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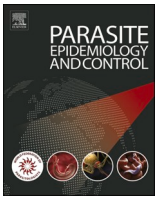

Asymptomatic *Plasmodium* infection among primary schoolchildren and *Anopheles*-mediated malaria transmission: A cross-sectional study in Ouidah; south-western Benin

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ABSTRACT

Understanding the contribution of asymptomatic *Plasmodium* carriers in malaria transmission might be helpful to design and implement new control measures. The present study explored the prevalence of asymptomatic and symptomatic *Plasmodium* infections (asexual and sexual stages) and the contribution of asymptomatic *P. falciparum* carriers to *Anopheles*-mediated malaria transmission in Ouidah (Benin). Thick and thin blood smears were examined from finger-prick blood specimens using light microscopy, and the density of both asexual and sexual stages of *Plasmodium* species was calculated. Infectivity of gametocyte-infected blood samples to *Anopheles gambiae* was assessed through direct membrane feeding assays. The prevalence of asymptomatic *Plasmodium* infections was 28.73% (289/1006). All the asymptomatic gametocyte-carriers (19/19), with gametocytaemia ranging from 10– 1200 gametocytes/μL of blood, were infectious to *An. gambiae* mosquitoes. The mean oocyst prevalences varied significantly ($\chi^2 = 16.42$, $df = 7$, $p = 0.02$) among laboratory mosquito strains (6.9– 39.4%) and near-field mosquitoes (4.9– 27.2%). Likewise, significant variation ($\chi^2 = 56.85$, $df = 7$, $p = 6.39 \times 10^{-10}$) was observed in oocyst intensity. Our findings indicate that asymptomatic *Plasmodium* carriers could significantly contribute to malaria transmission. Overall, this study highlights the importance of diagnosing and treating asymptomatic and symptomatic infection carriers during malaria control programmes.

Abbreviations: WHO, Word Health Organization; WBCs, White blood cells; ITNs, Insecticide-treated bed nets; IRS, Indoor residual spraying; DMFAs, Direct membrane feeding assays; *An.*, *Anopheles*; spp., species; s.s., sensu stricto; s.l., sensu lato; CX, Carbamates; OP, Organophosphates; G119S, Glycine substitution by Serine at codon 119; L1014F, Leucine substitution by Phenylalanine at codon 1014; DDT, Dichlorodiphenyltrichloroethane; PYR, Pyrethroids; USA, United States of America; MSaT, Mass Screening and Treatment; MDA, Mass Drug Administration; IPT, Intermittent Preventive Therapy; NMCP, National Malaria Control Programme.

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1. Introduction

Malaria remains a major public health issue in endemic countries struggling to control morbidity and mortality since the epidemiology of this disease is highly complex (Cui et al., 2012). In 2020, an estimated 228 million (95%) malaria cases worldwide occurred in the World Health Organization (WHO) African region (WHO, 2021). Strategies used to control malaria rely on the prevention of infections and case diagnosis coupled with antimalarial treatment. The most common interventions include (i) distribution of insecticide-treated bed nets (ITNs), (ii) indoor residual spraying (IRS), (iii) use of pyrimethamine-sulfadoxine for both asymptomatic and symptomatic pregnant women, (iv) seasonal chemoprevention in children aged under five years, with or without malaria symptoms, (v) use of Artemisinin-based Combination Therapy (ACT) for uncomplicated malaria (WHO, 2018) and (vi) malaria vaccine (RTS, S/AS_{01}) administration, recently recommended by WHO for children living in regions with moderate to high transmission (WHO, 2021). According to the WHO guidelines for the treatment of malaria in endemic countries, only the patients presenting a history of fever or a temperature $\geq$ 37.5 °C (symptomatic individuals) should be diagnosed and treated (WHO, 2015).

The most predominant malaria parasite *Plasmodium falciparum*, has a complex life cycle involving a human host and a mosquito vector in a favourable environment (Phillips et al., 2017). After releasing merozoites (erythrocyte invasion stages) into the human bloodstream, parasite densities can increase during the 48 h intraerythrocytic cycle, causing malaria symptoms. However, in some individuals living in endemic regions, immunity is acquired following repeated exposure to malaria parasites (Eldh et al., 2020). Although, immunity does not necessarily prevent infection but can limit parasite density and avert malaria symptoms leading to asymptomatic *Plasmodium* carriage (Tran et al., 2013).

In endemic countries, very few studies have investigated the determinants, spatial distribution and infectiousness of asymptomatic malaria carriers (Nguyen et al., 2018; Slater et al., 2019), which have been recognised to play a potential role in malaria transmission (Ciuca et al., 1963). As a result, data on asymptomatic plasmodial infections are not often considered in the design and implementation of national malaria control strategies (Hemingway et al., 2016).

Surveys performed in endemic countries using molecular tools have shown that asymptomatic *Plasmodium* carriers are common ($\geq$ 75%), whereas symptomatic infection carriers comprise only a fraction of infections despite the severity of malaria episodes (Bousema et al., 2014). In some cases, asymptomatic carriers could become symptomatic within days or weeks of initial detection (Nsobya et al., 2004), but most asymptomatic infections can persist for many months with variable parasite densities (Roucher et al., 2012).

