Malaria Burden and Associated Risk Factors in an Area of Pyrethroid-Resistant Vectors in Southern Benin

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Abstract. Malaria remains the main cause of morbidity and mortality in Benin despite the scale-up of long-lasting insecticidal nets (LLINs), indoor residual spraying, and malaria case management. This study aimed to determine the malaria burden and its associated risk factors in a rural area of Benin characterized by high net usage and pyrethroid-resistant mosquito vectors. A community-based cross-sectional survey was conducted in three districts in southern Benin. Approximately 4,320 randomly selected participants of all ages were tested for malaria using rapid diagnostic tests within 60 clusters. Risk factors for malaria infection were evaluated using mixed-effect logistic regression models. Despite high population net use (96%), malaria infection prevalence was 43.5% (cluster range: 15.1–72.7%). Children (58.7%) were more likely to be infected than adults (31.2%), with a higher malaria prevalence among older children (5–10 years: 69.1%; 10–15 years: 67.9%) compared with young children (<5 years: 42.1%); however, young children were more likely to be symptomatic. High household density, low socioeconomic status, young age (<15 years), poor net conditions, and low net usage during the previous week were significantly associated with malaria infection. Malaria prevalence remains high in this area of intense pyrethroid resistance despite high net use. New classes of LLINs effective against resistant vectors are therefore crucial to further reduce malaria in this area.

INTRODUCTION

During the past few decades, long-lasting insecticidal nets (LLINs), indoor residual spraying, and malaria case management. This study aimed to determine the malaria burden and its associated risk factors in a rural area of Benin characterized by high net coverage and pyrethroid-resistant mosquito vectors. A community-based cross-sectional survey was conducted in three districts in southern Benin. Approximately 4,320 randomly selected participants of all ages were tested for malaria using rapid diagnostic tests within 60 clusters. Risk factors for malaria infection were evaluated using mixed-effect logistic regression models. Despite high population net use (96%), malaria infection prevalence was 43.5% (cluster range: 15.1–72.7%). Children (58.7%) were more likely to be infected than adults (31.2%), with a higher malaria prevalence among older children (5–10 years: 69.1%; 10–15 years: 67.9%) compared with young children (<5 years: 42.1%); however, young children were more likely to be symptomatic. High household density, low socioeconomic status, young age (<15 years), poor net conditions, and low net usage during the previous week were significantly associated with malaria infection. Malaria prevalence remains high in this area of intense pyrethroid resistance despite high net use. New classes of LLINs effective against resistant vectors are therefore crucial to further reduce malaria in this area.

MATERIALS AND METHODS

Study area and design. A community-based cross-sectional survey was conducted between October and November 2019 in three districts (Cové, Zagnando, and Ouinhi) located in southern Benin. The survey was part of pre-intervention activities of a cluster randomized controlled trial designed to assess the impact of two novels dual-active ingredient LLINs (Royal Guard® net combining alpha-cypermethrin and pyriproxyfen; and Interceptor® G2 net incorporating alpha-cypermethrin and chlorfenapyr) for control of malaria transmitted by pyrethroid-resistant vectors, a part of the “New Nets Project”. The trial protocol has been fully described elsewhere.24 Malaria prevalence in the region was 36.5% in children under 5 years old according to the demographic health survey in 2018.7 The main vector control consists of the distribution of pyrethroid-only LLINs through universal coverage campaigns every 3 years (the most recent conducted in 2017) and routine service delivery to pregnant women during antenatal care and to children under 5 years during extended programs on immunization. An entomological
survey conducted concurrently with this study,\textsuperscript{25} showed that \textit{Anopheles coluzzii} and \textit{Anopheles gambiae} s.s. were the main malaria vectors and both were highly resistant to pyrethroids mediated by multiple resistance mechanisms mainly L1014F \textit{kdr} mutation (> 80%) and overexpressed mixed-function oxidases. The indoor and outdoor entomological inoculation rate (EIR) was 21.6 and 5.4 infected bites/person/month, respectively.\textsuperscript{25}

\textbf{Study population.} A census was carried out in June 2019 in the 123 villages of the study area. Houses were numbered, georeferenced, and information on the household’s residents (name, sex, age, number of sleeping areas and LLINs, relationship with the household head) were collected. Sixty clusters were identified with each cluster comprising of one village or a group of villages for an average of 200 households (approximately 1,200 residents). All resident adults or children over 6 months of age willing to participate and providing consent (or guardian’s consent) were eligible for the study.

