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Asymptomatic Submicroscopic *Plasmodium* Infection Is Highly Prevalent and Is Associated with Anemia in Children Younger than 5 Years in South Kivu/Democratic Republic of Congo

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Abstract. One of the most important problems in controlling malaria is the limited access to effective and accurate diagnosis of malaria parasitemia. In the Democratic Republic of Congo (DRC), malaria is one of the leading causes of morbidity and mortality. The purpose of this study was to assess the prevalence of anemia and the relationship with asymptomatic submicroscopic *Plasmodium* infection. A cross-sectional study was carried out among 1,088 apparently healthy children aged between 6 and 59 months selected at random in the health zone of Miti Murhesa in South Kivu/DRC. Capillary blood was obtained for hemoglobin (Hb) concentration measurement by Hemocue® Hb 301. Malaria detection was performed by microscopy and the loop-mediated isothermal amplification (LAMP) assay. Anemia was defined as Hb < 11g/dL. We applied the chi-square test for comparisons, and multiple logistic regression was used to identify the risk factors for anemia and submicroscopic *Plasmodium* infection. The prevalence of anemia was 39.6%, and the prevalence of parasitemia was 15.9% and 34.0% using microscopy and LAMP test, respectively. Submicroscopic *Plasmodium* infection was found in 22.3% of the children. The independent risk factors for anemia are *Plasmodium* infection, children younger than 24 months, low middle-upper arm circumference, and history of illness two weeks before. Otherwise, children with submicroscopic malaria infection have a significantly increased risk for anemia, with a need of transfusion. The prevalence of malaria infection was underestimated, when microscopy was used to diagnose malaria. Children with low parasitemia detected by LAMP but not by microscopy showed a significantly increased prevalence of anemia.

INTRODUCTION

In 2018, there were an estimated 228 million new malaria cases worldwide. Most cases were from sub-Saharan Africa (93%).[1] Each year, approximately 405,000 deaths are due to malaria.[1] Children younger than 5 years are particularly susceptible to malaria, with an average of one child dying every 2 minutes.[1,2] The Democratic Republic of Congo (DRC) is at present one of the most affected countries.[1] In the DRC, according to the recent Demographic and Health Survey organized in 2014, the prevalence of malaria was 23% in children younger than 5 years. In South Kivu (SK), this prevalence has been estimated to be 10%.[3] *Plasmodium falciparum* is the most prevalent malarial parasite in sub-Saharan Africa, accounting for 99.7% of estimated malaria cases in 2018.[1] It is estimated that anemia affects a quarter of the global population, including 293 million (47%) children younger than 5 years.[4–6] In the DRC, 47% of children aged between 6 and 59 months are affected by anemia: 20% showing a mild form, 25% a moderate form, and 2% a severe form. In SK, this prevalence has been estimated to be 27%.[3] Malaria and anemia remain public health problems, with high priority in the DRC, in SK in particular. It has been described that *P. falciparum* causes acute and chronic anemia in endemic areas.[7] The study carried out by Menendez et al.[8] in a high malaria transmission zone confirmed the role of *Plasmodium* in the etiology of anemia in children. *Plasmodium falciparum* may account for about 60% of all episodes of severe anemia in children.[8] Other authors demonstrated the contribution of malaria in the overall prevalence of anemia in young children.[9–13] The pathophysiological mechanisms of the emergence of anemia in malaria infection has been extensively studied. Briefly, there is an increased hemolysis of infected and uninfected red cells, inadequate erythropoiesis because of the high level of tumor necrosis factor and macrophage inhibitory factor production, intramedullary deposition of hemozoin, and low levels of interleukin-10.[14,15] The degree of malarial anemia depends on various factors, among properties of both hosts and parasites.[16] Immunity is the factor that most strongly determines whether a malaria infection produces symptoms. An individual's level of immunity to infection is determined by past exposure history and age. Increased immunity leads to improved control over parasite multiplication and decreased parasite density, which in turn lessens the severity of symptoms.[17] In younger children, especially in children younger than 6 months, the presence of maternal antibodies may decrease the prevalence of *Plasmodium* infection and therefore of asymptomatic infection. Older children (aged > 6 months), when the maternal protection decreases, are more prone to develop symptomatic or even severe symptomatic malaria episodes resulting in anemia.[18–21] Chronic subclinical infections tend to be associated with substantially lower parasite densities in contrast with acute symptomatic infections. These chronic infections may be "submicroscopic" and thus are not detected in a blood film or by rapid diagnostic tests (RDTs). Analytically more sensitive methods such as molecular analyses with polymerase chain reaction (PCR) are needed to detect the low parasitemia which can persist for months or years.[22] In regions of high transmission, children eventually acquire the ability to maintain a parasite density below the level that causes fever, but chronic or repeated infection may cause a state of chronic anemia.[15] Advances in molecular diagnostic techniques have revealed a larger reservoir of asymptomatic human malarial infections than previously recognized. Several studies have shown the relevance of molecular analysis to detect *Plasmodium* in asymptomatic children with low parasite density with less than five parasites/μL, not detectable with RDT or