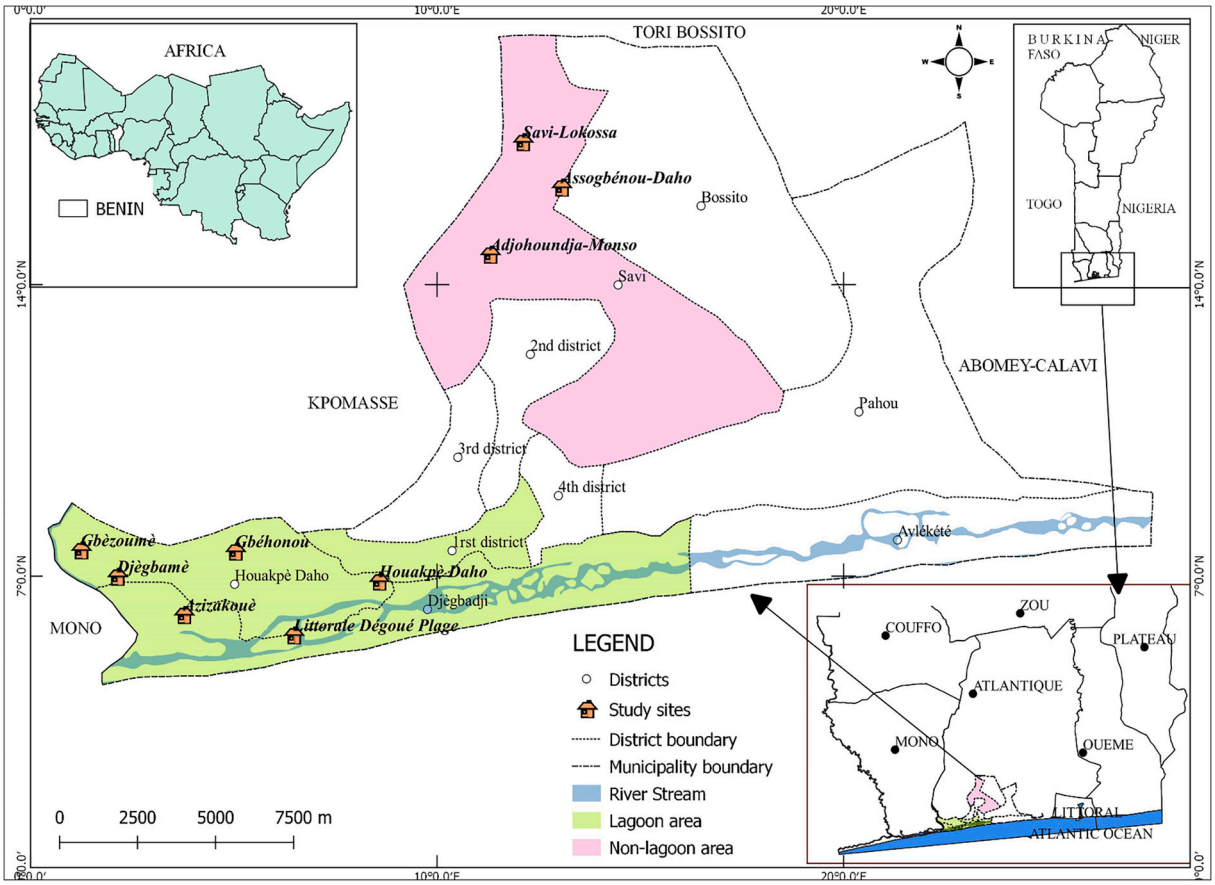


Fig. 1. Map of Ouidah showing the study sites.

Moreover, it has been demonstrated that a relatively high prevalence of asymptomatic *Plasmodium* infections could contribute to mosquito vectorial capacity according to the Ross-MacDonald model (Brady et al., 2016), which quantifies the expected number of secondary infections from a single *Plasmodium*-infected individual in a susceptible population (Smith et al., 2012).

A descriptive epidemiological survey conducted between 2007 and 2008 in the health district of Ouidah-Kpomassè-Tori Bossito (south-western Benin) among children aged 0 to 5 years indicated 21.8% annual prevalence rate of asymptomatic *P. falciparum* (both asexual blood forms and gametocytes) infections (Damien et al., 2010). Furthermore, it is known that the presence of gametocytes in the peripheral blood of the human host does not cause any clinical symptoms of malaria (Rovira-Vallbona et al., 2017). However, the transmission of gametocytes to mosquito vectors constitutes a bottleneck in the *Plasmodium* life cycle as only a few parasites ingested in mosquito bloodmeal survive in its midgut (Sinden, 2015). To our knowledge, little is known about the infectiousness of asymptomatic *P. falciparum* carriers to malaria-transmitting vectors in Benin. To provide valuable information for malaria control and elimination programmes, we investigated the infectious reservoir among schoolchildren in Ouidah, south-western Benin. The presence of asexual and sexual stages of *Plasmodium* species in asymptomatic and symptomatic carriers was examined. Direct membrane feeding assays were carried out to assess the infectivity of gametocyte-positive blood samples from asymptomatic individuals to different colonies of *An. gambiae*.

2. Materials and methods

2.1. Ethical approval

Approval for this study was obtained from the ethical committee of research of the Institute of Biomedical and Applied Science, Benin (CER-ISBA N° 88).