\textbf{Study procedures.} To achieve adequate participant enrolment, enhanced community sensitization activities were conducted before and during the survey with the support of hamlet leaders and community health workers. For malaria infection prevalence assessment, 72 individuals per cluster were randomly selected (total of 4,320 participants), stratified by age (< 5 years, 5–9 years, 10–14 years, ≥ 15 years) from each of the 60 clusters.

The survey included two components: 1) a household survey and 2) a clinical survey. During the household survey, a questionnaire was administered to obtain information on possible risk factors for malaria and sampled persons were tested for malaria using rapid diagnostic tests (Malaria Pf HRP2 Ag RDT, Carestart®).

\textit{Household survey.} Age-stratified individuals were randomly selected using two-stage cluster sampling from the census list generated during the registration activity. A maximum of two members was selected per house. The household questionnaire included information on gender, age, educational status and occupation, socioeconomic status, vector control measures used (number of nets, type and condition of nets, duration of usage), previous malaria cases, and care-seeking behaviors. The physical conditions of nets was assessed visually and categorized as good, average, and poor condition based on consensus between two field staff. We also assessed the presence of potential breeding sites such as water taps, open gutters, semi-open gutters, wells, puddles, septic tanks, or rivers in the proximity of the household.

\textit{Malaria testing.} Participants were tested with an rapid diagnostic test (RDT), regardless of symptoms. A temperature record was taken for all participants using an infrared thermometer. Symptomatic malaria infection was defined by a positive RDT plus fever (temperature ≥ 37.5°C) or a history of fever in the last 48 hours. Treatment was provided free of charge if they were found positive. Any participants with severe malaria or any diagnosed ailments that could not be treated by study staff were referred to the nearest health facilities.

Hemoglobin levels were measured with a Hemocue device in children aged 5 years old and under. Anemia was defined as a hemoglobin concentration below 110 g/L. Severe, moderate, and mild anemia were respectively defined as hemoglobin levels less than 70 g/L, between 70–99 g/L and 100–109 g/L.\textsuperscript{26}

Long-lasting insecticidal net coverage was assessed with the following indicators\textsuperscript{27,28}: 1) \textit{Household net ownership} defined as the proportion of households with at least one bed net; 2) \textit{Household net access} which is the proportion of households with enough nets for every two family members (based on one net for every two members); 3) \textit{Population net access} corresponding to the proportion of individuals with access to bed nets within the households (assuming that each bed net in a household can be used by two people), and 4) \textit{Population net use} representing the proportion of individuals who reported sleeping under a bed net the previous night. Net usage was also assessed during the previous week (net used 0–4 nights, i.e., few nights, 5–6 nights, i.e., most nights, 7 nights, i.e., all nights). The physical quality of the nets were also broadly assessed by eye, with field workers classifying nets as good, average, or poor, based on its condition.

Mosquito collections were undertaken across all villages, between September and October 2019. In each village, four houses were selected for mosquito sampling using human landing catches (HLCs). To ensure the supervision of mosquito collectors, the first house was randomly selected from the study census list, while the other three were chosen within a radius of 15–20 m around the first one. Four collectors were required per house (one indoor and one outdoor replaced by two others after 6 hours of collection). Details on entomological data collection as well as baseline characteristics have been fully detailed elsewhere.\textsuperscript{25}

\textbf{Data management and statistical analysis.} All data collected during the survey were recorded in electronic forms on smartphones installed with Open Data Kit (ODK) collect and analyzed with STATA version 16 (Stata Corp LP, College Station, TX).

Descriptive statistics and 95\% confidence intervals were used to summarize demographic data. Mixed-effect logistic regression models were used to assess risk factors with clusters included as a random effect. Variables with \( P \) values below 0.2 were included in multivariate analyses and were eliminated step by step using the backward selection procedure. Only variables whose \( P < 0.05 \) were retained in the final model. For variables with more than two categories, a \( P \) value of the global test is given. Spatial similarities in malaria prevalence and EIR were examined visually using QGIS, with “natural breaks” to display the most variation in the data, given the histogram for each variable. A linear regression was used to assess the association between cluster-level malaria infection prevalence and cluster-level entomological indicators (mosquito density, EIR).