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blood film.[23–30] This study is the first in this area using an ultrasensitive test in the mass screening to assess a role of a subclinical and submicroscopic *Plasmodium* infection in the etiology of anemia in children younger than 5 years. The main objective of this study was to explore the prevalence and relationship of malaria and anemia in children younger than 5 years, and more specifically, to investigate the relationship between submicroscopic *Plasmodium* infection and anemia in children living in the rural area of SK, DRC.

MATERIALS AND METHODS

Study location. The study was carried out in the health zone (HZ) of Miti Murhesa in the SK Province/DRC. South Kivu is situated in the eastern DRC and shares borders with Rwanda, Burundi, and Tanzania. Bukavu is the capital of the province. The landscape includes mountains (eastern north part), the central basin (western part), and the vast plains in the southern east part. We can distinguish a rainy season from September to May and a dry season from June to August. The province has 34 HZs. Miti Murhesa is a rural HZ located at 30 km north of Bukavu, situated between 1,500 and 2,000 m of altitude. However, because Miti Murhesa is situated at 1,500–2,000 km of altitude, the period of transmission may be shorter as this area may be considered as a mountain ecosystem.[31,32]

Study design. In this cross-sectional survey, we recruited 1,088 children using a systematic sampling method to select clusters (villages) in the HZ of Miti Murhesa. Data collection took place from April 5 to May 9, 2018 (end of the rainy season).

Sample size. The sample size for this study was based on a proportion in a single cross-sectional survey, and the estimate of the proportion was based on the prevalence of malaria in SK, which was estimated to be 10%.[3] The absolute precision was set at 2.5%, with 95% CI, and the design effect of 2 with 5% of missing. The sample size required for this study was found to be 1,161 children aged 6–59 months. In practice, we opted for 30 clusters (villages) as previously described,[33] with 38 observations per cluster (30 clusters × 38 observations). Finally, 1,088 children were selected from 30 villages.

Sampling method. A two-stage sampling process was used to determine the study participants. First, we randomly selected 30 villages using the systematic sampling method. The village chief informed us about the number of households. We divided the total number of households by 38 (the number of children to be included per village) to calculate the sampling interval. The first selected household was randomly selected between one and the sampling interval. Then, we continued based on the sampling interval. We included one child, aged 6–59 months, per household who was a permanent resident of Miti Murhesa and whose parents/guardians granted consent for study inclusion and blood sample collection. In households with more than one eligible child, the child was randomly selected. If there was no eligible child in a selected household, the household next was then chosen.