2.2. Study areas

The study was conducted in Ouidah municipality (south-western Benin) among schoolchildren in non-lagoon and lagoon areas (Fig. 1) selected from rural localities. Ouidah is located at 6°21′47″N–2°05′06″E, 7 m above sea level. There is a mosaic of swamps, lakes and lagoons in the surrounding areas, perennial and some of which become inundated during the rainy seasons. The climate is sub-equatorial, with temperatures ranging between 24 and 30 °C in the rainy season and between 23 and 33 °C in the dry season.

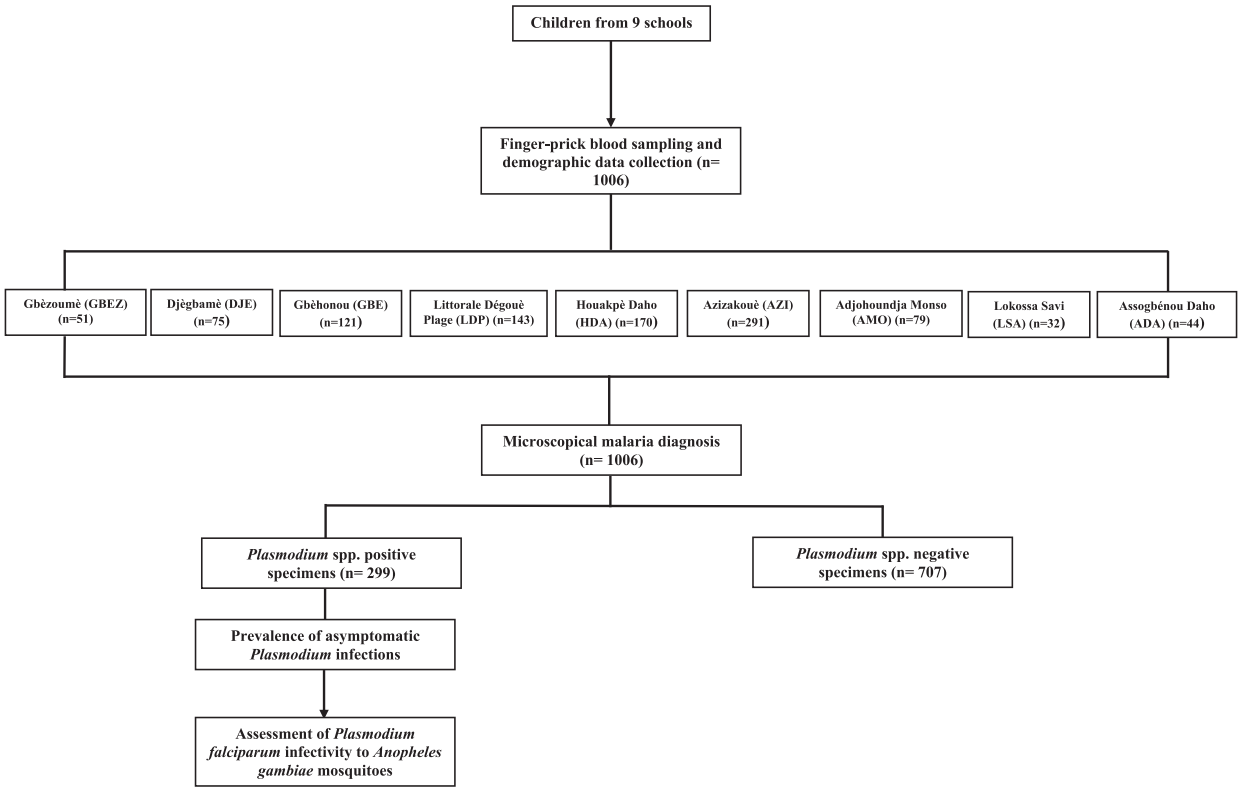


Fig. 2. Study design for determination of *Plasmodium* spp. carriers and experimental infections. n, number of participants.

2.3. Sample collection

A cross-sectional study was conducted from 5th to 28th June 2018 during the rainy season. All the children of all ages present at the selected schools and whose parents or legal guardians gave signed informed consent were enrolled in the study. For each participant, malaria symptoms such as chills, headaches, and some anthropometric parameters (age, gender and body temperature), were recorded, and a finger-prick blood sample was collected. Thick and thin blood smears were prepared. The slides were transferred to the laboratory to identify the *Plasmodium* infections on the same day. The study design for the determination of asymptomatic *Plasmodium* carriages and assessment of their infectivity to *Anopheles* mosquitoes is illustrated in Fig. 2.

2.4. Plasmodium spp. asexual and gametocyte detections

The slides were examined individually under light microscopy by three experienced clinical technicians. Densities of both asexual- and sexual-stage parasites assessed from thick blood smears were estimated using the standard diagnostic test procedures (Moody, 2002). Thin blood smears were used to identify the human pathogenic *Plasmodium* species in asexual and sexual stages (Strickland, 2000). Participants whose blood smears were detected positive for *Plasmodium* infections were referred to the local health centre and treated according to national recommendations (Ministry of Health of Benin, 2017). They were followed up and no clinical symptoms were observed in asymptomatic patients.