Risk factors assessed included characteristics at the household level (number of residents, head of the household gender and education, socioeconomic status, ethnic group, net ownership, and access), and individual level (age, gender, net usage). Household socioeconomic status was created by using principal component analysis with the following variables included: type of lighting, access to water, type of roof, type of floor, type of toilet, household head level of education, household crowding, and ownership of assets (motorbike, television, bike, radio, sheep, bed, phone).

\textbf{Ethical statement.} The study protocol was approved by the Benin Ministry of Health ethics committee (Reference N\textdegree 6/30/MS/DC/SGM/DRFMT/CNERS/SA) and by the institutional ethics review board of the London School of Hygiene and Tropical Medicine (N’16237). All participation was voluntary. Written informed consent was obtained from
an adult guardian in the household or given by the participant if over 18 years. Assent was sought for children over 10 years.

RESULTS

A total of 216,138 inhabitants in 54,143 households were identified as part of the census. In the baseline survey, 4,441 randomly selected participants from 3,027 households were included.

Sociodemographic characteristics of the study population. The mean number of people living in households was 5.3 (standard deviation [SD] ± 2.6) inhabitants. The number of sleeping spaces in the household was on average 2.7 (SD ± 1.3). The majority of heads of household had no formal education (71.4%) and the main ethnic group was “Fon” (77.2%). Mosquito breeding sites were observed close to 76.9% of households. Females represented 50.8% of the participants; children under 5 years old, 5–10 years, 10–15 years, and above to 15 years accounted for 16.5%, 14.4%, 13.6%, and 55.4%, respectively. Demographic, socioeconomic, and household characteristics of study participants are detailed in Table 1.

Household LLIN characteristics. Table 2 presents the characteristics of bed nets recorded in the households. The number of bed nets in the household was on average 2.5 (SD ± 1.3). Most of the nets found were insecticide-treated nets (96.5%). The majority of the bed nets were provided during the previous national campaign of 2017 (91%) with others supplied through public health routine activities (8%, from antenatal care visits and expanded program immunization). PermaNet® 2.0 was the most common LLIN type observed in the households (64.3%). Most bed nets had been used for more than 2 years (92.7%). The proportion of LLIN in poor condition was 37.9%. Household net ownership, household net access, population net access, and population net use were 95.8%, 55.9%, 79.5%, 95.8%, respectively. The majority of the participants (88.4%) declared to have slept under an LLIN every night during the previous week. Children under 5 years old had slept more often under a net every night the previous week in comparison to others (< 5 years: 93.1%; 5–10 years: 88%; 10–15 years: 84.9%; > 15 years: 87.3%).

Prevalence of malaria and analysis of potential risk factors. The prevalence of malaria infection was 43.5% (cluster range: 15.1–72.7%) (Table 3). Children less than 5 years old (malaria prevalence 42.1%), aged between 5–10 years (69.1%), and 10–15 years (67.9%) were most likely to be infected with malaria in comparison to adult participants (31.2%). However, among infected participants, the proportion of symptomatic cases was higher among children under 5 years (33.3%) in comparison to older children (5–10 years: 28.9%; 10–15 years: 23.7%) and adults (19.2%). The proportion of people presenting a fever or history of fever in the last 48 hours was 18.7% (829/4,441) of which 57.2% were malaria positive. Among children under 5 years old, anemia was present in 75.9% (cluster range: 33.3–100%).

Household population density, socioeconomic status, gender, ethnic group, age, households with enough LLIN, net use the previous week, and net usage duration were factors associated with malaria infection (Table 4). After adjusting for confounding factors, the risk of malaria infection was significantly

| Characteristics | Proportion or mean |
|-----------------|--------------------|
| **Cluster-level** |                   |
| Number of clusters | 60 |
| Number of households per cluster | Mean (range) 904 (82–5403) |
| Proportion of children under 10 years per cluster, % | Mean (range) 30.9 (25–35.2) |
| Cluster with high socioeconomic status, % (n) | Yes 62.7 (37) |
| **Household-level** |                   |
| Number of households enrolled | 3,027 |
| People living in the household | Mean (± SD) 5.3 (±2.6) |
| Number of sleeping spaces | Mean (± SD) 2.7 (±1.3) |
| Head of household gender, % (n) | Male 83.4 (2,525) |
| | Female 16.6 (502) |
| Head of household education level, % (n) | No education 71.4 (2,160) |
| | Primary education 17.2 (521) |
| | Lower secondary education 7.3 (220) |
| | Higher secondary education 2.7 (81) |
| | College/University 1.4 (45) |
| Ethnic group, % (n) | Fon 77.2 (2,336) |
| | Other* 22.8 (691) |
| Presence of potential breeding sites, % (n) | Yes 76.9 (2,209) |
| **Individual-level** |                   |
| Number of participants enrolled | 4,441 |
| Participant gender, % (n) | Male 49.1 (2,182) |
| | Female 50.8 (2,259) |
| Age, years % (n) | <= 5 16.5 (735) |
| | 5–10 14.4 (638) |
| | 10–15 13.6 (606) |
| | > 15 55.4 (2,462) |
| Education level, % (n) | Not in the age of education 16.5 (735) |
| | Not educated 52.5 (2,333) |
| | Educated 30.9 (1,373) |

