

Malaria among children under 10 years in 4 endemic health areas in Kisantu Health Zone: epidemiology and transmission

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Abstract

Introduction

The Democratic Republic of Congo (DRC) is the second most malaria-affected country globally with 21,608,681 cases reported in 2019. The Kongo Central (KC) Province has a malaria annual incidence of 163 cases/per 1000 inhabitants which are close to the national average of 153.4/1000. However, the malaria prevalence varies both between and within health zones in this province. The main objective of this study was to describe the epidemiology and transmission of malaria among children aged 0 to 10 years in the 4 highest endemic health areas in Kisantu Health Zone (HZ) of KC in DRC,

Methods

A community-based cross-sectional study was conducted from October to November 2017 using multi-stage sampling. A total of 30 villages in 4 health areas in Kisantu HZ were randomly selected. The prevalence of malaria was measured using a thick blood smear (TBS) and known predictors and associated outcomes were assessed. Data are described and associations determinants of malaria infection were analysed.

Results

A total of 1790 children between 0 and 10 years were included in 30 villages in 4 health areas of Kisantu HZ. The overall prevalence in the study area according to the TBS was 14.8% (95%CI:13.8-16.6; range:0-53). Mean sporozoite index in the study area was 4.3% (95%CI:2.6-6.6). The determination of Kdr-west resistance alleles showed the presence of both L1014S and L1014F with 14.6% heterozygous L1014S/L1014F, 84.4% homozygous 1014F, and 1% homozygous 1014S. The risk factors associated with malaria infection were sandy or wooden floors aOR: 15.8% (95%CI: 8.6-29.2) and a moderate or low income: 1.6 (95%CI:1.1-2.3).

Conclusion

Malaria prevalence differed between villages and health areas within the same health zone. The control strategy activities must be oriented by the variety in the prevalence and transmission of malaria in different areas.

Background

The Democratic Republic of the Congo (DRC) is, after Nigeria, the most affected malaria-endemic country with more than 29 million estimated cases annually¹. The malaria control strategies in DRC are based on early diagnosis and prompt treatment, and vector control. There are three key vector control interventions in the National Strategic Plan: long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and larval source management. The most widely implemented of these is the use of LLINs. LLINs are distributed for free through mass campaigns, school campaigns, and routine antenatal consultations².

According to the DRC National Malaria Control Program (NMCP) 2016–2020 strategic plan, 55.8% of children under 5 years of age sleep under LLINs². IRS is implemented by a small number of mining companies such as Tenke Fugurume Mining and some cement factories to protect their workers and neighboring communities². Control activities through larval source management campaigns are not currently implemented at a large scale.

Vector control interventions are threatened by the emergence of insecticide resistance. In DRC, the primary vector, *Anopheles gambiae* s.l is generally susceptible to bendiocarb (carbamate) and pirimiphos-methyl (organophosphate) but resistant to DDT (organochlorine), permethrin, deltamethrin, and alpha-cypermethrin(pyrethroids)^{3–7}. The impact of this resistance is undetermined, but in West Africa, resistance to pyrethroids resulted in a decrease in the efficacy of pyrethroid impregnated nets^{8–10}.

In DRC, the physical durability and effectiveness of nets impregnated with deltamethrin (DawaPlus 2.0) and α-Cypermethrin (Duranet) is only two years and not three years as recommended by WHO¹¹. Despite the frequent distribution of LLN during the past ten years, malaria transmission has remained stable in several areas of the DRC due, most probably for multiple reasons¹². Amongst different reasons, the increased resistance against the insecticides used in the LLIN could play an important role¹². Therefore, LLINs with other insecticides as synergistic mosquito nets was suggested¹³. We conducted a mixed entomological and epidemiological survey to identify the types of vectors and their resistance against some commonly used insecticides. At the same time, the disease transmission among the most vulnerable population groups also will be more clearly understood. These data will help to choose evidence-based and tailored interventions to successfully control malaria transmission.

Methods Study Design

Study area

This study was carried out in four most malaria endemic health areas in Kisantu health zone (HZ) in Kongo Central (KC), one of 26 provinces of DRC. Kisantu is located in Lukaya district covering a surface area of 2400 km² and a population of 153,188 inhabitants. In this area, the malaria incidence rate, in 2017, was 163/1000 Cases per person per year (DHIS2, 2017). The insecticide susceptibility testing conducted in the closest national entomological monitoring site, Kimpese (in the same province). In 2016 showed that *An. gambiae* s.l. was resistant to permethrin with 17% mosquito mortality in WHO susceptibility tests (95% CI: 11–26) and 76% to deltamethrin mortality (95% CI: 67–83)¹⁴.

Study design and Study population

A mixed entomological and epidemiological study was carried out in 4 health areas of Kisantu health zone. The epidemiological study consisted of a stratified multi stage cross-sectional study conducted in households with children aged 0 to 10 years. Informed and signed consent was obtained from the head

of household. In case of illiteracy, we used fingerprint (thumb) consent in the presence of an independent witness.

The stratification took into account the model used in the strategic plan for the fight against malaria. This model divides the country in 3 zones according to the parasitic infection: hypo-endemic (5%), holo-endemic (6–30%) and hyper-endemic zone (30%). The entomological study consisted of identifying species of *anopheles* and their susceptibility to some insecticide.