2.5. Assessment of asymptomatic gametocyte carrier's infectivity to Anopheles gambiae mosquitoes

2.5.1. Anopheles gambiae strains

Four established laboratory colonies of *An. gambiae* sensu stricto and four strains of near-field *An. gambiae* sensu lato collected in 2018 and kept in the insectary were used for the experimental infections. The different *An. gambiae* strains and their insecticide resistance profiles are summarized in Table 1. Briefly, the *An. gambiae* s.l. mosquitoes were collected from the areas of high insecticide resistance in Accra (Ghana) and Tiassalé (Ivory Coast) and were used as near-field strains. These near-field mosquitoes were kept under pressure of 0.05% deltamethrin and 0.1% bendiocarb according to the WHO protocol described in (WHO, 2016) to maintain their insecticide-resistance profiles. We used deltamethrin and bendiocarb insecticides because they were used respectively in ITNs (Green et al., 2009; N'Guessan et al., 2010) and IRS (Akogbeto et al., 2011; Protopopoff et al., 2013) for malaria vector control in African countries. Both laboratory and near-field mosquitoes were reared under the same insectary conditions ($27 \pm 2\,^{\circ}C$ ambient temperature and $80 \pm 8\%$ relative humidity). The different strains of *An. gambiae* were used in these experiments to assess the infectivity of asymptomatic *P. falciparum* gametocytes to the major African malaria vectors as a proxy for mosquito diversity occurring in natural settings.

2.5.2. Direct membrane feeding assays

Direct Membrane Feeding Assays (DMFAs) were performed as previously described (Alout et al., 2013) with minor modifications. The *P. falciparum* gametocyte-infected blood samples were collected from consenting participants by venipuncture into heparin tubes. Donor serums were replaced with heat-inactivated European naïve AB^{+} serum (Sigma-Aldrich, cat n°: H4522) to limit the potential transmission-blocking effects of human immunity (Gouagna et al., 2004a). Three (3) to four (4) day-old mosquito females, starved for twenty-four (24) hours (with access to water-moistened cotton wool), were allowed to feed on reconstituted blood for up to 20 min. All mosquito strains were fed with each gametocyte-infected blood sample. Afterwards, unfed mosquitoes were discarded, and the remainder were kept with 10% honey solution daily under insectary conditions.

2.5.3. Oocyst detection and counting

On the seventh day after experimental infections, the midguts of blood-fed mosquito females were individually dissected in a drop

Table 1
An. gambiae strains and their insecticide resistance profiles.

An. gambiae strains	Type of the strains	Known resistance mutations	Resistance profiles	References
Kisumu (Ki)	Laboratory colonies	None	Susceptible to all insecticides	(Shute, 1956)
KisKdr (Kd)		Homozygous for *kdr*^{R}(L1014F) mutation	Pyrethroids and DDT resistant	(Alout et al., 2013)
AcerKis (Ac)		Homozygous for *ace-1*^{R}(G119S) mutation	Carbamates and organophosphates resistant	(Djogbénou et al., 2007)
AcerKdrKis (Ak)		Homozygous for both *kdr*^{R}(L1014F) and *ace-1*^{R}(G119S) mutations	Pyrethroids and DDT, carbamates and organophosphates resistant	(Assogba et al., 2014, p. 20)
ASD	Near-field strains	Unknown	Deltamethrin resistant	(Mitchell et al., 2012)
ASB		Unknown	Bendiocarb resistant	
TSD		Unknown	Deltamethrin resistant	(Chouaibou et al., 2012)
TSB		Unknown	Bendiocarb resistant	

ASD, Accra selected with deltamethrin; ASB, Accra selected with bendiocarb; TSD, Tiassalé selected with deltamethrin; TSB, Tiassalé selected with bendiocab.

of 0.05% mercurochrome solution. The oocysts were then counted from each infected midgut under light microscopy with a magnification of 10×, and oocyst prevalence and intensity were assessed.

2.6. Statistical analysis

Children presenting malaria symptoms such as chills and headaches and body temperature ≥ 37.5 °C at enrolment were classified as symptomatic participants, and those with no history of fever within the past 48 h (temperature < 37.5 °C) and who did not show any other clinical signs of malaria before inclusion in the study, were classified as asymptomatic.

Descriptive statistics were used to present the proportions for all categorical variables. Asexual parasite density and gametocytaemia assessed from thick blood smears were interpreted as the parasite number detected per 500 white blood cells (WBCs). They were converted into the parasite number/μL of blood, assuming a WBC count of 8000 WBCs/μL of blood as previously described (Munyekenye et al., 2005). The result was obtained for each thick blood smear by calculating the arithmetic mean from asexual or/and sexual parasite densities of the first, second and third readings. A fourth reading was conducted if the ratio of results between the highest and lowest parasite densities was >1.5 or if both results were <300 parasites/μL and had a difference of >100 parasites/μL. *Plasmodium* species were determined from thin blood smears by counting the number of parasites detected per 5000 red blood cells (RBCs). The slide was negative if no parasites were seen after counting 500 WBCs on the thick blood smear.