SD = standard deviation.

* Other ethnic groups: Mahi, Holli, Yoruba, Goun.
higher with an increasing number of people living in the house (adjusted odds ratio [aOR] 1.32, 95% CI 1.05–1.69 for households having between three and seven members; and aOR 1.54, 95% CI 1.16–2.04 for households having more than three members in comparison to households with less than three members). People living in households with average (aOR 1.43, 95% CI 1.19–1.69) and low (aOR 1.44, 95% CI 1.16–1.79) socioeconomic levels were more at risk of malaria infection than those living in households with high socioeconomic status. The age groups at highest risk were children

### Table 2

| Characteristics                                    | Proportion or mean |
|-----------------------------------------------------|--------------------|
| **Household-level**                                |                    |
| Number of households enrolled                      | 3,027              |
| Household net ownership, % (n)                     | Yes (95.8, 2,902)  |
| Household net access, % (n)                         | Yes (55.9, 1,691)  |
| Population net access, % (95% CI)                  | Yes (79.5, 78.9–80.1) |
| Number of nets (any type) in the household         | Mean (±SD) 2.5 ± 1.3 |
| Number of LLIN in the household                    | Mean (±SD) 2.3 ± 1.3 |
| **Individual-level**                               |                    |
| Number of participants enrolled                    | 4,441              |
| Number of participants enrolled with a net         | 4,088              |
| Number of participants enrolled with an LLIN       | 3,949              |
| Origin of LLIN, % (n)                              |                    |
| National campaign (2017)                           | 91.0 (3,595)       |
| Healthcare facility (ANC)                          | 6.4 (251)          |
| Healthcare facility (EPI)                          | 1.6 (65)           |
| Public market                                      | 0.4 (16)           |
| Other                                              | 0.6 (22)           |
| Proportion of LLIN observed, % (n)                  |                    |
| Yes                                                | 99.0 (3,910)       |
| Type of LLIN, % (n)                                |                    |
| PermaNet 2.0                                       | 64.3 (2,489)       |
| Dawa plus                                          | 9.6 (374)          |
| Olyset Net                                         | 7.0 (271)          |
| Duranet                                            | 1.4 (53)           |
| Yorkool                                            | 0.5 (21)           |
| Other*                                             | 5.7 (221)          |
| Unknown                                            | 11.4 (443)         |
| Duration usage of LLIN, % (n)                      |                    |
| ≤ 1 year                                           | 7.2 (286)          |
| 2 years                                            | 64.3 (2,540)       |
| 3 years                                            | 21.1 (833)         |
| > 3 years                                          | 7.3 (290)          |
| LLIN conditions, % (n)                             |                    |
| Good                                               | 18.9 (732)         |
| Average                                            | 43.2 (1,671)       |
| Poor                                               | 37.9 (1,468)       |
| Population LLIN use, % (n)                         |                    |
| Yes                                                | 95.8 (3,782)       |
| Net usage the week before the visit, % (n)         |                    |
| All nights                                         | 88.4 (3,490)       |
| Most of the nights                                 | 8.6 (338)          |
| Few nights                                         | 3.1 (121)          |