Epidemiologic survey

Sample size

The number of children (n) included in the study was calculated using the following formula described by Kish, (1965) for proportion¹⁵.

$$\frac{Z_{\alpha-\frac{1}{2}}^2 \cdot p \cdot q}{d^2} \geq Deffxn$$

Where p represents the estimated prevalence of malaria among children ($p = 0.471$), q= (1-p) the proportion of children under five years who get negative blood smear. (Demographic and Health Survey II, DRC)¹⁶; $Z_{\alpha-\frac{1}{2}}$ Z is the level of confidence based on the normal distribution. Z is 1.96 if the alpha is 0.05 is in our calculation. d is the precision degree that we assumed at 5%; the design effect (deff) was assumed to 2. The minimal sample size computed was 765 children.

Sampling

In the first stage, 4 of the 15 Health areas within Kisantu Health Zone with the highest malaria prevalence were selected based on the 2017 parasitological surveys (Database of DLM 2017). In the second stage, 30 villages were selected randomly from the list of all the 72 villages in the four health areas using the Excel function *rand*. In the third stage, we conducted a census to collect a list of all children in these 30 villages between age of 0 and 10 years included. Then a systematic random sampling was used to identify children aged from 0 to 10 years per village as illustrated in the Fig. 1.

Data collection

A questionnaire was administered to each household of the selected children. Information on demographic characteristics (sex, age, literacy, occupation, and marital status of the parents/guardian), socio-economic status (of the parents/guardian) and related variables were collected. To assess the socio-economic level of the households, observations were made on household characteristics (i.e., material used for walls, roofs, and floors).

A thick blood smear (TBS) and a thin blood smear were taken from selected children in the households following WHO procedures¹⁷. Thick and thin smears were stained with 2% Giemsa, then stored in filing

boxes and read with microscopes at the Clinic of the University of Kinshasa. When the thick smear was positive, a thin smear was used for the species determination and to assess the parasite density of the sample. The thick and thin smears were independently read by two readers. A third assessment was performed in National Institute of Biomedical Research (NIRB) when a difference in TBS interpretation or a difference in parasite density in the thin blood smear of more than 15% was observed between the two readers. The result of the third reader which was the most experienced was taken into account.

Plasmodium infection prevalence was calculated using the formula: total children with positive (*Plasmodium* infection) TBS / total children tested. The infection prevalence by village was used to group villages according to the DRC NMCP's 2016–2020 strategic plans as: (i) hypo endemic $\leq 5\%$, (ii) meso-endemic 6–30% and (iii) hyper-endemic $> 30\%^2$.

Haemoglobin levels were measured on the spot in the field using a portable automated HemoCue®Hb 301 hemoglobinometer system according to the manufacturer instructions. Anaemia was classified as mild, moderate and severe when haemoglobin concentrations were 9.0–9.9g/dl, 7.0–8.9g/dl and 5.0–6.9g/dl respectively¹⁸.

The weight of children was measured using digital scales (Seca GmbH, Hamburg, and Germany) to the nearest 0.1 kg. The height was measured using a mobile measuring Seca mat (Seca GmbH, Hamburg, Germany) for children 0 to 99 centimetres and a recumbent length board for children of height > 99 centimetres to the nearest centimetre. The outcome variable was undernutrition indicated by wasting, stunting and underweight status in children 6 months of age. Stunting (height/age), wasting (weight/height) and underweight (weight/age) were defined as per WHO child growth standards. Children with Z scores less than two standard deviations (≤ 2 SD) below the median of the WHO child growth standards were classified as malnourished (stunted, wasted or underweight), minus 3 SD indicates severe stunting, wasting, and underweight¹⁹. Z scores of less than -2 SD were considered indicative of stunting²⁰.

Entomological Survey

Adults *Anopheles* was sampled from each of the same 30 villages in June 2018. We selected randomly six households per village and performed human landing catches (HLC) in three of the houses for one night per house to estimate the biting rate. In the 3 other selected households, indoor resting mosquitoes were collected from the walls, roof and furniture using a standard battery-powered Prokopack aspirators in the three remaining houses between 6:00 to 8:00 AM. A total of 180 households were included in this survey (90 HLC, 90 aspirations).

The collectors of the HLC only worked for 6 hours at a time, so four collectors per household per night were needed. Collectors were selected from amongst the local population; after written informed consent, they were enrolled and trained on the entomological activities. The collectors were referred to a health

center and were treated free-of-charge if any illness occurs during the HLC activities and up to three weeks after collections.

The collected mosquitoes were morphologically identified²¹ and subsequently stored in tubes with silica gel for later detection of sporozoites (CSP ELISA) and molecular analysis of alleles at the Kdr 1014 locus. Based on the biting rates and the *Plasmodium falciparum* prevalence, the *P. falciparum* entomological inoculation rate (PfEIR) were calculated this rate is a measure of exposure to infectious mosquitoes. It is usually interpreted as the number of *P. falciparum* infective bites received by an individual during a night or season (aPfEIR)²².

The sex and physiological status (fed, unfed, semi-gravid, and gravid) of the mosquitoes collected by Prokopack were determined. Subsequently they were dried on silica gel for the CSP by ELISA and the alleles of kdr 1014 locus, East and West together using the new developed technique from Huynd L²³. at CDC Atlanta further analysis.

Susceptibility Testing

Anopheles larvae and pupae were collected in all villages included in the study and brought to the laboratory where they were reared to adults. Adults *Anopheles* species aged 3–5 days were tested using WHO susceptibility tube tests against the diagnostic concentrations of deltamethrin 0.05%; permethrin 0.75%; alpha-cypermethrin 0.05%; DDT 4%; bendiocarb 0.1% and PBO 4% following WHO guidelines²⁴. After 24 hours, mosquitoes were scored as dead or alive, identified morphologically and stored in tubes with silica gel for subsequent molecular analysis. We corrected observed mortality using Abbott's formula when control mortality varied between 5 and 20%. We repeated experiments when control mortality was greater than 20%²⁵.