To analyse the infectivity of *P. falciparum* gametocytes to the *An. gambiae* mosquito strains, the data consisted of two response variables as follows:

- Infected status: Mosquito was termed "infected" (presence of at least one oocyst) or "not infected" (absence of oocyst) for each dissected mosquito midgut;
- Oocyst intensity: Number of oocysts counted on the infected mosquito midgut.

A *P. falciparum* gametocyte-infected blood sample was defined as infectious if it could successfully infect at least one mosquito, determined by at least one oocyst in its midgut.

GraphPad Prism software (version 8.0.2, San Diego, California, USA) was used to perform descriptive statistics. Variation in oocyst prevalence and intensity among mosquito strains was determined by a nonparametric Kruskal-Wallis test (McKight and Najab, 2010) using R statistical software version 3.4.4 (R Core Team, 2015). Statistical significance was set at $p < 0.05$.

3. Results

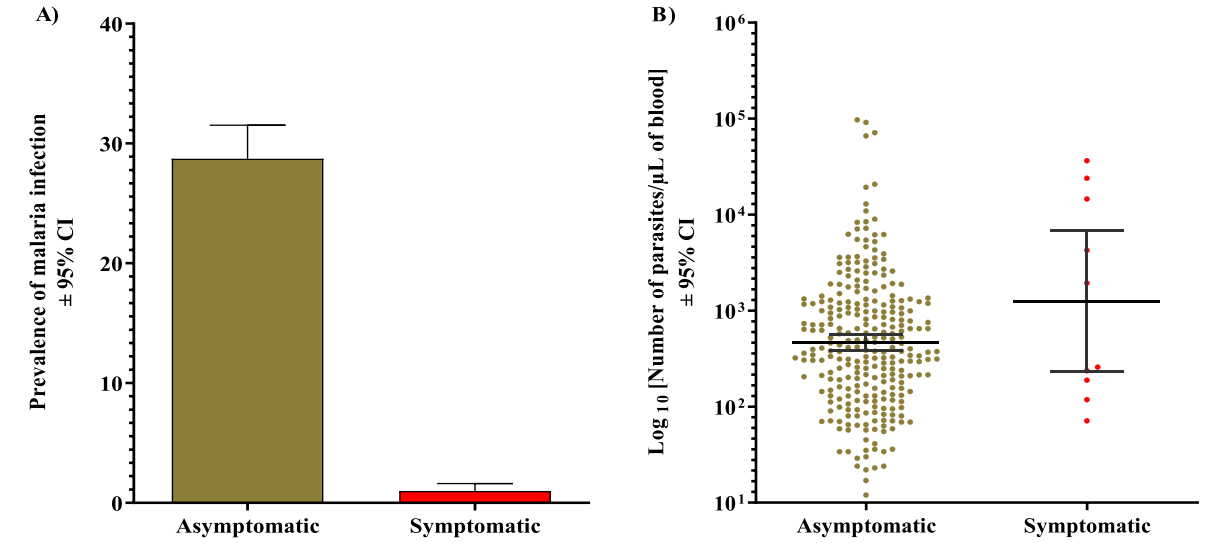
3.1. Study population and the overall prevalence of malaria infections

A total of 1006 participants from nine schools were included in the present study. The mean ages of asymptomatic and symptomatic participants were 9.5 and 9.0 years, respectively (Table 2). The overall prevalence of malaria infections was 29.72% (299/1006), largely predominant (26.24%) in lagoon areas (Fig. 3A). Out of these 299 participants, 289 were asymptomatic. Furthermore, the highest parasitaemias ranging from 12 to 97,304 parasites/μL of blood were observed in the asymptomatic carriers, while the few symptomatic patients displayed parasitaemias ranging from 71 to 36,521 parasites/μL of blood (Fig. 3 B).

Table 2
Proportions of *Plasmodium* spp., mean parasitaemia, and prevalence of gametocyte carriers by age, gender and whether symptomatic.

Participant groups	Total samples	*Plasmodium* spp. positive	Proportions of asexual parasites n(%)				Mean asexual parasite densities(p/μL)	Prevalences of gametocyte carriers, n (%)
			P. falciparum	*P. malariae*	*P. vivax*	*P. ovale*	*P. falciparum*	*P. falciparum*
Type of infection								
Asymptomatic	972	289	285(98.62)	2(0.69)	1(0.35)	1 (0.35)	2373	37(3.68)
Symptomatic	34	10	10(100)	0(0)	0(0)	0(0)	8203	1(0.10)
Age (years)								
4–7	294	71	71(100)	0(0)	0(0)	0(0)	1672	14(1.39)
8–11	440	152	148(97.37)	2(1.32)	1(0.66)	1 (0.66)	3229	15(1.49)
12–15	260	73	73(100)	0(0)	0(0)	0(0)	3200	8(0.80)
≥ 16	12	3	3(100)	0(0)	0(0)	0(0)	1366	1(0.10)
Gender								
Female	424	107	106(99.07)	0(0)	1(0.93)	0(0)	1221	15(1.49)
Male	582	192	189(98.44)	2(1.04)	0(0)	1 (0.52)	3420	23(2.29)

n, sample size.