Subject selection. A total of 1,088 children were recruited for the survey. The inclusion criteria were based on age (younger than 5 years), willingness of the parent/guardian of the child to participate in the study demonstrated by completion and signing/thumb printing of the consent form and willingness to provide samples required for the laboratory test, and general good health. We excluded all children with acute illness by measuring temperature ($T \geq 37.5$°C), children with significant morbidity (congenital heart disease, severe malformations, and cancer), children with psychomotor retardation, or severely sick children (with diarrhea, vomiting with dehydration, convulsion, and dyspnea); these children were referred to the health center for care.

Study questionnaire. A questionnaire was administered to the child's parent/guardian to collect data on demographic characteristics (gender, age, education level of the head of the family, occupation, and marital status), socioeconomic status–related variables (number of house occupants, house type, toilet type, and water source), preventive intervention (history of complementary food before 6 months, exclusive breastfeeding during 6 months, and use of insecticide-treated bed net (ITN) during the night before the survey), and morbidity (fever in the last 2 weeks and antecedent of transfusion).

Clinical evaluation and anthropometric measurements. The axillary temperature was measured using a digital thermometer, and fever was defined as temperature ≥ 37.5°C; pitting bilateral edema was noticed if present. Experienced healthcare personnel assessed the children's growth by measuring height (children aged > 2 years) or length (children aged ≤ 2 years), middle upper arm circumference (MUAC), and weight. Height/length and weight were measured nearest 0.1 cm and 0.05 kg, respectively. Duplicate measurements were taken using standardized methods and calibrated equipment,[34] and a third measurement was obtained if the difference between the first two measurements was greater than the allowable difference for that measure. Weight-for-age, height-for-age, and weight-for-height were expressed in *Z*-scores and calculated with the WHO Anthro (WHO Anthro Geneva version 3.2.2) for Stata software version 13.1 for Mac (StatCorp, College station, TX). Extreme outliers were excluded as follows: weight-for-age z-score (WAZ) ≤ −6 or ≥ 5, height-for-age z-score (HAZ) ≤ −6 or ≥ 5, and weight-for-height z-score (WHZ) ≤ −6 or ≥ 5. A child was identified as being malnourished if he or she scored < −2 SD for one of the anthropometric indices: HAZ (stunting), WAZ (underweight), and WHZ (wasting). If the corresponding indices were scored < −3 SD, the child was considered having severe undernutrition.

Blood collection and laboratory tests. Hemoglobin (Hb) was determined by finger prick by the Hemocue 301+ instrument (Angelhom, Sweden). The WHO criteria were used to define and classify anemia in children aged 6–59 months.[35] Anemia was defined as Hb < 11g/dL and classified into mild if Hb was in the range 10–10.9 g/dL, moderate if Hb was 7–9.9 g/dL, and severe if Hb was < 7 g/dL. Hemoglobin was adjusted for altitude as proposed by Sullivan KM et al.[36] A blood sample was collected by venipuncture into two tubes, one with ethylenediaminetetraacetic acid (EDTA) (4 mL) and one without anticoagulant (4 mL). The tubes were immediately stored in cooler boxes containing ice packs and were transported to the field laboratory of the Provincial Reference General Hospital of Bukavu for malaria microscopy and loop-mediated isothermal amplification (LAMP)–based illumigene malaria assay analysis. Slides were stained with 10% Giemsa and read by two independent, trained, and experienced microscopists. If necessary, a third independent microscopist was added to resolve discrepant results. A slide was considered negative after 200 microscopic fields were examined in the thick smear. The LAMP assay was performed using the illumipro-10™ incubator/reader, which is capable of testing 10 samples per run. The illumigene malaria amplification assay was used.

The illumigene assay is a qualitative in vitro diagnostic LAMP test for the detection of *Plasmodium* spp. DNA in human venous EDTA whole blood samples. The assay targets a region of the *Plasmodium* genome that is conserved across *P. falciparum, vivax*, *ovale*, *malariae*, and *knowlesi*.[37] The assay does not distinguish between the different *Plasmodium* species. The assay uses a simple filtration workflow (SMP-PREP™) to extract DNA from EDTA anticoagulated whole blood, a procedure relying on chemical lysis which produces amplifiable DNA within 10 minutes.[37,38] The change in turbidity associated with LAMP assays, due to the magnesium-pyrophosphate build up as a by-product, is measured by the illumipro-10 reader, and a qualitative result is determined.

