Malaria Parasite Species Composition of *Plasmodium* Infections Among Asymptomatic and Symptomatic School-Age Children in Rural and Urban Areas of Kinshasa, Democratic Republic of Congo

Sabin S. Nundu
Institute of tropical Medicine, Nagasaki University
Richard Culleton (culleton.richard.oe@ehime-u.ac.jp)
Ehime University https://orcid.org/0000-0001-8808-5771
Shirley V. Simpson
Institute of Tropical Medicine, Nagasaki University
Hiroaki Arima
Institute of Tropical Medicine, Nagasaki University
Jean-Jacques Muyembe
Institut National de Recherche Biomédicale
Toshihiro Mita
Juntendo University
Steve Ahuka
Institut National de Recherche Biomédicale: Institut National de Recherche Biomedicale
Taro Yamamoto
Nagasaki University

Research

Keywords: Plasmodium, malaria, school-age children, Democratic Republic Congo

Posted Date: April 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-389568/v1

Abstract

Background. Malaria remains a major public health concern in Democratic Republic of Congo (DRC), and school-age children are relatively neglected in malaria prevalence surveys and may constitute a significant reservoir of transmission. This study aimed to understand the burden of malaria infections in school-age children in Kinshasa/DRC.

Methods. 634 (427 asymptomatic and 207 symptomatic) blood samples were collected from a cross-sectional survey of school-age children aged 6 to 14 years both without and with malaria symptoms. Nested-PCR was performed for malaria parasite species typing.

Results. The overall prevalence of *Plasmodium* spp., *Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale* was 62.3, 58.1, 19.9 and 10.8% among asymptomatic whereas it was 94.4, 93.2, 12.6 and 15.9% in symptomatic children, respectively. All *Plasmodium* species infections were significantly more prevalent in the rural area compared to the urban area in asymptomatic infections ($p<0.001$). Living in a rural as opposed to an urban area was associated with a five-fold greater risk of asymptomatic malaria parasite carriage ($p<0.001$). Amongst asymptomatic malaria parasite carriers, 43% of children in the rural area were co-infected with two or more species with *P. falciparum* + *P. malariae* the most common (24%) whereas in the urban setting, fewer children carried co-infections (16%) with *P. falciparum* + *P. malariae* again the most common (9%). A fifth of rural-dwelling symptomatic children were co-infected with two or more species with *P. falciparum* + *P. ovale* the most common (14%), while a quarter of symptomatic children in the urban area carried multiple species, with co-infections of *P. falciparum* + *P. malariae* the most common (11%).

Conclusion. School-age children are at significant risk from both asymptomatic and symptomatic malaria parasite infections. Continuous systematic screening and treatment of school-age children in high-transmission settings across the country may reinforce malaria intervention measures.

Background

Despite a widespread reduction in malaria-asscociated morbidity and mortality in the past decade, malaria remains a major public health problem in sub-Saharan Africa. In 2018, 228 million estimated cases of malaria resulting in 405,000 deaths occurred worldwide. The African region accounted for 93% of cases and 94% of deaths, and *Plasmodium falciparum* was responsible for 99.7% of all cases (1).

It has been shown that in high transmission sites, symptomatic malaria occurs most often in children under five years old, whereas asymptomatic infections generally occur in older people, school-age children and adults that have acquired immunity against the disease due to repeated exposure (2–5). Systematic screening and treatment of asymptomatic individuals in high-transmission settings may facilitate malaria intervention measures and contribute to malaria elimination (6, 7).

School-age children are not usually covered by household-based cluster surveys and/or malaria interventions and so represent an untreated demographic that may harbour a significant parasite reservoir (8–13), thus posing a major challenge for malaria control, surveillance, and elimination strategies (8, 14–16). Even though school-age children rarely develop complicated forms of malaria, chronic infection among this group is an important major contributor to pathology, including anaemia, and thus may have profound consequences for

neuro-cognitive development and educational achievement including increased absenteeism, poor school performance, and cognitive disorders (17–22).

The Democratic Republic of Congo (DRC) is the second most malaria-affected country in the world after Nigeria (1). About 97% of its inhabitants live in perennial malaria transmission zones, in which transmission occurs for 8 to 12 months yearly (23). In 2018, the country accounted for 12% and 11% of all estimated malaria cases and deaths worldwide, respectively (1). Currently, all WHO recommended intervention measures are undertaken under the umbrella of the national malaria control program (NMCP), except for chemoprevention in children. The burden of malaria in school-age children in DRC is not well understood, as most malaria prevalence studies have focused on children under five years old.

Here, we assessed the burden of malaria among asymptomatic and symptomatic school-age children living in rural and urban areas in Kinshasa/DRC, to inform the design and employment of school-based malaria control interventions for malaria control in this underserved population.

Methods

Study design

A cross-sectional study was undertaken between October and November 2019 among school-age children aged 6 to 14 years in Kinshasa, DRC.

Study area and study population

The study was carried out in primary schools and health facilities in Kinshasa. Kinshasa is the capital city of the country with an area of 9, 965 km^2 (urban area: 600 km^2) located at 4˚19’30”S 15˚19’20”E. In 2017, its population was estimated at 12.7 million. The common language is Lingala (local language) and French (official language). It is at an altitude of 240 m above the sea level, with an annual temperature range of 10˚C to 38˚C (October: 17˚C to 35˚C, November: 18˚C to 37˚C) and an annual rainfall of 1482 mm. Kinshasa is subdivided into 24 communes and 35 health zones (HZ).

The study was conducted at primary schools and health facilities in the rural area of Mont-Ngafula 2 Health Zone (HZ) and the urban area of Selembao HZ. In selected schools, we included all children aged 6 to 14 years with body temperatures less than 37.5˚C during a physical examination and who did not have malaria-related symptoms in the two weeks prior to the survey. In selected health facilities, we included all outpatient children aged 6 to 14 years who exhibited fever and/or malaria-related symptoms within the three days prior to medical consultation and who had not taken antimalarial drugs prior to the consultation.

Sampling and Sample size determination

Both hospital-based and school-based surveys were conducted. A two-stage stratified cluster sampling protocol was performed to select two health zones (HZ) among 35 within Kinshasa and their constituent health facilities and primary schools. The sample size was determined using the standard statistical formula $n= (Z^2p (1-p)/d^2)$ considering 95% confidence interval, 50% estimated prevalence and 5% precision.

Based on this assumption, 634 school-age children (427 asymptomatic and 207 symptomatic) were included in this study.

Data collection

In schools, two visits were necessary for sample collection. The first visit consisted of the selection of primary classrooms of each primary grade followed by the distribution of written consent forms to all children belonging to the selected classroom for their parents/guardians. On the second visit, after obtaining written consent from parents/guardians, information related to sex, age, and body temperature were recorded and a physical examination carried out. An interview was conducted for asymptomatic school-age children from 4th to 6th primary grades using a semi-structured questionnaire to record information related to the history of fever and malaria-related symptoms and treatment; sociodemographic status of parents (parent marital status, number of father's partners, father's education level, mother's education level, family size) and information related to the use of mosquito bed nets and/or indoor insecticide spray within households.

The questionnaire was written in English by the research team, and later translated into French (official language). When children were unable to give correct answers to certain questions (questions related to the education level of parent, marital status, etc.), the question(s) were addressed to parents through telephone calls.

In health facilities samples were collected day by day depending on health facility attendance. Malaria-related symptoms and information related to gender, age, and body temperature were recorded followed by physical examination in health facilities after obtaining consent from outpatient parents/guardians.

Detection and identification of Plasmodium spp.