Parasite Infection Detection And Molecular Analysis

An. gambiae s.l. and *An. funestus* s.l. heads and thoraces were separated from the abdomen and *Plasmodium falciparum* sporozoite antigen was detected using ELISA according to standard protocols²⁶. We performed ELISA tests at the NIRB. We extracted DNA from legs and wings for PCRs to identify species and resistance mechanism (kdr-w). We used SINE PCR to identify the species of 200 mosquitoes morphologically identified as *An. gambiae* s.l.²⁷ Kdr resistance alleles 1014F were also determined by PCR on the same 200 mosquitoes using the protocol described by Huynd LY and al²³. The PCR assays were conducted at the Centers for Disease Control and Prevention (CDC) laboratory (Atlanta, GA, USA).

Data analysis

Stata version 13.0 was used for statistical analysis. Descriptive statistics were used to summarize the characteristics of the study population. Continuous variables were reported using means with standard

deviations when variables were normally distributed if not, medians with ranges were reported. Categorical variables were summarized in frequencies or percentage.

For the epidemiological study, the prevalence was calculated using all children who had a positive thick drop, over the total study population.

We used χ^2 test to search for the association between strata of malaria transmission and some characteristics of the households (sex, age, weight, size, temperature, and number of people living in the household, number of sleeping spaces, occupation and level of education of the head of household, house type and nutritional status). Statistically significant associations were thereafter assessed in a logistic regression analysis to determine factors independently associated with malaria transmission. The odds ratio (OR) with a corresponding 95% confidence interval was reported to quantify the strength of association. Significance was set at p-value of 0.05. Mapping of vector distribution and prevalence was also performed. Student's t test was used to compare means of dead anopheles according to the insecticide.

The following formulas were used to calculate entomological indicators:

- The sporozoite rate (SR) = (ELISA positive total number/ total number of *Anopheles* species tested) x 100
- Human biting rate (HBR) = total number of *Anopheles* species collected by HLC during a specific period / total number of man-nights
- Nightly entomological inoculation rate (EIR) = Nightly HBR x SR

Results

Study participants characteristics

A total of 1790 children were included in this study in 30 villages in the health zone of Kisantu. Nearly half of the children were female (51.4%) or aged under five years (51.7%) (Table 1).

Table 1
 General characteristics of the study population in the 4 areas in Kisantu
 Health Zone, Kongo-Central Province, 2018

Variables	n
Number of participants	1790
Demographic variables	
Male (n (%; 95%CI)	870 (48.6; 49.1–43.7)
Age (year; mean, SD)	5.1 ± 3.1
Age < 5 years (n (%; 95% CI)	925(51.7; 49.3–54.1)
Hb (gr/l) Mean ± SD	9.9 ± 1.7
Weight (kg) Mean ± SD	15.5 ± 5.8
Height (cm) Mean ± SD	100.5 ± 20.8
Temperature (°C) Mean ± SD	36.7 ± 0.5
Socio-economic status of the household	
Number of persons per household (Mean ± SD)	7.7 ± 2.8
Number of children below 10 years (Mean ± SD)	3.5 ± 1.5
Number of sleeping space (Mean ± SD)	2.8 ± 0.9
Profession of household head (n (%; 95% CI)	
Jobless	193 (10.8; 9.3–12.4)
Farming/fishing/hunting/animal rearer	1154 (64.5; 60.8–68.3)
Civil servant	197 (11.0; 9.5–12.6)
Independent	151 (8.4; 7.2–9.2)
Other	95 (5.3; 4.3–6.4)
Educational level of household head	
Unschooled	76 (4.3; 3.5–5.4)
Primary	1201 (68.8; 66.2–70.6)
Secondary	410 (23.4; 21.4–25.4)
Institute/University	67 (3.8; 2.9–4.9)
Main floor type in the house	

*100 children were brought back to the cause of age < 6 months

Variables	n
Cement/tiles	687(38.4; 36.1–40.7)
Sand/Bamboo	1103(61.6; 59.3–63.8)
Main external wall types	
Cement blocks	142(7.93; 6.7–9.3)
Mud blocks	1251(69.9; 66.1–73.8)
Mud plaster on wooden support	350(19.5; 17.6–21.7)
Mud walls /Straw	47(2.6; 1.9–3.5)
Main roofing material	
Zinc	791(44.2; 41.2–47.3)
Straw	741(41.4; 38.5–44.5)
Thatch	258(14.4; 12.7–16.2)
Nutritional*	
Stunting	
Normal/mild	967(54.0; 50.7–57.5)
Moderate/severe	608(34.0; 31.4–36.7)
Over	215(12; 10.5–13.7)
Wasting	
Normal/mild	1211(67.7; 63.9–71.5)
Moderate/severe	268(15; 13.3–16.8)
Over	215(12; 10.5–13.7)
Underweight	
Normal/mild	1050(58.7; 55.2–62.3)
Moderate/severe	461(25.8; 23.5–28.2)
Over	279(15.6; 13.8–17)
Malaria infection	
TBS positive	266(14.8; 13.3–16.6)
<i>Plasmodium falciparum</i>	244(93.5; 89.9–96.0)

*100 children were brought back to the cause of age < 6 months

Variables	n
<i>Plasmodium malariae</i>	10(3.8; 1.9–6.7)
<i>Plasmodium falciparum + malariae</i>	2(0.8; 0.1–2.5)
<i>Gametocyte plasmodium falciparum</i>	5(1.9; 0.7–4.2)

*100 children were brought back to the cause of age < 6 months

Socio-economically, 64.5% of the heads of the households were farmers, fishermen or cattle breeders, and the majority had a primary school education level. The great majority (69.9%) of the visited houses was built with mud walls. The most frequent roofing material was steel sheeting (44.2%).