We defined an infection as asymptomatic if *Plasmodium* parasites without fever were identified. Submicroscopic *Plasmodium* parasitemia was defined as a negative thick smear with a positive LAMP illumigene malaria assay. To define the group of submicroscopic *Plasmodium* parasitemia, we excluded all children who had concurrently positive thick smear and LAMP test and selected those with exclusively LAMP-positive tests.

Statistical analysis. Data were double-entered in Microsoft Excel (Microsoft 2016, Santa Rosa, CA), and analyses were performed using MedCalc® software version 9.4.2.0 (MedCalc, Mariakerke, Belgium). Continuous variables (Hb and age) were expressed as median ± interquartile range or mean ± SD, and categorical variables were summarized by proportions. The chi square test was used to compare proportions.

A multivariate logistic regression model, based on forward stepwise analysis, was used to determine adjusted odds for risk associated with anemia and submicroscopic *Plasmodium* infection.

TABLE 1
Baseline characteristics of the study population and its association with anemia in children

Variable	*N* (%)	Number of children with anemia, *n* (% = *n*/N)	*P*-value
Age-group (months)	1,088 (100)	431 (39.6)	
0–23	368 (33.8)	176 (47.8)	< 0.0001
24–59	720 (66.2)	255 (35.4)	
Gender	1,088 (100)	431 (39.6)	
Male	545 (50.1)	232 (42.6)	0.05
Female	543 (49.9)	199 (36.6)	
Education level of the head of the household	1,070 (100)	423 (39.5)	
Illiterate	350 (32.7)	142 (40.6)	
Primary	366 (34.2)	130 (35.5)	
Secondary and over	354 (33.1)	151 (42.7)	0.13
Household size	1,088 (100)	431 (39.6)	
1–5	366 (33.7)	152 (41.5)	0.002
6–10	627 (57.6)	257 (41.0)	
> 10	95 (8.7)	22 (23.2)	
History of complementary food before 6 months of age	1,088 (100)	431 (39.6)	
Yes	506 (46.5)	194 (38.3)	0.45
No	582 (53.5)	237 (40.7)	
Use of insecticide-treated bed net	1,088 (100)	431 (39.6)	
Yes	675 (62.0)	264 (39.1)	0.71
No	413 (38.0)	167 (40.4)	
History of transfusion	1,088 (100)	431 (39.6)	
Yes	205 (18.8)	93 (45.4)	0.07
No	883 (81.2)	338 (38.3)	
History of illness two weeks before	1,088 (100)	431 (39.6)	
Yes	742 (68.2)	326 (43.9)	< 0.0001
No	346 (31.8)	105 (30.3)	
Edema	1,088 (100)	431 (39.6)	
Yes	45 (4.1)	16 (35.6)	0.67
No	1,043 (95.9)	415 (39.8)	
Middle upper arm circumference (mm)	1,088 (100)	431 (39.6)	
< 125	210 (19.3)	123 (58.6)	< 0.0001
≥ 125	878 (80.7)	308 (35.1)	
WHZ	1,069 (100)	420 (39.3)	
<–2	93 (8.7)	53 (57.0)	0.0004
≥–2	976 (91.3)	367 (37.6)	
HAZ	1,055 (100)	415 (39.3)	
< –2	631 (59.8)	251 (39.8)	0.76
≥ –2	424 (40.2)	164 (38.9)	
WAZ	1,083 (100)	429 (39.6)	
< –2	367 (33.9)	161 (43.9)	0.04
≥ –2	716 (66.1)	268 (37.4)	
Thick smear	1,088 (100)	431 (39.6)	
Positive	173 (15.9)	119 (68.8)	< 0.0001
Negative	915 (84.1)	312 (34.1)	
Loop-mediated isothermal amplification test	1,057 (100)	424 (40.1)	
Positive	359 (34.0)	218 (60.7)	< 0.0001
Negative	698 (66.0)	206 (29.5)	

Ethical consideration. Ethical approval was obtained by the Institutional Ethical Committee of the Catholic University of Bukavu, UCB (Ref: UCB/CIE/NC/003/2017). Written informed consent was collected from the parents/guardians for all children before inclusion. All children with fever ($T \geq 37.5$), Hb < 10 g/dL, and MUAC < 125 mm were referred to the health center for care.