In both schools and health facilities, blood was collected from a finger prick for malaria rapid diagnostic tests, thin and thick film preparation, and spotted onto Whatman 903™ filter paper (Whatman plc, UK) for PCR analysis. Whatman 903™ filter papers containing blood samples were dried and stored in individual plastic bags containing desiccant and stored at -20˚C before transportation to Nagasaki University for PCR analysis. DNA was extracted using the QIAamp DNA Mini Kit® (Qiagen, USA) according to the manufacturer's instructions. DNA was eluted in 50 µL of the provided buffer.

A nested PCR using primers targeting the *Plasmodium* mitochondrial cytochrome c oxidase III (*cox3*) gene was performed using the protocol described by Isozumi *et al* (24) with minor modifications; in particular, the *P. vivax* specific primers were redesigned due to concerns regarding the non-specific binding of the originally described primers (**Table 1**). PCR products were visualized under UV light on 2% agarose gels run at 100 volts for 30 minutes and stained with Gel Red® solution for 30 minutes.

Data management and analysis

Data was double-entered and validated in EPI INFO version 3.5.1 and analysed using STATA version 14.2 (College Station. Texas, USA). Descriptive variables were analysed as proportions (qualitative variables) or by median/mean (continuous variables). Chi-square tests (or Fisher's exact tests when appropriate) and logistic regression analysis were used to assess associations between independent variables and *Plasmodium spp.*

infection prevalence. Odds ratios (ORs) and 95% confidence intervals (CIs) were derived. Significance was set at $p < 0.05$.

Ethical considerations

The study was approved by the ethics committees of the School of Public Health, Kinshasa University, DRC (Approval number: ESP/CE/042/2019) and the Institute of Tropical Medicine, Nagasaki University (Approval number: 190110208-2). Written informed consent was obtained from children's parents/guardians and assent from children ≥ 7 years old were sought. The written informed consent document was provided either in French (official language) or Lingala (local language) depending on the parent's educational background. All malaria positive cases from mRDTs were treated according to national malaria diagnosis and treatment guidelines.

Results

Description of study population

A total of 634 (210 asymptomatic and 105 symptomatic in the rural area; 217 asymptomatic and 102 symptomatic in the urban area) children aged 6 to 14 years old were included in this study.

Their median (interquartile range) age was 9 (7-11) and 8 (7-9) for asymptomatic and symptomatic children in the rural area, and 8 (7-10) and 9 (7-12) for asymptomatic and symptomatic children in the urban area, respectively. Children aged 6 to 9 years old accounted for 51% of the asymptomatic group and 78% of the symptomatic group in the rural area, and 68% of the asymptomatic group and 53% of the symptomatic group in the urban setting; half of the asymptomatic and the symptomatic group were females in the rural area, and about 55% of the asymptomatic group were females and 56% of the symptomatic group were males in the urban setting (**Additional file, Table S1**).

Information relating to sociodemographic characteristics of asymptomatic school-age children and malaria preventive measures

Among 227 (131 in the rural area and 96 in the urban area) asymptomatic school-age children who were interviewed, most of children (≥80%) lived together with their parents in both rural and urban areas, 60% had at least one mosquito bed net and 31% slept under a mosquito net the night before the interview in the rural area whereas 28% had at least one mosquito bed net and 25% slept under a mosquito net the night before in the urban area (**Additional file, Table S2**).

History of last fever and/or malaria-like symptoms

Among 131 children interviewed in the rural area, 54% reported a malaria episode in the three months prior to the survey. Only 34% went to a health facility, and the majority of these (82%) went to a health centre. Forty-four percent of children had self-medicated (of which 33% had used pyrimethamine/sulfadoxine and 11% artemisinin-based combination therapy) without a confirmed malaria diagnosis. Fifty-two percent of children missed classes and among these, 39% missed five days or more (**Figure 1**).

Among 96 children interviewed in the urban area, a 78% reported a malaria episode in the three months prior to the survey. Only 39% went to a health facility, and the majority of these (95%) went to a health centre. Seventeen

percent of children had self-medicated (of which 25% used Pyrimethamine/sulfadoxine, 25% used artemisinin-based combination therapy and 50% did not remember the drug's name) without a confirmed malaria diagnosis. Forty-four percent of children missed classes and among these, 38% missed five days or more (**Figure 1**)

Symptoms of outpatient school-age children at admission

All 207 children (105 in the rural area and 102 in the urban area) attending health facilities had fever symptoms (100%), followed by vomiting (37%), fatigue (28%) and diarrhoea (23%) in the rural area versus headache (72%), fatigue (50%), lack of appetite (49%) and abdominal pain (48%) in the urban setting as major symptoms (**Additional file, Table S3**)

Prevalence of Plasmodium spp. infections

The overall prevalence of *Plasmodium* spp., *Plasmodium falciparum,Plasmodium malariae and Plasmodium ovale* was 62%, 58%, 19% and 10% among asymptomatic children and 94%, 93%, 12% and 15% in symptomatic children, respectively. No *P. vivax* infections were observed.

All malaria parasite species were significantly more prevalent in the rural area compared to the urban setting in asymptomatic infections (p<0.001), whereas in symptomatic infections, *P. malariae* was significantly less prevalent in the rural area compared to the urban setting (7.6 versus 17.7%, p=0.03, **Table 1**).

Association age / gender with Plasmodium species infections

Figure 3 shows the distribution of the malaria parasite species infecting asymptomatic children in the rural and urban areas. The distribution of *P. falciparum* does not change with age in the in rural setting, while it increases with age in the urban setting. *Plasmodium malariae* and *P. ovale* infections are more common in older children in the rural area.

In the rural area, asymptomatic children aged 10 to 14 years were significantly more likely to be infected with *P. malariae* (38.5 versus 24.5%, p=0.03) and *P. ovale* (23.1 versus 10.4%, p=0.014) than those aged 6 to 9 years. In the urban area, however, older children were more likely to carry *P. falciparum* than younger children (50.7 versus 35.1%, p=0.029). There was no association between gender and any particular *Plasmodium* species infection iin either the rural or urban areas (**Additional file, Table S4a**). Older children were less likely to harbour single species (33.7 versus 57.6%) and more likely to harbour mixed species infections (44.2 versus 24.5 %), (p=0.02) infections in the rural area, whereas in the urban area older children were insignificantly more likely to harbour both single species (46.4 versus 33.8%) and mixed species infections (8.7 versus 6.8 %), (p=0.13) infections (**Additional file, Table S5a**)

In symptomatic children, there was no association between age or gender with particular malaria parasite infections in either the rural or the urban areas except for *P. ovale* which infected younger children more often in the urban setting (4.2 versus 22.2 %, p=0.009) (**Additional file, Table S4b**). Age and gender were not associated with carriage of single compared to mixed infections in either the rural or the urban areas (**Additional file, Table S5b**).

Predictors of asymptomatic malaria infection

Residence in the rural setting was associated with an increased risk of asymptomatic malaria parasite carriage. There was an approximately five times greater risk of asymptomatic carriage of malaria parasites for children living in the rural, as opposed to urban area ($p<0.001$). Other investigated factors were not associated with increased risk of asymptomatic malaria infection (**Additional file, Table S6).**

Discussion

This study aimed to measure the burden of *Plasmodium* spp. infections amongst asymptomatic and symptomatic school-age children living in rural and urban areas in Kinshasa, DRC.