The averages of the weight, height, and temperature of the children were 15.5 ± 5.8 kg; 100.5 ± 20.5 cm, and 36.7 ± 0.5 °C, respectively (Table 1). Regarding malnutrition, three types were observed in the children surveyed: 34% showed signs of delay of growth, 15% had an insufficient weight and 25.8% had a moderate or severe malnutrition. The median of the haemoglobin was 9.9 ± 1.7 g/dl (Table 1).

Plasmodium infection prevalence

The overall prevalence of *Plasmodium* infection according to the TBS was 14.8% (95CI:13.3–16.6) (Table 1). Fifteen of the villages were classified as hypo-endemic, twelve as meso-endemic and three as hyper-endemic (Fig. 3). *Plasmodium falciparum* was the primary *Plasmodium* species (93.5%; 95%CI 89.9–96.0). *Plasmodium malariae* represented 3.8% (CI 1.9–6.7 and 0.77% (CI 0.1–2.5) were mixed infections *P. falciparum* and *P. malariae*.

Main type of wall, the type of ground, the main type of domestic roof (straw and thatch) and the malnutrition were associated with *Plasmodium* infection. In multi-variate analysis, having sand or wood floors (aOR: 15.8; IC95%: 8.6–29.2) and having moderate or severe malnutrition (aOR: 1.6; IC95%:1.1–2.3) were associated to the arisen of plasmodium infection (Table 2).

Table 2

Bivariate and multivariate analysis determinants of epidemiological stratum (zone hypo/meso endemic and hyper endemic)

Variables	Bivariate analysis Crude OR (95% CI)	p value	Multivariate analysis Adjusted OR (95% CI)	p value
Wall type				
Mud walls/Straw	2.1(1.5–2.8)	0.001		
Cement block	1			
Floor type				
Sand/wood	15.7 (8.5–32.2)	0.001	15.8 (8. 6-29.2)	< 0.001
Cement/tiles	1			
Roofing type				
Straw	2.7(1.9–3.8)	0.001		
Thatch/wood	4.5(3.0-6.7)	0.001		
Zinc	1			
Wasting				
Moderate/severe	1.5 (1.1–2.2)	0.024	1.6 (1.1–2.3)	0.024
Over	1.9 (1.3–2.6)	0.001	1.9 (1.3–2.7)	< 0.001
Normal/mild	1			
Stunting				
Moderate/severe	1.5 (1.1–1.9)	0.010		
Over	1.3 (0.9-2.0)	0.198		
Normal/mild	1			
Underweight				
Moderate/severe	1.3 (1.0-1.8)	0.071		
Over	1.1 (0.7–1.6)	0.748		
Normal/mild	1			
Given the high collinearity between independent variables such as types of soil, roofing and walls, only the type of soil was taken into account in the model. And for nutritional status because of the multicollinearity between stunting, wasting and underweight, we included only wasting in the model.				

Entomological survey results

Anopheles mosquito composition, behaviour and human biting rate

Anopheles funestus s.l. was the most abundant (58.2%) species collected followed by the *Anopheles gambiae s.l.* (24.1%) (Table 3). Other *Anopheles* species represented respectively 12.8% for *An. nili*, 1.8% for *An. paludis* and 3.0% for *An. coustani*. A total of 1049 Culicinae were also captured. Both *An. gambiae s.l.* and *An. funestus s.l.* indoor biting behaviours were similar to those of outdoor biting (Figs. 2). They were active between 6:00 pm and 6:00 am. The maximal mean peak of *An. funestus s.l.* of 44 bites per person per night between 21:00–22:00 pm outdoor and of 50 bites per person per night between 23:00 pm and 0:00 am indoor.

Table 3

The number of specimens collected by species of Anopheles in the 4 areas in Kisantu Health Zone

MOSQUITOES	HLC		Aspiration	Total (%)
	IN	OUT		
<i>An. funestus s.l.</i>	234 (23.7)	303 (30.6)	35 (3.5)	572 (58.2)
<i>An. gambiae s.l.</i>	96 (9.7)	134 (13.5)	7 (0.7)	237 (24.1)
<i>An. coustani</i>	10 (1.0)	19 (1.9)	0	29 (3.0)
<i>An. nili</i>	52 (5.3)	74 (7.5)	0	126 (12.8)
<i>An. paludis</i>	7 (0.7)	11 (1.1)	0	18 (1.8)
Total				982 (100)

The highest human biting rates were recorded in the villages of Bokol, Kifua and Kinkuanga, with rates of 11.6, 21.3 and 9.6 bites per person per night, respectively. Overall, the mean sporozoite index in the Health Zone of Kisantu was 4.3% (Table 4),

Table 4
Mortality of *An. gambiae* in WHO tube tests with insecticides Entomological Inoculation Rate (HLC Indoors & Outdoors) in the 4 areas in Kisantu Health Zone

Entomological indicator	n (%; 95% CI)	Mean difference in mortality	P value
Insecticide susceptibility of <i>An. gambiae</i> s.s.			
Permethrin 0.75%	100(25; 16.5–33.5)	58	< 0.001
Permethrin 0.75% + PBO	100(83; 75.6–90.4)		
Deltamethrin 0.05%	100(62; 52.5–71.5)	37	< 0.001
Deltamethrin 0.05% + PBO	100(99; 97.1–100)		
a-Cypermethrin	100(48; 38.2–57.8)	44	< 0.001
a-Cypermethrin + PBO	100(92; 86.7–97.3)		
DDT 4%	100(11; 4.9–17.1)	18	< 0.0015
DDT 4% + PBO	100(29; 20.1–37.9)		
Bendiocarb	100(100; 98–100)	NA	NA
<i>Plasmodium falciparum</i> circumsporozoite			
<i>An. gambiae</i> s.l.	200(3,5; 1,5–6,8)		0.621
<i>An. funestus</i> s.l.	200(5; 2,6–8,7)		
total	400(4,3; 2,6–6,6)		
Human biting rate (p/nighth/p/person)			
<i>An. gambiae</i> s.l.	1.27		
<i>An. funestus</i> s.l.	2.98		
total	4.26		
Nightly entomological inoculation rate (p/nighth)			
<i>An. gambiae</i> s.l.	0.04		

Entomological indicator	n (%; 95% CI)	Mean difference in mortality	P value
<i>An. funestus</i> s.l.	0.15		
total	0.18.		