RESULTS

Baseline characteristics of study population. The baseline characteristics of the study population and their association with anemia are summarized in Table 1. A total of 1,088 children aged 6–59 months participated in this study, of whom 431 (39.6%) were anemic; 66.2% of children were aged between 24 and 59 months. Anemia was more prevalent among children younger than 24 months ($P < 0.001$). The proportion of boys was 50.1%, and anemia was more prevalent in males (42.6%) than in females (36.6%) ($P = 0.05$). No difference was observed in the education level of the head of the household, comparing anemic versus non-anemic children. The majority (57.6%) of children live in households with a people size of 6–10, but anemia was less prevalent in children from the household sizes exceeding 10 people ($P = 0.002$). There was no difference related to the addition of complementary food. The majority of children (62%) slept under ITN. No difference was observed between anemic and non-anemic children; 18.8% of children reported having received a transfusion in the past. In anemic children, a history of transfusion was more frequent than in non-anemic children, but the difference between the two groups was not significant. The majority of children (68.2%) reported an antecedent of illness during the 2 weeks preceding the survey. This was more frequent in anemic children than in non-anemic children ($P < 0.0001$). Bilateral pitting edema was observed in 4.1%, and edema was higher among non-anemic children (64.4%), but no association was observed. The prevalence of MUAC < 125 mm, wasting (WHZ < –2), stunting (HAZ < –2), and underweight (WAZ < –2) were seen, respectively, in 19.3%, 8.7%, 59.8%, and 33.9%. Middle upper arm circumference < 125 mm, wasting, and underweight were significantly associated with anemia. For the LAMP malaria test, 31 results were not available, and 11 results were invalid, and in 20 children, an insufficient amount of blood was collected to perform the analysis. Overall, in 15.9% children, a positive thick smear was found. Infected children had an increased chance to be anemic compared with noninfected children ($P < 0.0001$); 34.0% children had a positive LAMP test. Asymptomatic *Plasmodium* parasitemia was associated with anemia ($P < 0.0001$).

A Multiple logistic regression model for factors associated with anemia in children from Miti Murhesa, SK. In a multiple logistic regression model (Table 2), asymptomatic *Plasmodium* infection with positive thick smear and LAMP test increase the risk for anemia in a highly significant way with odds ratios (ORs) (2.59; 95% CI: 1.63–4.10 and 2.87; 95% CI: 2.04–4.01), respectively. Besides, age less than 24 months, history of illness 2 weeks before, and low MUAC increased the risk for anemia with OR, respectively, 2.17, 1.70, and 2.63. However, children living in the household with a size of more than 10 showed a decreased risk for anemia (OR = 0.78; 95% CI: 0.62–0.99).

TABLE 2
Multiple logistic regression model for factors associated with anemia in children

Variable	aOR (95% CI)	*P*-value
Age-group (months)		
6–23	2.17 (1.62–2.91)	< 0.0001
24–59	1	
Household size		
1–10	1	0.04
> 10	0.78 (0.62–0.99)	
History of illness 2 weeks before		
Yes	1.70 (1.25–2.29)	0.0005
No	1	
Middle upper arm circumference (mm)		
< 125	2.63 (1.88–3.69)	< 0.0001
≥ 125	1	
Thick smear		
Positive	2.59 (1.63–4.10)	< 0.0001
Negative	1	
Loop-mediated isothermal amplification malaria test		
Positive	2.87 (2.04–4.01)	< 0.0001
Negative	1	