Kinshasa, the capital city of DRC, constitutes an urban malaria facies where malaria prevalence is moderate, with an average of eight percent of the population infected at any given time in the city, with increase prevalence variations away from the city centre (23, 25). Malaria transmission rates are not homogenous throughout the city and depend on the population density and level of urbanization. The prevalence is highest in the more densely populated and less urbanized zones in the periphery (23, 26). Additionally, malaria infection usually follows a seasonal pattern regulated by mosquito population fluctuations controlled by climate (26, 27). This study was conducted at the beginning of the rainy season between October and November when conditions of temperature and humidity are favourable for malaria transmission. Temperature, humidity, and rainfall constitute important drivers of mosquito dynamics and malaria risk (27-29).

School-age children currently constitute a neglected group regarding the control and prevention of malaria in the DRC, which currently predominantly targets children under five years old and pregnant women (23). The prevalence of malaria parasite infections found in this study was 62.3% (80.0% in rural area and 45.2% in urban area) in asymptomatic children and 94.7% (97.2 % in rural area and 92.2% in urban area) in those children with symptoms suggestive of malaria. The high prevalence found in both asymptomatic and symptomatic children highlights the importance of malaria in this age group in Kinshasa.

At least nine out of ten children that presented at a health facility with fever or malaria-related symptoms were found to be infected with malaria parasites. The WHO recommends that anti-malaria treatment is given only after a positive confirmatory test for malaria, by means such as microscopy or RDT. However, both microscopy and RDTs carry some risk of false negative results, compromising treatment in some cases. Additionally, not all symptomatic children can attend health facilities despite the fact that the NMCP provide diagnosis and treatment free to all children attending health facilities for confirmatory test. This situation may be due to sub-optimal communication between NMCP health professionals and community members or due to a lack of available transportation fees for access to the health facility. In this study, among the children surveyed, nearly 66% (rural) and 62% (urban) of children did not go to health facilities for confirmatory testing in the last time they had malaria-like symptoms, and among them, 44% and 17% reported having self-medicated, respectively.

We observed a high prevalence of *Plasmodium* spp. infection in asymptomatic school-age children of 45% and 80% in the urban and rural settings, Our findings are similar to those found in a survey conducted in Lualaba, DRC (77%) (30), and in the national malaria survey in Cote d'Ivoire (63.3%) (31), whilst differing from a study conducted in Uganda (30%) (20). Studies conducted in Kenyan schools between 2008 and 2010 showed a prevalence of 4% with a range between 0 and 71% (32). Discrepancies in prevalence may be explained by differences in detection techniques used, differences in season of sampling, and transmission setting

differences (13, 20, 33). Malaria management policies regarding prevention and control measure also differ between countries and may be expected to account for some of the differences in prevalence observed.

In asymptomatic children, all *Plasmodium* species infections were significantly more prevalent in the rural area compared to the urban setting. There was a significant difference in malaria prevalence in children living in rural as opposed to urban areas, with the former five times more likely to be infected with malaria parasites. This finding is an agreement with numerous previous reports, and likely reflects the fact that the ratio of mosquitoes to humans is higher in rural areas than in urban areas (26, 27, 34).

It has been shown that, in urban areas, modern housing, high human population densities, awareness of use of insecticides and bed nets may reduce the risk of malaria transmission by reduction of human-mosquito contacts and individual biting rates (35, 36). An increase in the density of dwellings in older urban districts of Kinshasa has contributed to the scarcity of *Anopheles* breeding sites through elimination and pollution except in areas using urban agriculture and gardens (26). A relationship between areas close to agriculture fields and high malaria prevalence or transmission was found in Ghana, Uganda, Benin, and Cote d'Ivoire(37-42). Proximity to permanent larval habitats sites (43), inadequate social-health resources and lower health spending (44) may facilitate transmission in rural zones.

A study previously conducted in Kinshasa showed that in urban areas of the city, most of the mosquito nuisance is caused by *Culex quinquefasciatus* which accounted for 96% of the 121 bites/ person/night (b/p/n). The only malaria-vector mosquito detected was *Anopheles gambiae sensu stricto* which accounted for an average of 5.1 bites/person/night with a sporozoite rate of 1.86%, while in rural areas, mosquito nuisance is lower (20bites/person/night), and almost entirely due to six species of *Anopheles* including four vectors of malaria: *An. gambiae*, *An. funestus*, *An. nili* and *An. brunnipes* with mean sporozoite rates of 7.85%, 6.60%, 6.63% and 0.53% respectively. The study concluded that *Anopheles gambiae* had higher daily mean survival rates in rural areas (0.91) than in urban areas (0.78) leading to a greater malaria transmission rate in the former setting (27). This is supported by a meta-analysis which found that in sub-Saharan African cities, the mean annual entomologic inoculation rates (EIR) were 7.1 in city centers, 45.8 in periurban areas, and 167.7 in rural areas (45). However, apid and unprecedented urbanization in sub-Saharan African, combined with often increasing rates of poverty amongst city dwellers may increase the proportion of the malaria burden borne by urbanites (46).

We found no significant difference in *Plasmodium* spp. infections between genders in either asymptomatic and symptomatic children in both rural and urban areas. These findings agree with previous studies that demonstrated equal exposure between males and females to malaria risk at or below 12 years of age (47, 48).

We found that age, generally, was not associated with *Plasmodium* spp. infections. However, there was a significant association between age and asymptomatic *P. malariae* and *P. ovale* infections in the rural area, and *P. falciparum* infections in urban setting, with children aged 10 to 14 years more infected than those aged 6 to 9 years. Older children were also the group most likely to harbour more single and mixed infections than younger ones. The proportion of children infected with *P. falciparum* remained constant for all ages in the rural area, while it increased with age in the urban setting. *Plasmodium malariae* and *P. ovale* infections increased with age in the rural area while they did not do so in the urban setting. That difference may be due to age-related acquisition of parasite-tolerating immunity (49, 50). It has been shown that in malaria tropical facies, malaria

pre-immunity starts building up around 10 years (23). It may also reflect the relative force of infection of the species, with that of P. falciparum being higher than the other two species in the rural setting.

The presence of *P. ovale* and *P. malariae*, co-infected with *P. falciparum*, highlights the impact of those two parasites in asymptomatic and chronic malaria infection. *Plasmodium malariae* and *P. ovale* are not usually associated with severe malaria but *P. malariae* may be responsible for chronic nephrotic syndrome which can be fatal (51), and chronic infections that can last for years (52), even after leaving endemic regions (44, 53). *Plasmodium ovale* is responsible for relapses after months or even years without symptoms due to the presence of hypnozoites (54-60) and it has been shown to cause severe disease and even death on occasion (61-63). Younger school children become sicker with malaria and receive treatment more often, while older children, due to increasing immunity, are more likely to be asymptomatic and receive treatment less often (26).

Our data are in agreement with previous reports that show that *P. malariae* is much less likely to be observed in mixed species infections with *P. falciparum* in symptomatic malaria infections when the transmission rate of malaria is high (64, 65). The reason for this is currently unclear. It is possible that there is a protective effect of mixed infection with *P. malariae* on the severity of the disease caused by *P. falciparum*, perhaps mediated through cross-immunity. It is also possible that in symptomatic *P. falciparum* infections, this species competitively excludes co-infecting species due to increased parasitaemia. This exclusion could result from within-host competition for resources (66). Or through host-immune mediated mechanisms in which the innate immune response triggered by the high parasitaemia *P. falciparum* disproportionately affects the less dominant of the species in the co-infection. A further possibility is that the nested PCR methodology used here to determine parasite species may miss the less common of co-infecting species when the disparity between them is large as is likely in symptomatic *P. falciparum* infected patients.