The Entomological Inoculation Rate (EIR) per night was estimated at 0.04 infectious bites per night for *An. gambiae* s.l. and 0.15 for *An. funestus* s.l. and one person could receive from both vectors 0.18 infectious bites per night.

Insecticide resistance profile and molecular analysis results

The mosquito populations tested were resistant with insect mortality rates to permethrin of 25% (95%CI:17–34), deltamethrin of 62% (95%CI:53–72%), alpha-cypermethrin of 48% (95%CI: 38–58), DDT of 11% (95%CI:5–17%) and fully susceptible to bendiocarb (100%) (Table 4). However, results obtained with PBO pre-exposure had higher mortality rates: for permethrin 83% (95%CI: 76–90), deltamethrin 99% (95%CI: 97–100%), alpha-cypermethrin 92% (95%CI:87–97), DDT 29% (95%CI:20–38).

The PCR results showed that all *Anopheles gambiae* s.l. mosquitoes analysed for the species identification were *Anopheles gambiae* s.s. In those samples, 14.6% are heterozygous (*kdr-east* (*L1014S*) /*kdr-west* (*L1014F*)), 84.4% are homozygotes *kdr-west* (*L1014F*), and ~ 1% are *kdr-east* (*L1014S*). The allele frequency was 0.99 for Kdr-west and 0.16 for kdr west-east. (Table 5).

Table 5

Genotypes and allele frequency of the 1014F and 1014S *kdr* mutation in the Kisantu Health Zone.

<i>kdr</i>	Genotype						F(<i>kdr</i>)
	Number tested	Proportion (%)	SS	RR	RS	Did not amplify	
<i>kdr-west</i> (<i>L1014F</i>)	200	84.4	2	197	0	1	0.99
<i>kdr-east</i> (<i>L1014S</i>)	200	1	168	31	0	1	0.16
<i>Kdr – wes/east</i>	200	14.6					

RR: homozygous resistant, SS: homozygous sensitive, RS: Heterozygote resistant, F (*kdr*): allele frequency of resistant Kdr gene.

Discussion

Plasmodium baseline prevalence found by TBS was 14.8% in children between 0–10 years old. This study showed disparities in infection prevalence between villages in the same health zone. The

heterogeneity in malaria prevalence was expected, as malaria prevalence varies with a number of epidemiological and environmental factors, including land use, soil type and hydrography^{28,29}. Further, we observed that living in a house with a sand floors and having malnutrition is a risk factor for *Plasmodium* stratum epidemiological infection.

The sporozoite index found in this study is similar to that reported in other studies³⁰. From the species point of view no difference was found between *An. gambiae* and *An. funestus*. Both *An. gambiae* s.l. and *An. funestus* s.l., were found in the study site with a predominance of *An. funestus* s.l³. The presence of several rivers and swamps, typical ecology for *An. funestus* might explain the predominance of this species in Kisantu. Similar results were observed in Kinshasa⁵ and elsewhere in the country including Kongo Central³⁰. *An. gambiae* s.l. and *An. funestus* s.l. had a mean biting peak indoor and outdoor between 22:00–23:00 PM and 23:00–00:00 AM respectively. The similar bite peak was observed in Pawa village (Haut Uele) sentinel site 2017 through of the President Malaria Initiative (PMI) entomological monitoring activities³⁰. The EIR was estimated at 1.2 per person per night, for *An. gambiae* s.l. and 4.5 per person per night for *An. funestus* s.l.

The Phenotypic resistance to pyrethroids was observed in *An. gambiae* s.l. populations. This resistance to pyrethroids could have a negative impact on the efficacy of LLINs impregnated with pyrethroid^{3,5}. Susceptibility was noted with bendiocarb, which could open the possibility for controlling the pyrethroid resistant mosquitoes through indoor residual spraying. A recovery of deltamethrin susceptibility of *An. gambiae* s.l. observed after pre-exposure to PBO, showed the likely implication of metabolic resistance mediated by oxidases⁵. A genetic resistance against commonly used insecticides in LLN, predominantly in the east, was showed in this study. This situation could maintain an important transmission and calls for the current strategy to be reviewed. These results are similar to those found by some authors at the Nord Ubangi site in DRC showing the high frequency of 1014F in *An. gambiae* s.s. populations³¹.

Malaria control strategy in DRC is based on the detection, treatment and prevention. The prevention is based mainly on intermittent preventive treatment (IPT) and LLINs. The observed resistance of *An. gambiae* s.s could have a negative impact in LLINs efficacy³². On one hand the resistance level of the vectors should be carefully surveyed but also the development of an alternative strategy such as the deployment of LLINs combining with PBO. The study noticed that 33% of children were malnourished with a twice risk of developing malaria infection. These results are like those found in Kinshasa by Maketa and al, the authors found that malnourished children were almost twice as likely (OR: 1.8, p = 0.01) to develop malaria compared to healthy children^{19,33}.