### Table 3

| Characteristics                                    | Proportion % (n) |
|-----------------------------------------------------|-----------------|
| Number of participants enrolled, n                  | 4,441           |
| History of fever in the past 48 hours               | Yes (12.6, 562) |
| Fever* at the time of visit                         | Yes (8.0, 356)  |
| Hemoglobin test performed among children under 5 years | Yes (99.5, 731) |
| Anemia† among children under 5 years old           | Yes (75.9, 555) |
| Malaria rapid diagnostic test performed            | Yes (99.7, 4,428) |
| Malaria infection                                  | Yes (43.5, 1,924) |
| Malaria infection by age category                   |                 |
| ≤ 5 years                                          | 42.1 (309)       |
| 5–10 years                                         | 69.1 (440)       |
| 10–15 years                                        | 67.9 (410)       |
| > 15 years                                         | 31.2 (765)       |
| Proportion of symptomatic cases‡ among infected per age |                 |
| ≤ 5 years                                          | 33.3 (103/309)   |
| 5–10 years                                         | 28.9 (127/440)   |
| 10–15 years                                        | 23.7 (97/410)    |
| > 15 years                                         | 19.2 (147/765)   |
| Proportion of malaria among participants with fever or history of fever in the past 48 hours | Yes (57.3, 474) |

* Fever: infrared frontal temperature ≥ 37.5 °C.
† Hemoglobin (Hb) < 110 g/L.
‡ Symptomatic cases: defined by the presence of malaria infection without fever or history of fever in the last 48 hours.
under 5 years (aOR 1.66, 95% CI 1.38–1.99), between 5 and 10 years (aOR 5.31, 95% CI 4.33–6.53) and 10–15 years (aOR 4.88, 95% CI 3.96–6.03) in comparison to participants over 15 years. Participants who reported sleeping under nets a few nights (< 4 nights) the previous week were also more likely to be infected (aOR 1.95, 95% CI 1.29–2.94) in comparison to those who slept under nets every night. Also, household members using nets in poor condition were more likely to be infected (aOR 1.99, 95% CI 1.39–2.94) in comparison to those sleeping under nets in good condition.

We observed a similar spatial distribution of malaria vector density and the prevalence of infection within clusters (Figure 1), with both indicators being high in the north of the study area. At the cluster level, increased malaria prevalence was associated with higher human biting rate and EIR (Supplemental Figure 1, Supplemental Table 1) although R squared values were very low suggesting not a strong relationship.

**DISCUSSION**

This study examined risk factors for malaria infection in an area of intense transmission and the presence of pyrethroid-resistant vector mosquitoes. There is limited cross-sectional data available for this part of Benin and the current study represents a detailed analysis of the epidemiological picture, and compares it to the entomological outcomes reported in the same area.

Our data showed a high prevalence of malaria infection (43.5%) despite the high household net ownership and population net use. These findings are consistent with previous studies conducted in Benin. Despite there being several years since the last universal net campaign, net ownership, access, and use remained very high. The concerningly high prevalence, despite high net use, may be partially explained by the high level of pyrethroid resistance in the local malaria vectors. Indeed, entomological surveys conducted simultaneously to this study have confirmed the scale of pyrethroid resistance with mosquitoes mortality rates at 24, 48, and 72 hours postexposure lower than 40% for alpha-cypermethrin and permethrin insecticides and a frequency of the L1014F kdr mutation over 80%. Several studies have also highlighted the increasing role of pyrethroid-resistant *Anopheles* vectors in malaria transmission. However, this observation might be also related to the age and condition of the nets. In fact, we observed in this study that individuals using nets in poor condition were 1.37-fold more likely to be infected than those sleeping under nets in good condition. As the condition of the nets were only assessed by eye, rather than using the WHO hole assessment criteria, this finding should not be overinterpreted.

Children under 15 years were most likely to be infected with malaria, with children aged between 5 and 15 years at higher risk than those under 5 years. Traditionally, children aged under 5 years have been thought to be at the highest risk of malaria infection. Changes in infection burden by age group have also been reported elsewhere in SSA. This has important implications for targeting interventions and surveillance in SSA. Several studies have previously suggested that reduced transmission might lead to increased...
susceptibility to malaria infection among older children due to lower acquired immunity. However, good malaria case management in children under 5 years old might also partially explain the shift in infection burden. Despite infection being higher in older children, the most severe health consequences continue to mainly affect the youngest children. Indeed, 33.3% of the youngest children (<5 years) tested for malaria had fever whereas 28.9%, 23.7%, and 19.2% of infected children between 5–10 years, 10–15 years, and adults (>15 years) presented malaria-related morbidity, respectively. Furthermore, even though more asymptomatic, older children and adults are important drivers of malaria infection as they might not seek treatment and remain a reservoir for infection, undermining elimination efforts. They should, therefore, be considered for malaria public health interventions.