We did not detect *P. vivax* in any child in this study. This may be due to the high prevalence of the Duffy blood-group-negative phenotype amongst the children surveyed (67), or due to a lack of circulation of the *P. vivax* parasites in these areas. However, Kavunga *et al* (68) describe *P. vivax* in children under five living Kinshasa and North-Kivu in DRC emphasizing the need for continued monitoring of this species among Duffy blood-group-negative populations (49, 50).

Based on the high prevalence of malaria parasites in this study, we advocate integration of the WHO T3 (Test-treat-track) strategy in schools, including the training of teachers in health education, particularly regarding the use of mosquito bed nets (69). It has been shown that net coverage is relatively low in school-age children (12). In this study, use of mosquito bed net was not associated with plasmodium infection prevalence maybe due to small number of interviewed children. However, the coverage of use of mosquito bed net among them was low. Only 46% of children surveyed had mosquito bed nets in their houses but 38% of these did not use a net the previous night.

It is important to enhance control measures for asymptomatic malaria parasite carriage amongst school-age children to protect them against conditions such as chronic anaemia, absenteeism, reduced school performance, and other complications (17, 19). We found that 45% of children surveyed had previously missed classes due to malaria and of these, 40% had missed five days or more of school, illustrating the high educational burden of the disease amongst this age group.

The government should consider revising its policy regarding malaria control if the target of a 75% reduction in malaria mortality and incidence is to be achieved by 2025 (70). Regarding the global technical strategy for malaria 2016-2030 initiated by the WHO, the DRC is currently working on strategies to entirely implement the first pillar, ensuring universal access to malaria prevention, diagnosis, and treatment. Furthermore, the DRC ministry of health is exploring for mechanisms and strategies for control and prevention which will have positive impacts on malaria elimination in the country. We advocate the inclusion of school-age children in country-wide malaria survey, control, and prevention. We believe that malaria elimination in the DRC may only be possible if all age groups are included in intervention strategies, not just children under five and pregnant women. Thus, the implementation of malaria preventive treatment in younger and older children will help to reduce the burden of malaria in these susceptible groups.

It has been shown that continuous systematic malaria screening and treatment of asymptomatic individuals, such as school-aged children, in high-transmission settings may reinforce malaria intervention measures (7, 71, 72). Moreover, school-based malaria prevalence surveys (are easier to conduct and are cost-effective for more defined assessments at the local level, including routine longitudinal malaria surveillance as it is a reliable indicator of malaria burden and transmission intensity in a defined community (30, 73).

Conclusion

There is a need to include school-age children in malaria control, surveillance, and elimination strategies. Therefore, a continuous systematic school-based prevention, screening, and treatment of children in high-transmission settings may strengthen malaria intervention measures.

Declarations

Ethics approval and consent to participate

Surveys protocols were submitted to the ethics committees of the School of Public Health, Kinshasa University, DRC and the Institute of Tropical Medicine, Nagasaki University. Approvals were granted under the following reference numbers: The ethic committee of the School of Public Health, Kinshasa University, DRC (Approval number: ESP/CE/042/2019); the ethic committee of the Institute of Tropical Medicine, Nagasaki University (Approval number: 190110208-2). Written consent was taken from parents/guardians. Data was used with high confidentiality and no names were recorded.

Consent for publication

Consent for publication was obtained from the parents/guardians of each child during the study.

Availability of data and materials

The datasets used and/or analysed during the current study are available from

the first author (SSN) on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Japan International Cooperation Agency and the Joint Usage / Research Center on Tropical Disease, Institute of Tropical Medicine, Nagasaki University (2020-Ippan-14, 2020-Ippan-23).

Author contributions

Conceptualization: SSN, TY, RC

Data curation: SSN, RC

Formal analysis: SSN, RC

Investigation: SSN, TY, HA

Methodology: SSN, RC, TY, TM

Contributed materials: RC

Supervision: RC, TY, TM, SA, JJM

Writing-original draft: SSN

Writing review & editing: SSN, RC, TY, TM, SVS, HA, SA

Laboratory works: SSN, SVS, HA, RC

Acknowledgments

We thank the authorities of the Kinshasa Provincial Health Inspectorate and Institut National de Recherche Biomédicale (INRB) for facilitation. Special thanks and gratitude to Dr. Mamie Lau, the Head of Selembao Health Zone; Dr. Edo Molamba the Head of Mongafula-2 Health Zone as well as the Directors of the primary schools Ecole chretienne Edmond and Cité des aveugles for supervision. We also thank Dr. Akintije Simba Calliope for contributing to the discussion of this study.

References

1. World_Health_Organization. World malaria report 2019. https://wwwwhoint/publications/i/item/9789241565721. 2019;Accessed on 2 Feb 2020.
2. Day KP, Marsh K. Naturally acquired immunity to Plasmodium falciparum. Immunol Today. 1991;12(3):A68-A71.
3. Grobusch MP, Kremsner PG. Uncomplicated Malaria. In: Compans RW, Cooper MD, Honjo T, Koprowski H, Melchers F, Oldstone MBA, et al., editors. Malaria: Drugs, Disease and Post-genomic Biology. 295. Berlin, Heidelberg: Springer Berlin Heidelberg; 2005. p. 83-104.

4. Snow RW, Omumbo JA, Lowe B, Molyneux CS, Obiero JO, Palmer A, et al. Relation between severe malaria morbidity in children and level of Plasmodium falciparum transmission in Africa. Lancet. 1997;349(9066):1650-4.
5. Vafa M, Troye-Blomberg M, Anchang J, Garcia A, Migot-Nabias F. Multiplicity of Plasmodium falciparum infection in asymptomatic children in Senegal: relation to transmission, age and erythrocyte variants. Malar J. 2008;7(1):17.
6. Crowell V, Briët OJ, Hardy D, Chitnis N, Maire N, Di Pasquale A, et al. Modelling the cost-effectiveness of mass screening and treatment for reducing Plasmodium falciparum malaria burden. Malar J. 2013;12:4.
7. Kern SE, Tiono AB, Makanga M, Gbadoé AD, Premji Z, Gaye O, et al. Community screening and treatment of asymptomatic carriers of Plasmodium falciparum with artemether-lumefantrine to reduce malaria disease burden: a modelling and simulation analysis. Malaria Journal. 2011;10:210.
8. Alves FP, Gil LHS, Marrelli MT, Ribolla PEM, Camargo EP, Da Silva LHP. Asymptomatic Carriers of Plasmodium spp. as Infection Source for Malaria Vector Mosquitoes in the Brazilian Amazon. J Med Entomol. 2005;42(5):777-9.
9. Baliraine Frederick N, Afrane Yaw A, Amenya Dolphine A, Bonizzoni M, Menge David M, Zhou G, et al. High Prevalence of Asymptomatic Plasmodium falciparum Infections in a Highland Area of Western Kenya: A Cohort Study. J Infect Dis. 2009;200(1):66-74.
10. Bousema JT, Gouagna LC, Drakeley CJ, Meutstege AM, Okech BA, Akim IN, et al. Plasmodium falciparum gametocyte carriage in asymptomatic children in western Kenya. Malar J. 2004;3:18.
11. Drakeley CJ, Akim NIJ, Sauerwein RW, Greenwood BM, Targett GAT. Estimates of the infectious reservoir of Plasmodium falciparum malaria in The Gambia and in Tanzania. Trans R Soc Trop Med Hyg. 2000;94(5):472-6.
12. Noor AM, Kirui VC, Brooker SJ, Snow RW. The use of insecticide treated nets by age: implications for universal coverage in Africa. BMC Public Health. 2009;9:369.
13. Pullan RL, Bukirwa H, Staedke SG, Snow RW, Brooker S. Plasmodium infection and its risk factors in eastern Uganda. Malaria Journal. 2010;9:2.
14. Bottius E, Guanzirolli A, Trape J-F, Rogier C, Konate L, Druilhe P. Malaria: even more chronic in nature than previously thought; evidence for subpatent parasitaemia detectable by the polymerase chain reaction. Trans R Soc Trop Med Hyg. 1996;90(1):15-9.
15. Greenwood BM. Asymptomatic malaria infections — Do they matter? Parasitol Today. 1987;3(7):206-14.
16. Laishram DD, Sutton PL, Nanda N, Sharma VL, Sobti RC, Carlton JM, et al. The complexities of malaria disease manifestations with a focus on asymptomatic malaria. Malar J. 2012;11(1):29.
17. Clarke SE, Jukes MC, Njagi JK, Khasakhala L, Cundill B, Otido J, et al. Effect of intermittent preventive treatment of malaria on health and education in schoolchildren: a cluster-randomised, double-blind, placebo-controlled trial. Lancet. 2008;372(9633):127-38.
18. Fernando SD, Rodrigo C, Rajapakse S. The 'hidden' burden of malaria: cognitive impairment following infection. Malar J. 2010;9:366.
19. Lalloo DG, Olukoya P, Olliaro P. Malaria in adolescence: burden of disease, consequences, and opportunities for intervention. Lancet Infect Dis. 2006;6(12):780-93.

