https://doi.org/10.1186/s12936-017-1823-y

Peprah S, Tenge C, Genga IO et al (2019) A cross-sectional population study of geographic, age-specific, and household risk factors for asymptomatic *Plasmodium falciparum* malaria infection in western Kenya. Am J Trop Med Hyg 100:54–65. https://doi.org/10.4269/ajtmh.18-0481

Plowe CV, Djimde A, Bouare M et al (1995) Pyrimethamine and proguanil resistance-conferring mutations in *Plasmodium falciparum* dihydrofolate reductase: polymerase chain reaction methods for surveillance in Africa. Am J Trop Med Hyg 52:565–568. https://doi.org/10.1016/S0140-6736(19)31097-9

PNLP (2015) Directives nationales de prise en charge des cas de paludisme, Edition révisée

Savi de Tove YS, Hounto AO, Alao MJ et al (2017) Prevalence of *Plasmodium falciparum* parasitaemia in exclusively breastfed children aged 0–6 month in the Ouidah Kpomassè-Tori-Bossito health region in Benin. J Bacteriol Parasitol 09:0–5. https://doi.org/10.4172/2155-9597.1000332

Schneider P, Bousema JT, Gouagna LC et al (2007) Submicroscopic *Plasmodium falciparum* gametocyte densities frequently result in mosquito infection. Am J Trop Med Hyg 76:470–474. https://doi.org/10.4269/ajtmh.2007.76.470

Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). Biometrika 52:591–611. https://doi.org/10.1093/biomet/52.3-4.591

WHO (2019) Global malaria programme. WHO Regional Office for Africa. vailable from: https://www.who.int/news-room/fact-sheets/detail/malaria.

WHO (2016) World malaria report 2016

WHO (2019) The role of laboratory diagnosis to support malaria disease management Focus on the use of rapid diagnostic tests in areas of high transmission.Report of a who technical consultation, 25-26 October 2004

WHO (2015) Guidelines for the treatment of malaria, Third edition

WHO (2019) Malaria terminology global malaria programme. Available from: http://www.who.int/malaria

WHO (2020) World Malaria Report 2020

P Winskill PG Walker RE Cibulskis AC Ghani 2020 Prioritizing the scale-up of interventions for malaria control and elimination Malar J 1–11 https://doi.org/10.1186/s12936-019-2755-5

Woodford J, Collins KA, Odedra A et al (2020) An experimental human blood-stage model for studying *Plasmodium malariae* infection. J Infect Dis 221:948–955. https://doi.org/10.1093/infdis/jiz102

V Yman G Wandell DD Mutemi et al 2019 Persistent transmission of Plasmodium malariae and Plasmodium ovale species in an area of declining Plasmodium falciparum transmission in eastern Tanzania PlosNeg Trop Dis 1–16 https://doi.org/10.1371/journal.pntd.0007414

C Zinsou AB Cherifath 2017 The malaria testing and treatment landscape in Benin Malar J 16 https://doi.org/10.1186/s12936-017-1808-x