Socioeconomic status was also a factor significantly associated with malaria infection. Indeed, a recent systematic review has shown that an improved socioeconomic status would reduce dramatically the burden of malaria in SSA. Moreover, participants not sleeping under nets usually were more likely to be infected. This is in line with other studies highlighting the importance of population net usage despite the access.

We observed a similar spatial distribution of malaria infection and malaria vector density and EIR over the clusters, highlighting the usefulness of both indicators, as well as a loose association between increasing malaria prevalence and increasing mean human biting rate and EIR. Throughout the trial, we will assess the relationship between the indicators over season to see how each net type affects them.

There is little information in Benin regarding the effect of increasing LLIN use and increasing insecticide resistance on malaria vector control and impact on malaria infection. These baseline results suggested that pyrethroid LLIN provide, at best, only partial protection against malaria and the importance of trialing novel LLIN technologies and approaches.

The development and impact of insecticide resistance on malaria outcomes are key indicators to monitor. Long-lasting insecticidal nets remain a cornerstone of malaria control in Africa; however, the progress achieved so far is seriously threatened by pyrethroid-resistant malaria vectors. The increasing insecticide resistance in malaria vectors could have dramatic implications for malaria control and requires urgent remedial actions. It is, therefore, crucial to assess new classes of LLINs effective against resistant vectors to sustaining malaria reduction. Piperonyl butoxide-treated insecticidal nets have recently proven their effectiveness in malaria reduction in East Africa, though evidence of their epidemiological impact in West Africa is lacking. Other such novel generations of ITNs (pyrethroid-pyriproxyfen
and pyrethroid-chlorfenapyr ITNs are currently under evaluation in communities in Benin as part of the “New Nets Project, which aims to accelerate the impact of these new technologies across SSA.22,23

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REFERENCES

1. Bhatt S et al., 2015. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature 526: 207–211.
2. World Health Organization, 2020. World Malaria Report 2020: 20 Years of Global Progress and Challenges. Geneva, Switzerland: WHO; 2019. License: CC BY-NC-SA 3.0 IGO, 299.
3. World Health Organization, 2021. World Malaria Report 2021. Geneva, Switzerland: WHO; 2021—License: CC BY-NC-SA 3.0 IGO, 322.
4. Akogbeto MC et al., 2020. Lessons learned, challenges and outlooks for decision-making after a decade of experience monitoring the impact of indoor residual spraying in Benin, West Africa. Malar J 19: 45.
5. Kleinschmidt I et al., 2015. Design of a study to determine the impact of insecticide resistance on malaria vector control: a multi-country investigation. Malar J 14: 282.
6. National System of Health Information and Management (SNIGS), 2021. National Health Statistics 2020. Ministry of Health, Benin. 277. Available at: https://sante.gov.bw/assets/ressources/pdf/Annuaire_2020_MS_VF%20(1).pdf. Accessed June 15, 2022.
7. National Institute of Statistical and Economic Analysis (INSAE), 2019. Demographic Health Survey in Benin, 2017–2018: Key Indicators. Cotonou, Bénin et Rockville, MD: INSAE et ICF.
8. Chander F, Darrier F, Manga L, Akogbeto M, Faye O, Mouchet J, Guillet P, 1999. Status of pyrethroid resistance in Anopheles gambiae sensu lato. Bull World Health Organ 77: 230–234.
9. Ranson H, Lissenden N, 2016. Insecticide resistance in African Anopheles mosquitoes: a worsening situation that needs urgent action to maintain malaria control. Trends Parasitol 32: 187–196.
10. Djogbénou L, Pasteur N, Bio-Bangana S, Baidet T, Irish SR, Akogbeto M, Weill M, Chade F, 2010. Malaria vectors in the Republic of Benin: distribution of species and molecular forms of the Anopheles gambiae complex. Acta Trop 114: 116–122.
11. Akogbeto M, Yakoubou S, 1999. 1999. Resistance of malaria vectors to pyrethrins used for impregnating mosquito nets in Benin, West Africa. Bull Soc Pathol Exot 92: 123–130.
12. Yadouloue AW et al., 2010. Insecticide resistance status in Anopheles gambiae in southern Benin. Malar J 9: 83.
13. Aïkpon R, Sézolin M, Osse R, Akogbeto M, 2014. Evidence of multiple mechanisms providing carbamate and organophosphate resistance in field An. gambiae population from Atacora in Benin. Parasit Vectors 7: 568.
14. Gnanouoven V et al., 2015. Malaria vectors resistance to insecticides in Benin: current trends and mechanisms involved. Parasit Vectors 8: 223.
15. Ngoufo C, N’Guesan R, Fagbohoujn J, Subramanian K, Odjo A, Fongnikin A, Akogbeto M, Weetman D, Rowland M, 2015. Insecticide resistance profile of Anopheles gambiae from a phase II field station in Còvé, southern Benin: implications for the evaluation of novel vector control products. Malar J 14: 464.
16. Ngoufo C, N’Guesan R, Fagbohoujn J, Subramanian K, Odjo A, Fongnikin A, Akogbeto M, Weetman D, Rowland M, 2015. Insecticide resistance profile of Anopheles gambiae from a phase II field station in Còvé, southern Benin: implications for the evaluation of novel vector control products. Malar J 14: 464.
17. Akogbeto M, Yakoubou S, 1999. 1999. Resistance of malaria vectors to pyrethrins used for impregnating mosquito nets in Benin, West Africa. Bull Soc Pathol Exot 92: 123–130.
18. Bradley J et al., 2017. Insecticide-treated nets provide protection against malaria to children in an area of insecticide resistance in Southern Benin. Malar J 16: 225.
19. Tokponnon FT et al., 2019. Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: evidence from health facility data from Benin. Malar J 18: 37.
20. Kleinschmidt I et al., 2018. Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: a WHO-coordinated, prospective, international, observational cohort study. Lancet Infect Dis 18: 640–649.
21. Lindsay SW, Thomas MB, Kleinschmidt I, 2021. Threats to the effectiveness of insecticide-treated bednets for malaria control: thinking beyond insecticide resistance. Lancet Glob Health 9: e1325–e1335.
22. N’Guesan R, Odjo A, Ngoufo C, Malone D, Rowland M, 2016. A chlorfenapyr mixture net interceptor® G2 shows high efficacy and wash durability against resistant mosquitoes in West Africa. PLoS ONE 11: e0165925.
23. Ngoufo C, Agevo A, Fagbohoujn J, Fongnikin A, Rowland M, 2020. Efficacy of Royal Guard, a new alpha-cypermethrin and pyrthropinox treated mosquito net, against pyrethroid-resistant malaria vectors. Sci Rep 10: 12227.
27. Seyoum D, Spaybroeck N, Duchateau L, Brandt P, Rosas-Aguirre A, 2017. Long-lasting insecticide net ownership, access and use in southwest Uganda: a community-based cross-sectional study. *Int J Environ Res Public Health* 14: E1312.