20. Nankabirwa J, Wandera B, Kiwanuka N, Staedke SG, Kamya MR, Brooker SJ. Asymptomatic Plasmodium infection and cognition among primary schoolchildren in a high malaria transmission setting in Uganda. Am J Trop Med Hyg. 2013;88(6):1102-8.
21. Thuilliez J. Fever, malaria and primary repetition rates amongst school children in Mali: Combining demographic and health surveys (DHS) with spatial malariological measures. Soc Sci Med. 2010;71(2):314-23.
22. Thuilliez J, Sissoko MS, Toure OB, Kamate P, Berthélemy J-C, Doumbo OK. Malaria and primary education in Mali: A longitudinal study in the village of Donéguébougou. Soc Sci Med. 2010;71(2):324-34.
23. Agency_for_International_Development US. PRESIDENT'S MALARIA INITIATIVE DEMOCRATIC REPUBLIC OF THE CONGO Malaria Operational Plan FY 2018. https://wwwpmigov/docs/default-source/default-document-library/malaria-operational-plans/fy-2018/fy-2018-democratic-republic-of-the-congo-malaria-operational-planpdf?sfvrsn=5. 2018;Accessed on 2 Des 2020.
24. Isozumi R, Fukui M, Kaneko A, Chan CW, Kawamoto F, Kimura M. Improved detection of malaria cases in island settings of Vanuatu and Kenya by PCR that targets the Plasmodium mitochondrial cytochrome c oxidase III (cox3) gene. Parasitol Int. 2015;64(3):304-8.
25. Kazadi W, Sexton JD, Bigonsa M, W'Okanga B, Way M. MALARIA IN PRIMARY SCHOOL CHILDREN AND INFANTS IN KINSHASA, DEMOCRATIC REPUBLIC OF THE CONGO: SURVEYS FROM THE 1980S AND 2000. The American Journal of Tropical Medicine and Hygiene Am J Trop Med Hyg. 2004;71(2_suppl):97-102.
26. Ferrari G, Ntuku HM, Schmidlin S, Diboulo E, Tshefu AK, Lengeler C. A malaria risk map of Kinshasa, Democratic Republic of Congo. Malaria journal. 2016;15:27.
27. Coene J. Malaria in urban and rural Kinshasa: the entomological input. Med Vet Entomol. 1993;7(2):127-37.
28. Bomblies A. Modeling the role of rainfall patterns in seasonal malaria transmission. Clim Change. 2012;112(3):673-85.
29. Stresman GH. Beyond temperature and precipitation: ecological risk factors that modify malaria transmission. Acta Trop. 2010;116(3):167-72.
30. Swana EK, Yav TI, Ngwej LM, Mupemba BN, Suprianto, Mukeng CK, et al. School-based malaria prevalence: informative systematic surveillance measure to assess epidemiological impact of malaria control interventions in the Democratic Republic of the Congo. Malar J. 2018;17(1):141.
31. Yapi RB, Hürlimann E, Houngbedji CA, Ndri PB, Silué KD, Soro G, et al. Infection and co-infection with helminths and Plasmodium among school children in Côte d'Ivoire: results from a National Cross-Sectional Survey. PLoS Negl Trop Dis. 2014;8(6):e2913.
32. Gitonga CW, Karanja PN, Kihara J, Mwanje M, Juma E, Snow RW, et al. Implementing school malaria surveys in Kenya: towards a national surveillance system. Malar J. 2010;9(1):306.
33. Kabatereine NB, Standley CJ, Sousa-Figueiredo JC, Fleming FM, Stothard JR, Talisuna A, et al. Integrated prevalence mapping of schistosomiasis, soil-transmitted helminthiasis and malaria in lakeside and island communities in Lake Victoria, Uganda. Parasites & Vectors. 2011;4(1):232.
34. Ménard D, Barnadas C, Bouchier C, Henry-Halldin C, Gray LR, Ratsimbasoa A, et al. Plasmodium vivax clinical malaria is commonly observed in Duffy-negative Malagasy people. Proc Natl Acad Sci U S A. 2010;107(13):5967-71.

35. Austin KF, Noble MD, Mejia MT. Gendered vulnerabilities to a neglected disease: A comparative investigation of the effect of women's legal economic rights and social status on malaria rates. Int J Comp Sociol. 2014;55(3):204-28.
36. Popovici J, Roesch C, Rougeron V. The enigmatic mechanisms by which Plasmodium vivax infects Duffy-negative individuals. PLoS Pathog. 2020;16(2):e1008258.
37. Clark TD, Greenhouse B, Njama-Meya D, Nzarubara B, Maiteki-Sebuguzi C, Staedke SG, et al. Factors Determining the Heterogeneity of Malaria Incidence in Children in Kampala, Uganda. The Journal of Infectious Diseases. 2008;198(3):393-400.
38. Govoetchan R, Gnanguenon V, Azondékon R, Agossa RF, Sovi A, Oké-Agbo F, et al. Evidence for perennial malaria in rural and urban areas under the Sudanian climate of Kandi, Northeastern Benin. Parasites & Vectors. 2014;7(1):79.
39. Klinkenberg E, McCall PJ, Wilson MD, Amerasinghe FP, Donnelly MJ. Impact of urban agriculture on malaria vectors in Accra, Ghana. Malaria Journal. 2008;7(1):151.
40. Noble MD, Austin KF. Rural Disadvantage and Malaria in Less-Developed Nations: A Cross-National Investigation of a Neglected Disease. Rural Sociol. 2016;81(1):99-134.
41. Norris DE. Mosquito-borne Diseases as a Consequence of Land Use Change. EcoHealth. 2004;1(1):19-24.
42. Soulama I, Nébié I, Ouédraogo A, Gansane A, Diarra A, Tiono AB, et al. Plasmodium falciparum genotypes diversity in symptomatic malaria of children living in an urban and a rural setting in Burkina Faso. Malar J. 2009;8:135.
43. World_Health_Organization. Gender, Health and Malaria. https://wwwwhoint/gender-equity-rights/knowledge/gender-health-malariapdf?ua=1. 2007;Accessed on 6 Feb 2020.
44. Hay SI, Guerra CA, Tatem AJ, Atkinson PM, Snow RW. Urbanization, malaria transmission and disease burden in Africa. Nat Rev Microbiol. 2005;3(1):81-90.
45. Yadouléton A, N'Guessan R, Allagbé H, Asidi A, Boko M, Osse R, et al. The impact of the expansion of urban vegetable farming on malaria transmission in major cities of Benin. Parasites & Vectors. 2010;3(1):118.
46. Stoler J, Weeks JR, Getis A, Hill AG. Distance threshold for the effect of urban agriculture on elevated self-reported malaria prevalence in Accra, Ghana. Am J Trop Med Hyg. 2009;80(4):547-54.
47. Afrane YA, Klinkenberg E, Drechsel P, Owusu-Daaku K, Garms R, Kruppa T. Does irrigated urban agriculture influence the transmission of malaria in the city of Kumasi, Ghana? Acta Trop. 2004;89(2):125-34.
48. Matthys B, Vounatsou P, Raso G, Tschannen AB, Becket EG, Gosoniu L, et al. Urban farming and malaria risk factors in a medium-sized town in Cote d'Ivoire. Am J Trop Med Hyg. 2006;75(6):1223-31.
49. Keiser J, Utzinger J, Caldas de Castro M, Smith TA, Tanner M, Singer BH. Urbanization in sub-saharan Africa and implication for malaria control. Am J Trop Med Hyg. 2004;71(2 Suppl):118-27.
50. Robert V, Macintyre K, Keating J, Trape JF, Duchemin JB, Warren M, et al. Malaria transmission in urban sub-Saharan Africa. Am J Trop Med Hyg. 2003;68(2):169-76.
51. Eiam-Ong S. Malarial nephropathy. Semin Nephrol. 2003;23(1):21-33.
52. Greenwood BM, Bradley AK, Greenwood AM, Byass P, Jammeh K, Marsh K, et al. Mortality and morbidity from malaria among children in a rural area of The Gambia, West Africa. Trans R Soc Trop Med Hyg. 1987;81(3):478-86.