Submicroscopic *Plasmodium* infection. Table 3 summarizes the demographic characteristics of the study population and its association with submicroscopic *Plasmodium* infection. The prevalence of submicroscopic *Plasmodium* infection was found to be 22.3%. Submicroscopic *Plasmodium* infection was higher in children aged 24 months and older ($P = 0.02$). Asymptomatic submicroscopic *Plasmodium* infection was higher in boys than in girls ($P = 0.004$). Children who did not receive complementary feeding before 6 months were more prone to have submicroscopic *Plasmodium* infection ($P = 0.01$). A history of transfusion was associated with submicroscopic *Plasmodium* infection with $P = 0.005$. Anemia was significantly associated with submicroscopic *Plasmodium* infection ($P < 0.0001$).

In the multiple logistic regression model (Table 4), the risk factors predicting submicroscopic *Plasmodium* infection included anemia, age, male gender, and a history of transfusion. The absence of extra feeding before 6 months of age was also associated with submicroscopic *Plasmodium* infection.

Anemia increases the finding of submicroscopic *Plasmodium* infection by 2.75 (95% CI: 1.96–3.85, $P < 0.0001$). Otherwise, children younger than 24 months were less at risk for submicroscopic *Plasmodium* infection (OR = 0.56; 95% CI: 0.39–0.80, $P = 0.001$). Finally, a history of transfusion in the past and male gender predict a submicroscopic *Plasmodium* infection (OR = 1.75; 95% CI: 1.16–2.63, $P = 0.007$) (OR = 1.59; 95% CI: 1.15–2.22, $P = 0.005$), respectively, whereas the extra feeding before 6 months shows a decreased risk for submicroscopic *Plasmodium* infection (OR = 0.64; 95% CI: 0.46–0.90, $P = 0.01$).

DISCUSSION

In this study, we examined the prevalence of anemia and focused on the relationship of submicroscopic *Plasmodium* infection and anemia in children younger than 5 years living in the rural area of SK/DRC. We found that anemia was most prevalent in children younger than 2 years. By contrast, submicroscopic *Plasmodium* infection was most prevalent in older children (aged 3–5 years). Nevertheless, anemia and submicroscopic *Plasmodium* infection strongly correlated

TABLE 3
Baseline characteristics of study population and its association with submicroscopic *Plasmodium* infection (PI)

Variable	*N* (%)	Submicroscopic PI, *n* (% = *n*/*N*)	*P*-value
Age-group (months)	898 (100)	200 (22.3)	
6–23	326 (36.3)	59 (18.0)	0.02
24–59	572 (63.7)	141 (24.7)	
Gender	898 (100)	200 (22.3)	
Male	434 (48.3)	115 (26.5)	0.004
Female	464 (51.7)	85 (18.3)	
Education level of the head of household	882 (100)	197 (22.3)	
Illiterate	291 (33.0)	54 (18.6)	0.15
Primary	295 (33.4)	73 (24.7)	
Secondary and over	296 (33.6)	70 (23.6)	
Household size	898 (100)	200 (22.3)	
1–5	305 (34.0)	68 (22.3)	0.54
6–10	508 (56.6)	117 (23.0)	
> 10	85 (9.4)	15 (17.6)	
History of complementary food before 6 months of age	989 (100)	200 (22.3)	
Yes	421 (46.9)	78 (18.5)	
No	477 (53.1)	122 (25.6)	0.01
Use of insecticide-treated bed net	898 (100)	200 (22.3)	
Yes	563 (62.7)	120 (21.3)	0.41
No	335 (37.3)	80 (23.9)	
History of transfusion	898 (100)	200 (22.3)	
Yes	147 (16.4)	46 (31.3)	0.005
No	751 (83.6)	154 (20.5)	
History of illness 2 weeks before	898 (100)	200 (22.3)	
Yes	604 (67.3)	140 (23.2)	0.39
No	294 (32.7)	60 (20.4)	
Edema	898 (100)	200 (22.3)	
Yes	36 (4.0)	5 (13.9)	0.30
No	862 (96.0)	195 (21.7)	
Middle upper arm circumference (mm)	898 (100)	200 (22.3)	
< 125	167 (18.6)	39 (23.4)	0.78
≥ 125	731 (81.4)	161 (22.0)	
WHZ	885 (100)	198 (22.4)	
< –2	72 (8.1)	16 (22.2)	0.90
≥ –2	813 (91.9)	182 (22.4)	
HAZ	869 (100)	190 (21.9)	
< –2	517 (59.5)	105 (20.3)	0.20
≥ –2	352 (40.5)	85 (24.1)	
WAZ	895 (100)	200 (22.3)	
< –2	301 (33.6)	64 (21.3)	0.63
≥ –2	594 (66.4)	136 (22.9)	
Anemia	898 (100)	200 (22.3)	
Yes	309 (34.4)	103 (33.3)	< 0.0001
No	589 (65.6)	97 (16.5)	