28. Roll Back Malaria Monitoring & Evaluation Reference Group, 2018. *Household Survey Indicators for Malaria Control*. 43. Available at: https://www.malariasurveys.org/documents/Household%20Survey%20Indicators%20for%20Malaria%20Control_FINAL.pdf. Accessed June 15, 2022.

29. Damien GB, Djennontin A, Rogier C, Corbel V, Bangana SB, Chandra F, Akogbeto M, Kindé-Gazzard D, Massougbdji A, Henry M-C, 2010. Malaria infection and disease in an area with pyrethroid-resistant vectors in southern Benin. *Malar J* 9: 380.

30. Nahum A et al., 2010. Malaria incidence and prevalence among children living in a peri-urban area on the Coast of Benin, West Africa: a longitudinal study. *Am J Trop Med Hyg* 83: 465–473.

31. Wang S-J, Lengeler C, Smith TA, Vounatsou P, Akogbeto M, Tan-Tan N et al., 2016. Age, spatial, and temporal variations in hospital admissions with malaria in Kili County, Tanzania: a longitudinal, observational cohort study. *Malar J* 9: 45.

32. Matowo NS et al., 2021. An increasing role of pyrethroid-resistant *Anopheles funestus* in malaria transmission in the Lake Zone, Tanzania. *Sci Rep* 11: 13457.

33. Rehman AM et al., 2011. How much does malaria vector control quality matter: the epidemiological impact of holed nets and inadequate indoor residual spraying. *PLoS ONE* 6: e19205.

34. Mogeni P et al., 2016. Age, spatial, and temporal variations in hospital admissions with malaria in Kilifi County, Kenya: a 25-year longitudinal observational study. *PLoS Med* 13: e1002047.