53. Siala E, Khalfaoui M, Bouratbine A, Hamdi S, Hili K, Aoun K. [Relapse of Plasmodium malariae malaria 20 years after living in an endemic area]. Presse Med. 2005;34(5):371-2.
54. Bottieau E, Van Gompel A, Peetermans WE. Failure of primaquine therapy for the treatment of Plasmodium ovale malaria. Clin Infect Dis. 2005;41(10):1544-5.
55. Centers_for_Disease_Control_and_Prevention. About Malaria. https://wwwcdcgov/malaria/about/diseasehtml. 2019;Accessed on 28 November 2020.
56. Chin W, Coatney GR. Relapse activity in sporozoite-induced infections with a West African strain of Plasmodium ovale. Am J Trop Med Hyg. 1971;20(6):825-7.
57. Collins WE, Jeffery GM. A retrospective examination of sporozoite-induced and trophozoite-induced infections with Plasmodium ovale: development of parasitologic and clinical immunity during primary infection. Am J Trop Med Hyg. 2002;66(5):492-502.
58. Garnham PC, Bray RS, Cooper W, Lainson R, Awad FI, Williamson J. The pre-erythrocytic stage of Plasmodium ovale. Trans R Soc Trop Med Hyg. 1955;49(2):158-67.
59. Jeffery GM, Young MD, Wilcox A. The Donaldson strain of malaria. 1. History and characteristics of the infection in man. Am J Trop Med Hyg. 1954;3(4):628-37.
60. Nathwani D, Currie PF, Smith CC, Khaund R. Recurrent plasmodium ovale infection from Papua New Guinea--chloroquine resistance or inadequate primaquine therapy? J Infect. 1991;23(3):343-4.
61. Facer CA, Rouse D. Spontaneous splenic rupture due to Plasmodium ovale malaria. Lancet. 1991;338(8771):896.
62. Groger M, Fischer HS, Veletzky L, Lalremruata A, Ramharter M. A systematic review of the clinical presentation, treatment and relapse characteristics of human Plasmodium ovale malaria. Malar J. 2017;16(1):112.
63. Lau YL, Lee WC, Tan LH, Kamarulzaman A, Syed Omar SF, Fong MY, et al. Acute respiratory distress syndrome and acute renal failure from Plasmodium ovale infection with fatal outcome. Malar J. 2013;12:389.
64. Abdulraheem MA, Ernest M, Ugwuanyi I, Abkallo HM, Nishikawa S, Adeleke M, et al. High prevalence of Plasmodium malariae and Plasmodium ovale in co-infections with Plasmodium falciparum in asymptomatic malaria parasite carriers in Southwest Nigeria. medRxiv. 2021:2021.01.14.21249635.
65. Black J, Hommel M, Snounou G, Pinder M. Mixed infections with Plasmodium falciparum and P malariae and fever in malaria. Lancet. 1994;343(8905):1095.
66. Tang J, Templeton TJ, Cao J, Culleton R. The consequences of mixed-species malaria parasite co-infections in mice and mosquitoes for disease severity, parasite fitness, and transmission success. bioRxiv. 2019:761791.
67. Miller LH, Mason SJ, Clyde DF, McGinniss MH. The resistance factor to Plasmodium vivax in blacks. The Duffy-blood-group genotype, FyFy. N Engl J Med. 1976;295(6):302-4.
68. Kavunga-Membo H, Ilombe G, Masumu J, Matangila J, Imponge J, Manzambi E, et al. Molecular identification of Plasmodium species in symptomatic children of Democratic Republic of Congo. Malar J. 2018;17(1):334.

69. World_Health_Organization. Scaling up diagnostic testing, treatment and surveillance for malaria. https://wwwwhoint/malaria/publications/atoz/test_treat_track_brochurepdf. 2012;Accessed on 25 Nov, 2020.
70. World_Health_Organization. GLOBAL TECHNICAL STRATEGY FOR MALARIA 2016–2030. https://appswhoint/iris/bitstream/handle/10665/176712/9789241564991_engpdf?sequence=1. 2015;Accessed on 2 Des 2020.
71. Clarke SE, Rouhani S, Diarra S, Saye R, Bamadio M, Jones R, et al. Impact of a malaria intervention package in schools on Plasmodium infection, anaemia and cognitive function in schoolchildren in Mali: a pragmatic cluster-randomised trial. BMJ Glob Health. 2017;2(2):e000182.
72. Cohee LM, Opondo C, Clarke SE, Halliday KE, Cano J, Shipper AG, et al. Preventive malaria treatment among school-aged children in sub-Saharan Africa: a systematic review and meta-analyses. Lancet Glob Health. 2020;8(12):e1499-e511.
73. Brooker S, Kolaczinski JH, Gitonga CW, Noor AM, Snow RW. The use of schools for malaria surveillance and programme evaluation in Africa. Malar J. 2009;8:231.