Conclusion

In this selected Health areas, malaria infection prevalence was high in some villages compared to the other in the health zone of Kisantu. House type was highlighted as a risk factor of having malaria infection. Malnutrition was associated with malaria endemicity. Malaria transmission vector is most *An.*

funestus and *An. gambiae*. These vectors were resistant to pyrethroids but more susceptible when PBO was used in pre-exposure phase. The policy against malaria regarding the LLINs should be based on the evidence of metabolic resistance and the biting period including the peak which is around 23h00.

Abbreviations

DRC: The Democratic Republic of the Congo; LLINs: long-lasting insecticidal nets; IRS: indoor residual spraying; NMCP: National Malaria Control Program; PBO: piperonyl butoxide; HZ: health zone; KC: Kongo Central; TBS: thick blood smear; HLC: human landing catches; SR: sporozoite rate; HBR: Human biting rate; HLC: Human Landing Catch; EIR: Nightly entomological inoculation rate.

Declarations

Ethics approval and consent to participate

The study received the approval (ESP/CE/061/2016) from the ethics committee of the School of Public Health of the University of Kinshasa, DRC. After a detailed explanation of the purpose of the study, written informed consent was obtained from parents or guardian and mosquito collectors. All febrile participants received an antipyretic and those with a positive RDT were treated according to national treatment guidelines.

Consent for publication

Not applicable

Availability of data and material

All relevant data supporting the findings of this study are included within the paper and their supporting information files.

Competing interests

All authors declare that they have no competing interests.

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Authors' contributions

PL, JPV, FW and GI participated in the conception and design of the study, GI and BM performed the fieldwork and wrote the first draft manuscript, PM, VM, LM and SL participated in the development of the computer software, the analysis, interpretation of data and revision of manuscript, JRM, AF and LM analyzed, interpreted the data and revised the manuscript, SRI, SL and WVB participated in the revision of manuscript. All authors read and approved the final version of the manuscript.

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References

1. World Health Organization (2021) World Malaria report 2021. ISBN 978-92-4-004049-6 (electronic version) CC BY-NC-SA 3.0 IGO; <https://creativecommons.org/licenses/by-nc-sa/3.0/igo>.
2. République Démocratique du Congo MdISPPNdLCIPP (2016) Plan Stratégique National de Communication 2017–2020. https://www.thecompassforsbc.org/sites/default/files/project_examples/DRC%20SBCC%20strategy%202017-2020%20final%2011-15-2017%5B2%5D.pdf.
3. Wat'senga F, Manzambi EZ, Lunkula A, Mulumbu R, Mampangulu T, Lobo N, Hendershot A, Fornadel C, Jacob D, Niang M, Ntoya F, Muyembe T, Likwela J, Irish SR, Oxborough RM (2018) Nationwide insecticide resistance status and biting behaviour of malaria vector species in the Democratic Republic of Congo. *Malar J* 17: 129. doi:10.1186/s12936-018-2285-6 [doi];10.1186/s12936-018-2285-6 [pii]
4. Lynd A, Oruni A, Van't Hof AE, et al. Insecticide resistance in *Anopheles gambiae* from the northern Democratic Republic of Congo, with extreme knockdown resistance (kdr) mutation frequencies revealed by a new diagnostic assay. *Malar J*. 2018;17(1):412. doi:10.1186/s12936-018-2561-5
5. Riveron JM, Watsenga F, Irving H, Irish SR, Wondji CS. High Plasmodium Infection Rate and Reduced Bed Net Efficacy in Multiple Insecticide-Resistant Malaria Vectors in Kinshasa, Democratic Republic of Congo. 2018;217(May 2015). doi:10.1093/infdis/jix570
6. Nardini L, Hunt RH, Dahan-Moss YL, et al. Malaria vectors in the Democratic Republic of the Congo: the mechanisms that confer insecticide resistance in *Anopheles gambiae* and *Anopheles funestus*. *Malar J*. 2017;16(1):448. doi:10.1186/s12936-017-2099-y
7. Kanzaa JPB, El Fahime E, Alaoui S, et al. Pyrethroid, DDT and malathion resistance in the malaria vector *Anopheles gambiae* from the democratic Republic of Congo. *Trans R Soc Trop Med Hyg*. 2013;107(1):8–14. doi:10.1093/trstmh/trs002
8. Guessan RN, Corbel V, Akogbeto M, Rowland M. Reduced Efficacy of Insecticide-treated Nets and Indoor Residual Spraying for Malaria Control in Pyrethroid Resistance Area, Benin. 2007;13(2):199–206.