pointing to a prominent role of the submicroscopic malaria infection in anemia, particularly in the age-group of 3–5 years. In general, almost 40% of children were anemic. This prevalence is in agreement with data previously described in the region. Two independent studies described the prevalence of anemia in the same region up to 36%[3] and 46.6%.[39] A third study found the prevalence of 58.6% and 35.4% in the age-groups 6–23 months and 24–59 months,[40] respectively. In a demographic investigation performed in 2007,[41] anemia was found up to 60%, decreasing towards 36% in 2013. Our present data confirm that anemia remains a major health problem for the SK region. Regarding the prevalence of *Plasmodium* infection, surprisingly, in our study, a marked different score was found when compared with previous results. Our study shows a higher prevalence of malaria up to 16% and 34% using thick smear and LAMP malaria test, respectively. This is far above the scores found in previous studies performed in the same region.[3,39] In 2014, a survey described the prevalence of malaria in SK of 12% and 10% with RDTs and thick smear, respectively. Differences may be due to the sensitivity of the tests used in the two surveys, and to seasonal differences. The LAMP test is more sensitive, but thick smear examinations also showed a higher prevalence than was previously reported. The same survey in 2014 showed 10.4% positive cases when PCR was applied.[42] This is surprisingly not higher than the findings by microscopy and RDT, and was also far below the numbers we found with the LAMP assay. Our study was performed at the end of the rainy season, associated with higher malaria transmission. A temporal change in transmission associated with climate change could also explain in part a rise in the prevalence of malaria.[31,32] One more reason could be an increased mosquito resistance to insecticides. We did not find any difference in the prevalence of parasitemia between children who used or did not use ITN. We should take into consideration that observed results might be explained by an inferior quality of the ITN because of a too long storage time. Four other studies, at least, did find a protective effect of ITN.[43–46] However, Gitonga et al.[44] in Kenya described that in

TABLE 4
Multiple logistic regression model for factors associated with submicroscopic *Plasmodium* infection in children

Variable	aOR	*P*-value
Age-group (months)		
6–23	0.56 (0.39–0.80)	0.001
24–59	1	
Gender		
Male	1.59 (1.15–2.22)	0.005
Female	1	
History of complementary food before 6 months of age		
Yes	0.64 (0.46–0.90)	0.01
No	1	
History of transfusion		
Yes	1.75 (1.16–2.63)	0.007
No	1	
Anemia		
Yes	2.75 (1.96–3.85)	< 0.0001
No	1	

some zones, the protective effect of ITN could not be confirmed. Recently, a study conducted in North Ubangi/DRC has demonstrated resistance of *Anopheles gambiae* to deltamethrin, permethrin, and dichlorodiphenyltrichloroethane.[47] Mosquito resistance to insecticides has been described elsewhere in the DRC.[48] Other factors playing a role in the rise of malaria are resistant to antimalarial drugs, the climate change, and the migration of people in the context of civil war.[32,49–52]