Tables

Table 1. Primer sequences and PCR conditions for *Plasmodium* spp. and *Plasmodium* genotyping amplification

Species	Primer name	Primer sequence (5' to 3')	Reaction components	Cycling conditions
	Outer			
Plasmodium	MtU.F	CTCGCCATTTGATAGCGGTTAACC	- One *Taq* 2X Master Mix with standard buffer: 12.5µL - 10µM forward primer: 0.5µL - 10µM reverse primer: 0.5µL - Nuclease free water: 6.5µL - DNA template: 5uL	94˚C/30s; 40 cycles of 94˚C/30s, 63˚C/1min, 68˚C/1min; 68˚C/5mins; 12˚C -∞
	MtU.R	CCTGTTATCCCCGGCGAACCTTC	- 25uL reaction volume	
	Inner			
P. falciparum	MtNst_falF	GAACACAATTGTCTTATTCGTACAATTATTC		
	MtNst_falR	CTTCTACCGAATGGTTTATAAATTCTTTC		
P. malariae	MtNst_malF	CTAGCTTTGTACACAAATTAATTCGTCTAC		
	MtNst_malR	CTTTATAAGAATGATAGATATTTATGACATA		
P. ovale	MtNst_ov2F	ATTATTGTCAAATATAAGTACTTTAATC		
	MtNst_ov2R	GGTTGAAGTTTATGATACTAATAATC		
P. vivax	Pv_For1	TATTATTGTCTATACTAGATACTATAG		
	Pv_Rev	CTATATTTTCATCATTAGTATCAGGA		
Plasmodium	MtUnst.F	GTAAACATGCwGTCATACATGATGCAC		
(Nested)				
	MtUnst.R	CCCCGGCGAACCTTCTTACCGT		

	- 10µM reverse primer: 0.5µL - Nuclease free water: 9.5µL - DNA template: 2uL 2x diluted outer PCR product - 25uL reaction volume

Table 2. Comparison of *Plasmodium* infection prevalence between rural and urban areas among asymptomatic and symptomatic school-age children

	Asymptomatic infection					Symptomatic infection				
	Rural area (N=210)		Urban area (N=217)			Rural area (N= 105)		Urban area (N= 102)		
Malaria infections	**Number**	**%)**	**number**	**%**	**p-value**	**Number**	**%**	**number**	**%**	**p-value**
Plasmodium species										
Plasmodium spp.	168	80.0	98	45.2	**<0.001**	102	97.1	94	92.2	0.11
P. falciparum	161	76.7	87	40.0	**<0.001**	101	96.2	92	90.2	0.09
P. malariae	66	31.4	19	8.8	**<0.001**	8	7.6	18	17.7	**0.03**
P. avale	35	16.7	11	5.1	**<0.001**	19	18.1	14	13.7	0.39
Type of Plasmodium infection										
Single infection	96	45.7	82	37.8	**<0.001**	80	76.2	70	68.3	0.10
Mixed infection	72	34.3	16	7.4	**<0.001**	22	21	24	23.5	0.22

Table 3. Proportion of *Plasmodium* species composition in asymptomatic and symptomatic school-age children by location

	Rural area (N=270)				Urban area (N=192)			
	Asymptomatic (N= 168)		Symptomatic (N= 102)		Asymptomatic (N= 98)		Symptomatic (N= 94)	
Type of Plasmodium infection								
Single infection	96	57.1	80	78.4	82	83.7	70	74.5
Mixed infection	72	42.9	22	21.6	16	16.3	24	25.5
***Plasmodium* species composition**								
P. falciparum	89	53.0	79	77.4	73	74.5	68	72.3
P. malariae	3	1.8	0	0.0	5	5.1	2	2.1
P. ovale	4	2.4	1	1.0	4	4.1	0	0.0
P. falciparum+P. malariae	41	24.4	3	3.9	9	9.2	10	10.6
P. falciparum+ P. ovale	9	5.4	14	13.7	2	2.0	8	8.5
P. malariae+P.ovale	0	0.0	0	0.0	2	2.0	0	0.0
P. falciparum+P.malariae+P. ovale	22	13.1	4	3.9	3	3.1	6	6.4

Figures

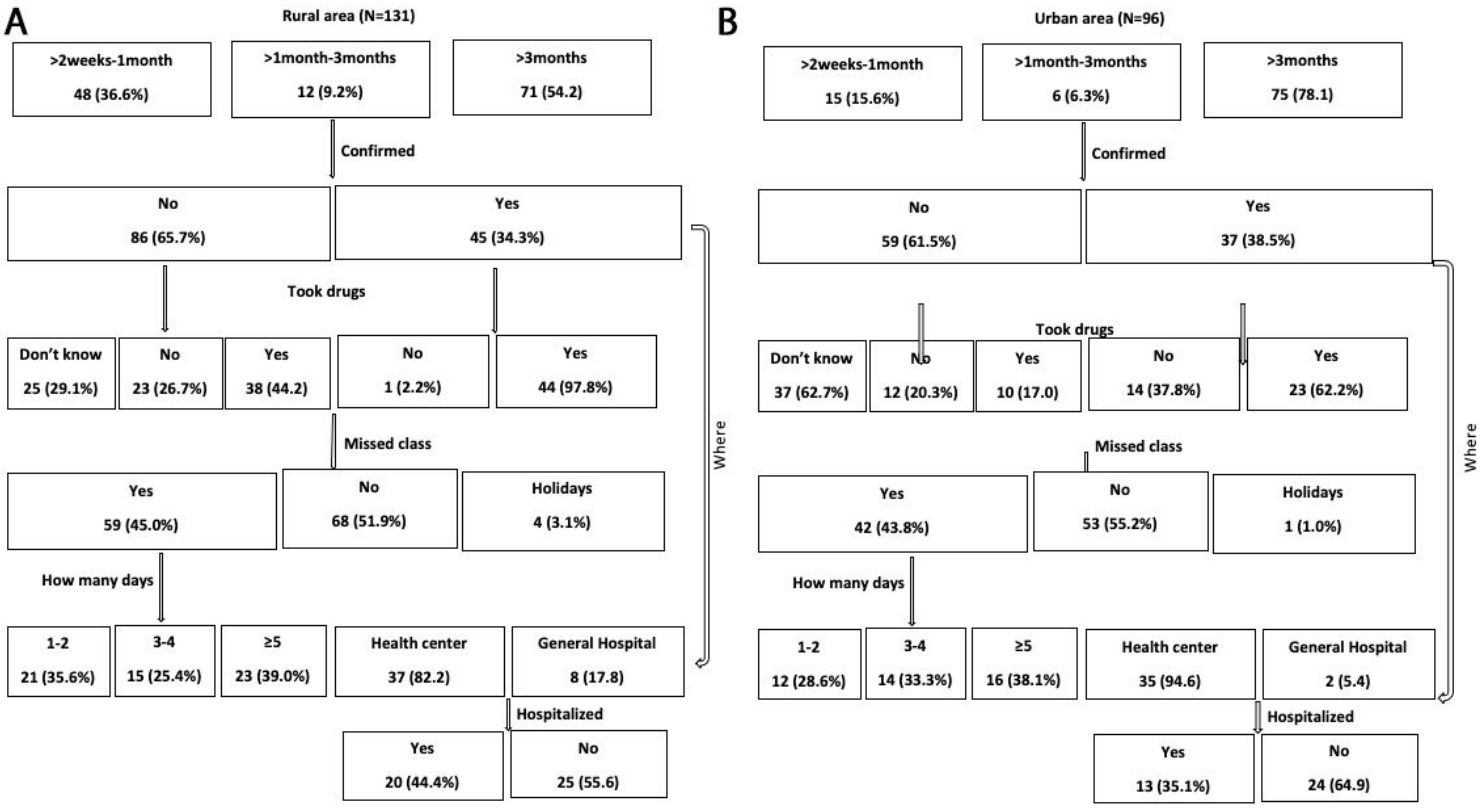

Figure 1

History of malaria-related symptoms among asymptomatic children in the rural (A) and urban (B) areas