Study areas			Total
Lagoon	25.35	0.89	**26.24**
Non-lagoon	3.38	0.1	**3.48**

Fig. 3. Prevalence of *Plasmodium* spp. infections (panel A) and level of parasitaemias on a log scale (panel B) in each participant group. For panel A, the non-bold numbers in the table represent the prevalence (%) of the overall number of *Plasmodium*-infected children from each study area. For the parasitaemia plots (panel B), points are offset horizontally where overlapping and the error bars indicate 95% confidence intervals (CIs) around the means.

3.2. *Plasmodium species and gametocyte carriage in asymptomatic infections*

Detailed data on the proportion of each *Plasmodium* species and the prevalence of gametocyte carriers in each participant group are summarized in Table 2. Among asymptomatic infections, the proportion of *P. falciparum* mono-infection was higher (98.62%) when compared to those of other *Plasmodium* species detected, namely *P. malariae*, *P. vivax* and *P. ovale*. Moreover, 13% (37/289) of asymptomatic infections harboured *P. falciparum* gametocytes with a density ranging from 10 to 1200 gametocytes/µL of blood

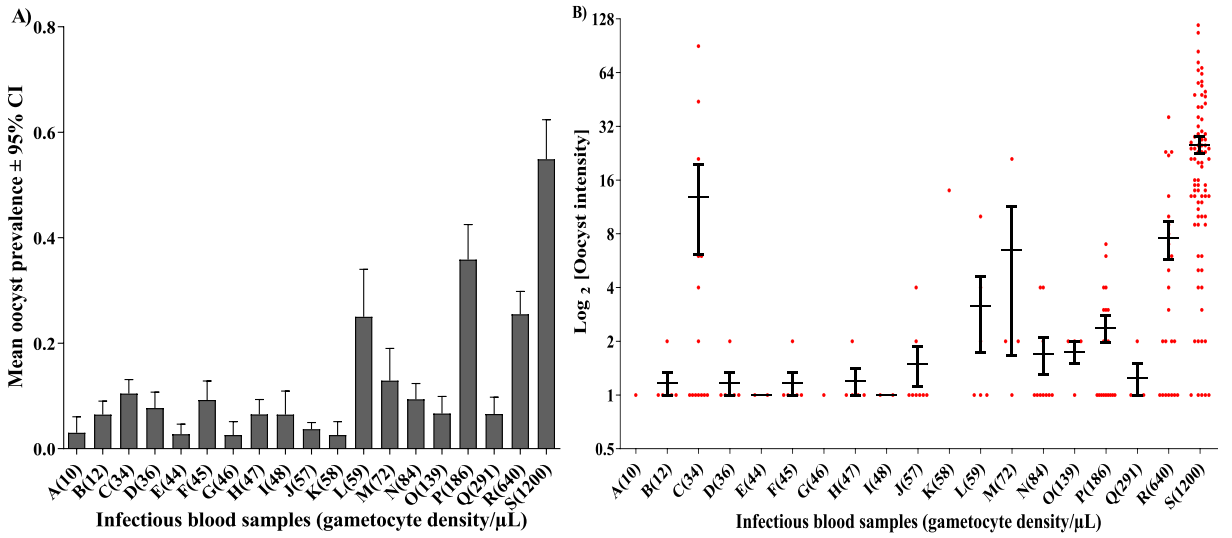


Fig. 4. Infectivity of asymptomatic carriers to *An. gambiae*. Panel A shows the prevalence of oocyst-infected mosquito females for each asymptomatic donor of the blood sample. The oocyst numbers per infected midgut are presented as the scatter dot plots and the mean oocyst intensity with 95% confidence intervals (CIs) for each infectious sample (panel B). Letters A-S denote the blood donors with the corresponding gametocytaemias in brackets. In the oocyst intensity plots on a log scale (panel B), points are offset horizontally where overlapping.