9. Agossa FR, Gnanguenon V, Anagonou R, et al. Impact of Insecticide Resistance on the Effectiveness of Pyrethroid-Based Malaria Vectors Control Tools in Benin: Decreased Toxicity and Repellent Effect. Bogyo M, ed. *PLoS One*. 2015;10(12):e0145207. doi:10.1371/journal.pone.0145207
10. Gnanguenon V, Azondekon R, Oke-agbo F, et al. Evidence of man-vector contact in torn long-lasting insecticide-treated nets. 2013.
11. Mansiangi P, Umesumbu S, Etewa I, et al. Comparing the durability of the long – lasting insecticidal nets D in northwest Democratic Republic of Congo. *Malar J*. 2020;1–18. doi:10.1186/s12936-020-03262-0
12. Janko MM, Irish SR, Reich BJ, et al. Articles The links between agriculture, *Anopheles* mosquitoes, and malaria risk in children younger than 5 years in the Democratic Republic of the Congo : a population-based, cross-sectional, spatial study. *Lancet Planet Heal*. 2018;2(2):e74-e82. doi:10.1016/S2542-5196(18)30009-3
13. Levitz L, Janko M, Mwandagalirwa K, et al. Effect of individual and community – level bed net usage on malaria prevalence among under – fives in the Democratic Republic of Congo. *Malar J*. 2018;1–8. doi:10.1186/s12936-018-2183-y
14. President's Malaria Initiative (2016) The President's Malaria Initiative (PMI) Project. Indoor Residual Spraying Task Order Six. The Democratic Republic of Congo. Entomological Monitoring 2016 final report November 01, 2015-October 31, 2016.
15. Kish L (1965) Sampling Organization and Groups of Unequal Sizes. *Am Sociol Rev* 30: 564–572.
16. Ministère du Plan et Suivi de la Mise en œuvre de la Révolution de la Modernité (MPSMRM), Ministère de la Santé Publique (MSP) et ICF. *République Démocratique Du Congo*; 2014.
17. Centers for Disease Control and Prevention (CDC) (2020) Diagnostic Procedures Blood-Specimens. <https://www.cdc.gov/dpdx/diagnosticprocedures/blood/specimenproc.html>.
18. World Health Organization (2011) Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. World Health Organization, Geneva
19. World Health Organization. (2006) WHO child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight -for-height and body mass index-for-age: methods and development
20. World Health Organization (2007) WHO Child Growth Standards: Head circumference-for-age, arm circumference-for-age, triceps skinfold-for-age and subscapular skinfold-for-age: Methods and development.
21. Gillies M, Coetzee M (1968) A supplement of the Anophelinae of Africa south of sahara (Afrotropical Region). *S Afr Inst Med Res* 54.
22. Kilama M, Smith DL, Hutchinson R, et al. Estimating the annual entomological inoculation rate for *Plasmodium falciparum* transmitted by *Anopheles gambiae* s. l. using three sampling methods in three sites in Uganda. 2014;1–13.
23. Huynh LY, Sandve SR, Hannan LM, Van Ert M, Gimning JE (2007) Fitness cost of pyrethroid insecticide resistance in *Anopheles gambiae*. In: Annual Meeting of the Society for the Study of

Evolution, Christchurch, New Zealand

24. World Health Organization. (2016) World Health Organization recommended long-lasting insecticidal mosquito nets.
25. Fleming R, Retnakaran A. Evaluating Single Treatment Data Using Abbott's Formula With Reference to Insecticides. *J Econ Entomol.* 1985;78(6):1179–1181. doi:10.1093/jee/78.6.1179
26. Wirtz RA, Ballou WR, Schneider I, Chedid L, Gross MJ, Young JF, Hollingdale M, Diggs CL, Hockmeyer WT (1987) *Plasmodium falciparum*: immunogenicity of circumsporozoite protein constructs in *Escherichia coli*. *Exp Parasitol* 63: 166–172
27. Santolamazza F, Calzetta M, Etang J, et al. Distribution of knock-down resistance mutations in *Anopheles gambiae* molecular forms in west and west-central Africa. *Malar J.* 2008;7:1–8. doi:10.1186/1475-2875-7-74
28. Alemu K, Worku A, Berhane Y. Malaria Infection Has Spatial, Temporal, and Spatiotemporal Heterogeneity in Unstable Malaria Transmission Areas in Northwest Ethiopia. 2013;8(11):1–10. doi:10.1371/journal.pone.0079966
29. Mouchet J, Camevale P, Coosmans M, Fontenille D, Ravaonjanahary C, Richard A, Robert V (1993) Typologie du paludisme en Afrique. *Cahiers Santé* 3: 220–238.
30. President's Malaria Initiative (2018) The President's Malaria Initiative (PMI) Project. Indoor Residual Spraying Task Order Six. The Democratic Republic of Congo. Entomological Monitoring 2017 final report November 01, 2016–October 31, 2017.
31. President's Malaria Initiative (2021) The PMI VectorLink Project, The Democratic Republic of the Congo, Sud Ubangi PBO Net Study. Rockville, MD. The PMI VectorLink Project, Abt Associates Inc.
32. Fadel AN, Ibrahim SS, Tchouakui M, et al. A combination of metabolic resistance and high frequency of the 1014F kdr mutation is driving pyrethroid resistance in *Anopheles coluzzii* population from Guinea savanna of Cameroon. *Parasit Vectors.* 2019:1–13. doi:10.1186/s13071-019-3523-7
33. Maketa V, Mavoko HM, da Luz RI, Zanga J, Lubiba J, Kalonji A, Lutumba P, Van Geertruyden JP (2015) The relationship between Plasmodium infection, anaemia and nutritional status in asymptomatic children aged under five years living in stable transmission zones in Kinshasa, Democratic Republic of Congo. *Malar J* 14: 83. 10.1186/s12936-015-0595-5 [doi];10.1186/s12936-015-0595-5 [pii]