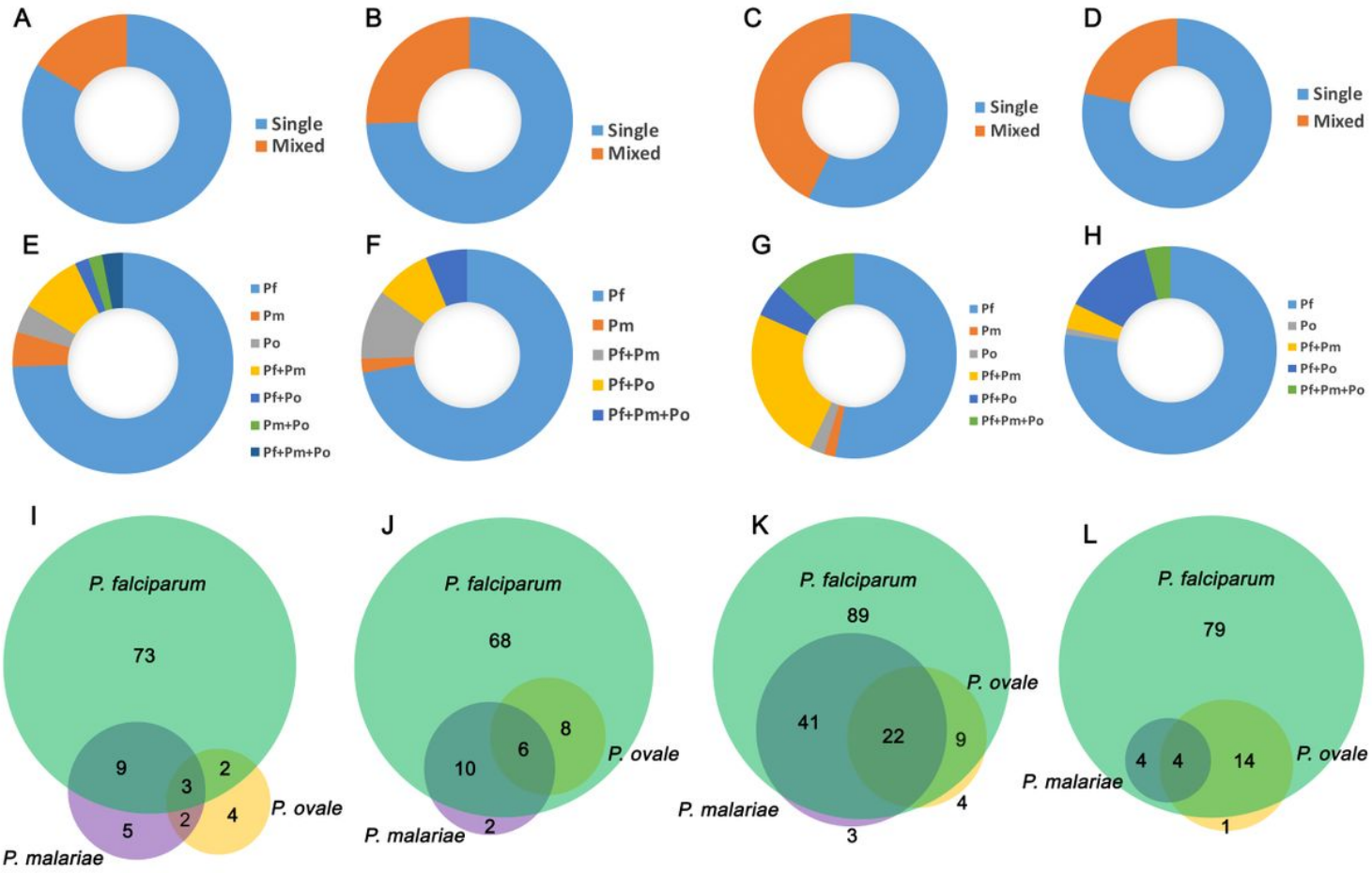

Figure 2

Species composition of Plasmodium infections in 98 asymptomatic (Panels A, E and I) and 94 symptomatic (B, F, and J) children in the urban Selembao health zone (HZ), and 168 asymptomatic (Panels C, G and K) and 102 symptomatic (Panels D, H, and L) children in the rural Mont-Ngafula 2 HZ. (A) Proportion of single and mixed infection in asymptomatic children in Selembao HZ. (B) Proportion of single and mixed infection in symptomatic children in Selembao HZ. (C) Proportion of single and mixed infection in asymptomatic children in Mont-Ngafula 2 HZ (D). Proportion of single and mixed infection in symptomatic children in Mont-Ngafula 2 HZ. (E) Malaria parasite species composition in asymptomatic children in Selembao HZ. (F) Malaria parasite species composition in symptomatic children in Selembao HZ. (G) Malaria parasite species composition in asymptomatic children in Mont-Ngafula 2 HZ. (H) Malaria parasite species composition in symptomatic children in Mont-Ngafula 2 HZ. (I) Prevalence of each of three malaria parasite species assayed (Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale) in asymptomatic children in Selembao HZ. (J) Prevalence of each of three malaria parasite species assayed (Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale) in symptomatic children in Selembao HZ. (K) Prevalence of each of three malaria parasite species assayed (Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale) in asymptomatic carriers in Selembao HZ. (L) Prevalence of each of three malaria parasite species assayed (Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale) in symptomatic carriers in Selembao HZ.

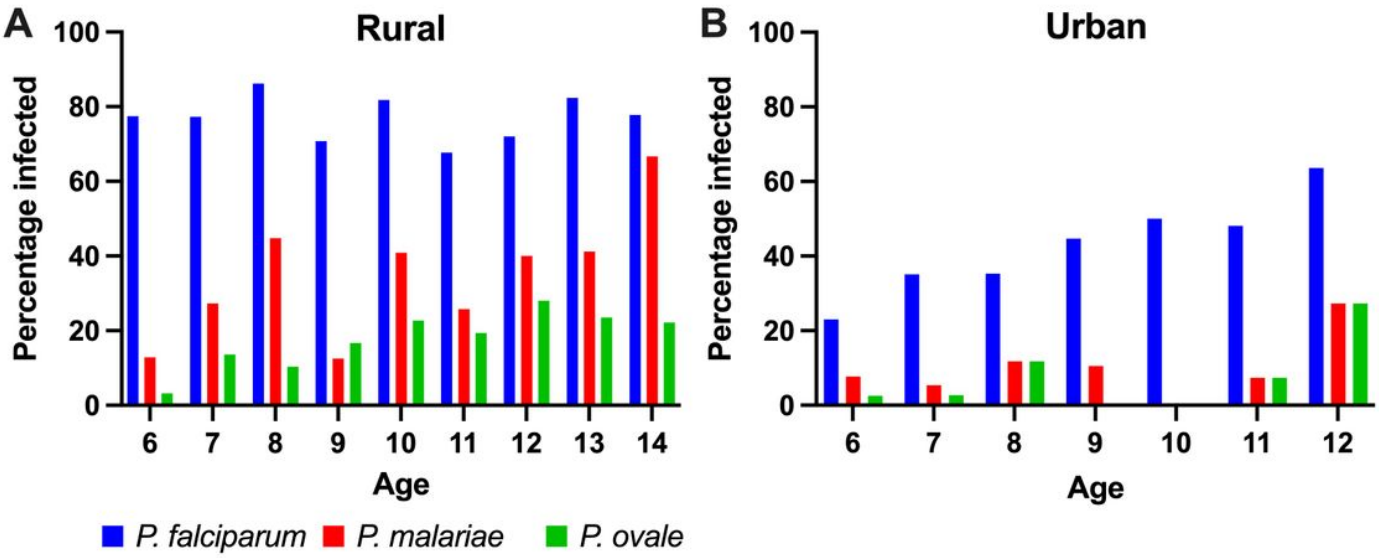

Figure 3

Species composition of Plasmodium infections stratified by age in asymptomatic school-age children living in rural (Panel A, n=210) and urban areas (Panel B, n=214). The number of children aged 13 and 14 years was very low in the urban area and excluded from the figure.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalInformationchecked.xlsx