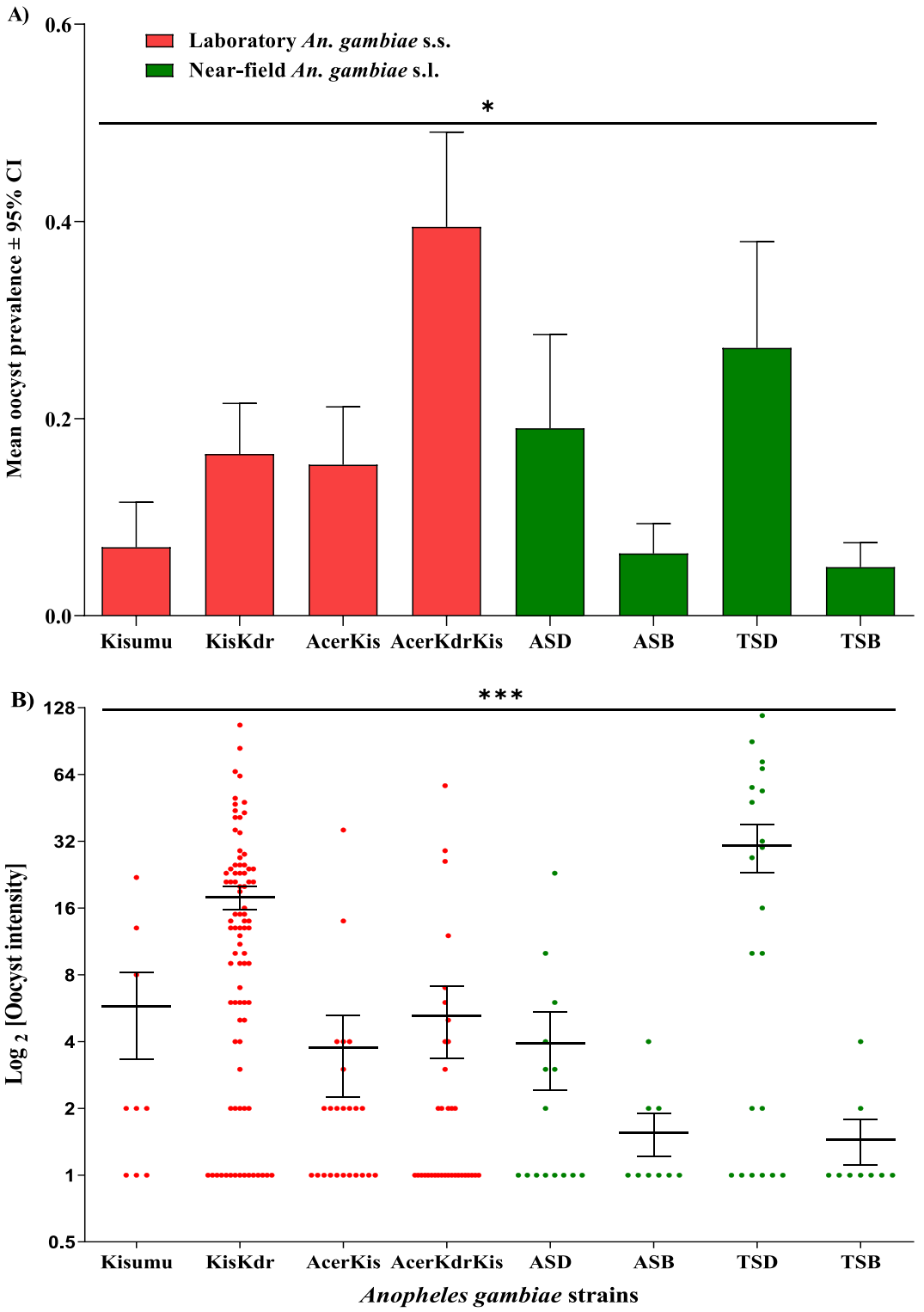


Fig. 5. Oocyst prevalences and intensities for each *An. gambiae* strain. Panels A and B show respectively the mean prevalence and number of oocysts per infected midgut for each *An. gambiae* strain. In Panel B, error bars show 95% confidence intervals (CIs) around the means and the dot plots are offset horizontally where overlapping. ASD, ASB, TSD and TSB denote respectively Accra Selected with Deltamethrin, Accra Selected with Bendiocarb, Tiassalé Selected with Deltamethrin and Tiassalé Selected with Bendiocarb.* and *** indicate respectively, $p = 0.02$ and $p = 6.39 \times 10^{-10}$.

(Fig. 4A). No co-infection of *Plasmodium* species was identified microscopically in either asymptomatic or symptomatic individuals diagnosed.

3.3. Infectivity of asymptomatic P. falciparum gametocytes to An. gambiae

Nineteen *P. falciparum* gametocyte-infected blood samples were successfully collected from asymptomatic carriers for experimental infections. The remaining gametocyte carriers did not consent to donate the blood. A total of 1486 *An. gambiae* females were dissected after the DMFAs to assess the presence of oocyst. All the asymptomatic gametocyte-infected blood samples were successfully capable of infecting mosquitoes. The oocyst prevalence and intensity assessed from each infectious gametocyte carrier (encoded A to S) are presented in Fig. 4A, B. When pooled the data from all replicates (infectious blood donors), the mean oocyst prevalences (ranging from 6.9- 39.4% and 4.9- 27.2% in the laboratory and near-field *An. gambiae*, respectively) varied significantly among mosquito strains ($\chi^2 = 16.42$, $df = 7$, $p = 0.02$) (Fig. 5A). Of the 898 laboratory mosquitoes dissected (Kisumu, KisKdr, AcerKis and AcerKdrKis), 16.37% (147/898) of individuals were found positive for *Plasmodium* infection. Meanwhile, of the 588 near-field mosquitoes dissected (ASD, ASB, TSD and TSB), 9.52% (56/588) of individuals carried *Plasmodium* parasites. These data were used to determine the intensity of oocyst infection, which varied significantly among mosquito strains ($\chi^2 = 56.85$, $df = 7$, $p = 6.39 \times 10^{-10}$) (Fig. 5B).

4. Discussion

The asymptomatic *Plasmodium* infections constitute a persistent threat to malaria elimination efforts (Lindblade et al., 2013). Herein, we investigated the prevalence of microscopic *Plasmodium* spp. infections (asexual and sexual blood stages) and the ability of asymptomatic *P. falciparum* gametocyte carriers to infect mosquitoes in mesoendemic malaria areas of Ouidah, south-western Benin.