Figures

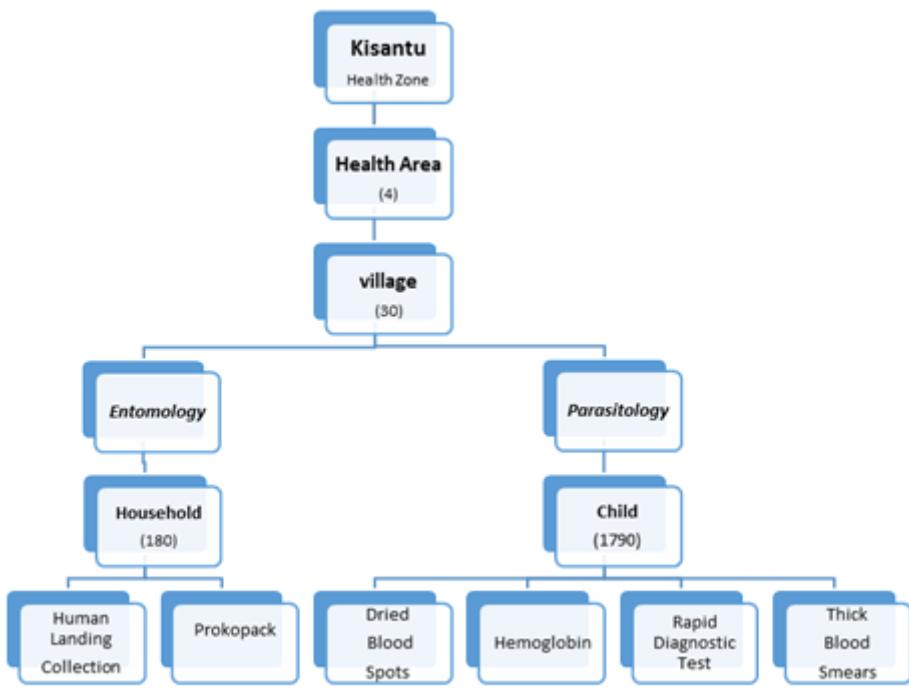


Figure 1

Design illustrating the entomological and epidemiological baseline data collection Kisantu 2018.

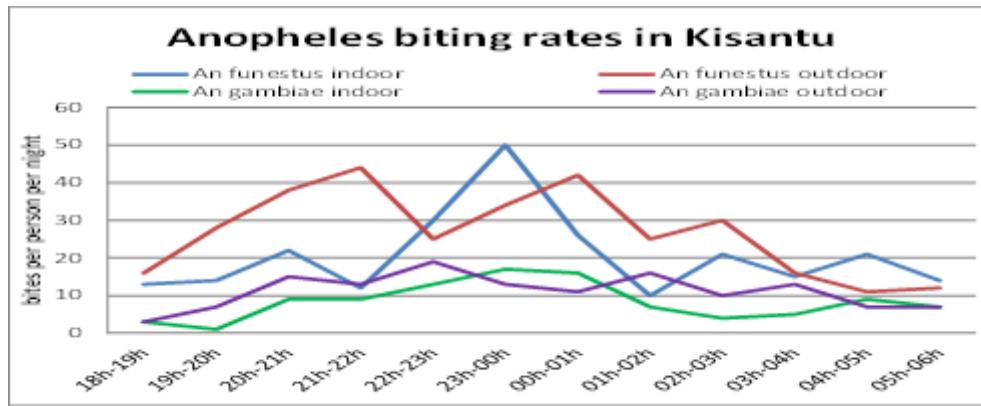


Figure 2

Assessment of An. funestus and An. gambiae s.l. activity based on the human landing collection, Kisantu 2018.

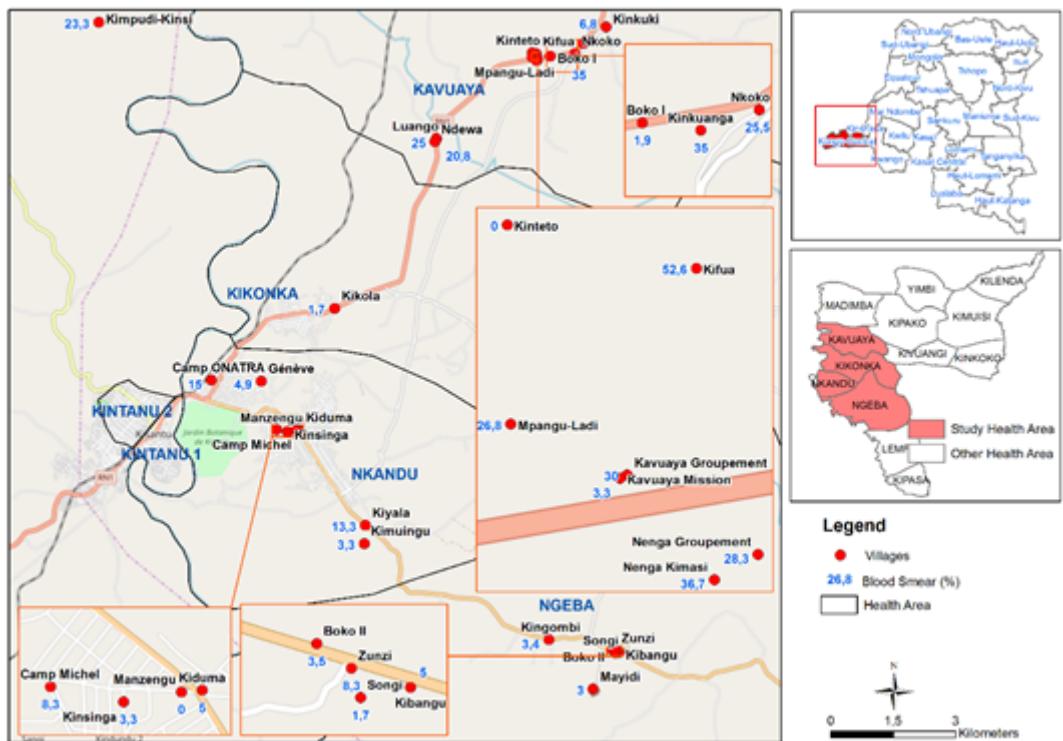


Figure 3

Local variation of malaria infection prevalence by villages in the 4 areas in Kisantu Health Zone, Kongo-Central Province.

Supplementary Files

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