35. Bouyou-Akotet MK et al., 2009. Evidence of decline of malaria in the general hospital of Libreville, Gabon from 2000 to 2008. *Malar J* 8: 300.

36. West PA et al., 2013. Malaria risk factors in north west Tanzania: the effect of spraying, nets and wealth. *PLoS ONE* 8: e56578.

37. Woolhouse ME, 1998. Patterns in parasite epidemiology: the peak shift. *Parasitol Today Pers Ed* 14: 428–434.

38. Snow RW, Marsh K, 2002. The consequences of reducing transmission of *Plasmodium falciparum* in Africa. *Adv Parasitol* 52: 235–264.

39. Nkumama IN, O’Meara WP, Osier FHA, 2017. Changes in malaria epidemiology in Africa and new challenges for elimination. *Trends Parasitol* 33: 128–140.

40. Roca-Feltner A, Cameiro I, Smith L, Schellenberg JRA, Greenwood B, Schellenberg D, 2010. The age patterns of severe malaria syndromes in sub-Saharan Africa across a range of transmission intensities and seasonality settings. *Malar J* 9: 282.

41. Paton RS et al., 2021. Malaria infection and severe disease risks in Africa. *Science* 373: 926–931.

42. Andolina C et al., 2021. Sources of persistent malaria transmission in a setting with effective malaria control in eastern Uganda: a longitudinal, observational cohort study. *Lancet Infect Dis* 21: 1568–1578.

43. Cohee LM, Nankabirwa Ji, Greenwood B, Djimde A, Mathanga DP, 2021. Time for malaria control in school-age children. *Lancet Child Adolesc Health* 5: 537–538.

44. Coelho ME, Cohee LM, Buchwald AG, Nyambalo A, Kubale J, Seydel KB, Mathanga D, Taylor TE, Lauber MK, Wilson ML, 2018. Simulation models predict that school-age children are responsible for most human-to-mosquito *Plasmodium falciparum* transmission in southern Malawi. *Malar J* 17: 147.

45. degaree A, Fennie K, Degaree D, Chennupati S, Madhivanan P, 2019. Improving socioeconomic status may reduce the burden of malaria in sub-Saharan Africa: a systematic review and meta-analysis. *PLoS ONE* 14: e0211205.

46. Yang G, Kim D, Pham A, Paul CJ, 2018. A meta-regression analysis of the effectiveness of mosquito nets for malaria control: the value of long-lasting insecticide nets. *Int J Environ Res Public Health* 15: 546.

47. Ebenezer A, Noutcha AEM, Okiwelu SN, 2016. Relationship of annual entomological inoculation rates to malaria transmission indices, Bayelsa State, Nigeria. *J Vector Borne Dis* 53: 46–53.

48. Smith DL, Dushoff J, Snow RW, Hay SI, 2005. The entomologi- cal inoculation rate and *Plasmodium falciparum* infection in African children. *Nature* 438: 492–495.

49. Tokponnon FT et al., 2014. Impact of long-lasting, insecticidal nets on anaemia and prevalence of *Plasmodium falciparum* among children under five years in areas with highly resistant malaria vectors. *Malar J* 13: 76.

50. Protopopoff N, Rowland M, 2018. Accelerating the evidence for new classes of long-lasting insecticide-treated nets. *Lancet Lond Engl* 391: 2415–2416.

51. Yahouédo GA, Cornelie S, Djègbè I, Ahlonsou J, Aboubakar S, Soares C, Akogbeto M, Corbel V, 2016. Dynamics of pyrethroid resistance in malaria vectors in southern Benin following a large scale implementation of vector control interventions. *Parasit Vectors* 9: 385.

52. Sovi A et al., 2020. Resistance status of *Anopheles gambiae* s.l. to insecticides following the 2011 mass distribution campaign of long-lasting insecticidal nets (LLINs) in the Plateau Department, south-eastern Benin. *Malar J* 19: 26.

53. Protopopoff N et al., 2018. Effectiveness of a long-lasting piperonyl butoxide-treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid-resistant mosquitoes: a cluster, randomised controlled, two-by-two factorial design trial. *Lancet* 391: 1577–1588.

54. Staedke SG et al., 2020. Effect of long-lasting insecticidal nets with and without piperonyl butoxide on malaria indicators in Uganda (LLINEUP): a pragmatic, cluster-randomised trial embedded in a national LLIN distribution campaign. *Lancet Lond Engl* 395: 1292–1303.