Our study revealed a malaria infections prevalence of 29.72% (299/1006), among which 28.73% were asymptomatic. Studies from Gabon (Nkoghe et al., 2011), Rwanda (Nyirakanani et al., 2018), Nigeria (Oladeinde et al., 2014) and Uganda (Njama-Meya et al., 2004) have reported respectively 6.2%, 12.2%, 25.91% and 17% prevalence of microscopic *Plasmodium* spp. infections in different age groups of asymptomatic carriers. Although the present work was not powered to compare data between different studies, our findings showed a relatively high prevalence of asymptomatic malaria infections among schoolchildren, which might be more prevalent in general populations because of age-acquired immunity to malaria in adults. Furthermore, since submicroscopic *Plasmodium* spp. detection using polymerase chain reaction (PCR) techniques has been shown to have a greater sensitivity than microscopic diagnostic (Sattabongkot et al., 2018), the prevalence of asymptomatic malaria infections we recorded is likely a significant underestimate of that occurring in the study populations. Also, a molecular detection could reveal the presence of *Plasmodium* spp. co-infections.

Furthermore, we assessed the infectivity of gametocytes carried by asymptomatic patients to *An. gambiae*. All the asymptomatic *P. falciparum* gametocyte-infected blood samples were able to infect all *An. gambiae* strains used in this study (mean oocyst prevalence of 13.7%) with a significant variation in both oocyst prevalence and intensity among mosquito strains. A previous study has revealed that *An. gambiae* s.l. is the principal vector of malaria parasites in Ouidah, Benin (Damien et al., 2010). Herein, we had the advantage of using various laboratory and near-field strains of *An. gambiae* mosquitoes reared under well-controlled insectary conditions. Thus, in our study areas, the asymptomatic gametocyte carriers are likely to serve as a human-to-mosquito transmission reservoir of *P. falciparum* malaria.

We also observed that the infectivity level of these asymptomatic malaria patients depends on the type (laboratory/near-field colony, insecticide susceptibility status) of *An. gambiae* mosquitoes. A previous study from western Kenya reported that 53.3% (32/60) of *P. falciparum* gametocyte-infected blood samples from asymptomatic carriers were able to infect a laboratory colony of *An. gambiae* sensu stricto with 12% oocyst prevalence (Gouagna et al., 2004b). Further, Ouédraogo et al. (Ouédraogo et al., 2009) carried out a study in the northwest of Ouagadougou (Burkina Faso) and found that 68.2% (15/22) of asymptomatic individuals with microscopically detectable *P. falciparum* gametocytes successfully infected a locally colony-reared *An. gambiae* sensu stricto resulting in 13.2% oocyst prevalence. Different specific factors related to mosquito vectors, *Plasmodium* parasites and human carriers could explain the relative differences in infectivity of gametocyte-infected blood samples from asymptomatic individuals from one study area to another.

However, there is a lack of knowledge about the accurate determinants that lead to the occurrence of asymptomatic *Plasmodium* infections in endemic countries, and consequently, the real contribution of these parasite carriers to malaria transmission is still poorly understood. Indeed, in a systematic review, Hassanpour and colleagues (Hassanpour et al., 2017) emphasised that the current knowledge of the proper role of asymptomatic plasmodial infections in malaria transmission cannot yet allow an optimal elimination strategy because of the lack of prospective cohort studies on the one hand and the significant differences in the factors such as parasite species, climate and mosquito vector species between the existing studies on the other hand. Therefore, in-depth knowledge of internal and external accurate factors that determine asymptomatic plasmodial infections' occurrence and spatio-temporal dynamics might help change the paradigm to successfully draw the line against malaria and accurately move toward malaria elimination goal in endemic countries.

Benin's malaria control program targets only symptomatic patients for diagnosis and treatment, a policy termed "passive malaria surveillance" (Ministry of Health of Benin, 2017). However, the asymptomatic *Plasmodium* carriers who are neither diagnosed nor treated could contribute to maintaining malaria transmission in human populations, as outlined in this study. Karl and her team have shown that low *Plasmodium* gametocytaemia carriage, most relevant in asymptomatic individuals, sustains a stable and persistent malaria transmission in endemic areas using a mathematical modelling approach (Karl et al., 2011). Therefore, passive malaria surveillance may be a waste of resources since it alone cannot help substantially reduce malaria transmission in high endemic settings.

Therefore, any attempt at malaria elimination should target symptomatic carriers and a larger population, including individuals without any malaria symptoms, since they could harbour a high density of both asexual and sexual *Plasmodium* parasites, as reported in this study. Overall, our work highlights a transmission reservoir of *P. falciparum* malaria in Ouidah (Benin) and consequently provides insights into the relative needs of the National Malaria Control Programme (NMCP) to change the paradigm for controlling this devastating disease.

5. Conclusion

To achieve the ultimate goal of malaria elimination, it is crucial to implement or strengthen the measures such as accurate monitoring of asymptomatic malaria cases, Mass Screening and Treatment (MSaT), Mass Drug Administration (MDA), Malaria Vaccine Implementation Programmes (MVIP) and Intermittent Preventive Therapy (IPT) alongside an effective vector control.

Author contributions

AAM, LD, CJA and LSD, conceived and designed the study. AAM, LD, CJA and LSD, carried out the, AAM and LSD, analysed the data, AAM, OYD, AOH, MJD, DW and LSD, wrote the manuscript. All authors have seen and approved the final and submitted version of the manuscript.

Declaration of Competing Interest

The authors have declared that there is no competing of interest.

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