Risk factors for anemia. A forward stepwise multiple regression analysis was used to identify the factors predicting anemia. Age, household size less than 10, history of illness 2 weeks before, MUAC < 125 mm, and *Plasmodium* infection remained significant for prediction of anemia. Children younger than 24 months were at increased risk of anemia, which is in agreement with previous results.[39,40] This can be due to the fact that many other causes of anemia play an important role such as increased requirement of iron in this period of rapid child growth, inadequate iron intake, and repeated infections. Malnourished children are at increased risk of micronutrient deficiencies and are more likely to suffer from impaired immune function with more infections, also parasitic infections.[6,21,53] These considerations may explain that children who reported a history of illness in the previous 2 weeks are more prone to anemia. The pathophysiology of anemia during an acute phase of infection or inflammation is not clearly established, but studies have demonstrated that children with acute infection experience a significant drop in Hb.[54–56] Importantly, we found a clear relationship between asymptomatic *Plasmodium* infection and anemia. Our findings are consistent with previous studies in sub-Saharan Africa concerning anemia.[21,44,45,57,58] Other findings, such as the correlation of weight and MUAC, are expected. In the logistic regression analysis, weight disappears and only MUAC remains, indicating that MUAC is the most important one. To our surprise, the biggest households showed the lowest anemia prevalence: one idea is that socioeconomic status may play a role.

Submicroscopic *Plasmodium* infection and risk factors. Multiple logistic regression analyses showed that independent variables predicting asymptomatic submicroscopic *Plasmodium* infection were age, anemia, gender, absence of complementary feeding before 6 months, and history of transfusion. Increasing age predicted submicroscopic *Plasmodium* infection. Our finding was consistent with other studies in Africa.[58,59] Increasing age has been clearly linked to lower parasite densities. In older children, more infection tend to be submicroscopic; this is probably because of a greater immunity in older children induced by cumulative exposure and developed immunity than in younger children who are more prone to develop symptomatic malaria.[17,19,20,59,60] In our study, we found a relationship between submicroscopic *Plasmodium* infection and anemia. Children with anemia were 2.7 times more prone (95% CI: 1.96–3.85) to have a submicroscopic *Plasmodium* infection. This finding is consistent with the study in Rwanda, where 28% of children with submicroscopic *Plasmodium* infection were anemic.[29] In general, asymptomatic, especially submicroscopic *Plasmodium* infection, has received less attention than symptomatic malaria. Submicroscopic carriers were found to be a source of human to mosquito transmission.[59,61] A history of transfusion also predicted submicroscopic *Plasmodium* infection. This illustrates that children at younger age may have overt malaria, needing transfusion, and remain asymptomatic carriers in the long term. Our study shows that children receiving exclusive breastfeeding during the first 6 months of life are at increased risk of submicroscopic *Plasmodium* infection. These results are in contrast with the studies conducted in Kinshasa/DRC[62] and in Malawi,[63] in which exclusive breastfeeding was associated with reduced risk of malaria infection in young children, whereas in Uganda, the protective effect of exclusive breastfeeding was not demonstrated.[64] The WHO recommends exclusive breastfeeding for 6 months. Breastfeeding brings clear benefits for child health by reducing morbidity and mortality from infectious diseases.[65]

CONCLUSION

The strong association between malaria and anemia is well known. This is also true in the SK region of the DRC. Importantly, anemia is also strongly associated with asymptomatic submicroscopic malaria, especially in malnourished children aged younger than 3 years and who were ill shortly before. The submicroscopic infection is most relevant in boys in the age-group of 24–59 months. In our study, the use of ITN did not offer a clear protection against malaria. This is a controversial finding, needing further exploration. It may be recommended to offer preventive malaria treatment in this age-group, and this is even more meaningful if the child is anemic, even in the absence of clinical malaria symptoms or negative thick smears. Because this study is cross-sectional and not longitudinal, we cannot make any predictions on the clinical outcome.

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