

Prevalence of *Plasmodium Falciparum* Parasitaemia in Exclusively Breastfed Children Aged 0-6 Month in the Ouidah Kpomassè -Tori-Bossito Health Region in Benin

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Abstract

Exclusive breastfeeding would reduce susceptibility to malaria according to several studies. The objective of this study was to assess *Plasmodium falciparum* (*P. falciparum*) parasitaemia in children under 6 months exclusively breastfed in southern Benin.

A descriptive cross-sectional study was conducted in 2014 from February to August. We randomly selected 106 exclusively breastfed children aged from 0 to 6 months in the Ouidah-Kpomassè-Tori-Bossito health region (OKT) in southern Benin. 24 h recall method was used to identify exclusively breastfed children. *Plasmodium* was detected by a thick drop, blood smear and a real time quantitative Polymerase Chain Reaction (qPCR).

The children average age was 2.2 months. The sex ratio (M/F) was 1. The prevalence of *P. falciparum* malaria was 0.9%. One child had fever and positive parasitaemia with gamétocytes confirmed by qPCR at 27 Cycle threshold (Ct). This child was 4.2 months old and slept under mosquito bed net. There was no asymptomatic parasitaemia among all the children.

The prevalence of *P. falciparum* parasitaemia in exclusively breastfed children, that was lower than the average prevalence in southern Benin, the practice of breastfeeding should be encouraged and strengthened. Future studies with objective measure of breast milk could help to better classify exclusive breastfeeding.

Keywords: *Plasmodium falciparum*; qPCR in real time; Breastfeeding; 24 h recall

Introduction

Despite measures in place, the majority of malaria cases (85-90%) recorded in sub-Saharan Africa is in children under five years of age [1]. The age 0 to 6 month range (0-6) is often excluded from malaria studies, resulting in little documented literature [2-4]. As a result, young children aged 0-6 months were considered to be relatively protected from malaria infection due to the presence of foetal hemoglobin [5] and the transfer of maternal antibodies to the newborn [6]. For some authors, these antibodies would simply be biomarkers of the mother's exposure to *Plasmodium*. [7]. Research has shown, recently, that malaria is present in this age group with a prevalence ranging from 3,7 to 41,6% depending on ecological areas and malaria facies in Africa. [8]. In Benin, the prevalence was 7.8% in children under 12 month in the area of Ouidah Kpomassè Tori-Bossito Bossito (OKT) [9].

During the first six months of a young child's life, exclusive breastfeeding (EBF) is a global public health recommendation. Despite the documented evidence of the benefits of breast milk [10,11], EBF is rarely practiced. Global estimates show that 64% of mothers in developed countries do not meet the standards of best breastfeeding practice [12]. In Benin, breastfeeding is widespread, but only 33% of babies are exclusively breastfed and 21.3% of newborns in southern Benin have received food before breastfeeding [13]. Some studies have found a reduced risk of malaria in exclusively breast-fed infants [14,15]. According to Kassim et al. breast milk lactoferrin would inhibit *in vitro* *P. falciparum* [16]. Wouldn't mothers who are aware of EBF practice, reduce the risk of malaria in early childhood in a mesoendemic area such as OKT in Benin? This is the subject of the present study, which proposes to determine the prevalence of malaria parasitaemia in children exclusively breastfed in the OKT zone in southern Benin.

Materials and Method

Study area and design

A descriptive cross-sectional study was conducted from February to August 2014 in the most frequented health facilities (Tokpadomé, Kindji, Pahou and Ouidah) of the region of Ouidah Kpomassè Tori-Bossito (OKT), a mesoendemic region of malaria in the South of Benin. These health facilities made at least weekly breastfeeding sessions during antenatal clinic and vaccination sessions.

For the large representation of the results, the population of the OKT zone, on the community place and the Mother-Child Pairs (MCPs) during the immunization and postnatal consultation sessions were informed on the study by “neighborhood announcements” and by health workers respectively. Written information on the modalities of the study was given to the mothers during awareness sessions.

Sampling and inclusion criteria

The sample size calculated by the Schwartz formula using the prevalence of *Plasmodium falciparum* infection (7.8%) obtained in the OKT health region in children under 12 months [8], gave a total of 110 children under 12 months. We used this sampling size calculation for children from 0 to 6 month for the present study.

Sample size calculation (S):

$Z=1.96$ for 95% confidence level

$p=0.78$ (Prevalence of *Plasmodium falciparum* infection in OKT)

$c=0.05$ (confidence interval)

$$S = \frac{Z^2 \times p \times (1 - p)}{c^2}$$

$S=110$ children

All MCPs and those who met the eligibility criteria were enrolled into the study.

Inclusion criteria included: 1) exclusively breastfeeding according to 24 h recall. 2) Resided for at least 6 months in the OKT area. 3) Not taken anti-malarial in the last four weeks prior to the survey. 4) HIV negative serologic status. 5) Hemoglobin level greater than 7 g/dl. 6) Agree and sign inform consent form. Finally 106 MCPs have been included in this study.

Training of field workers and data collection

Before the actual survey, training was organized by two nutrition specialists from the International Atomic Energy Agency (IAEA) on the purpose, measurement techniques, and the questionnaire. The questionnaire was tested outside the OKT area, which enabled the validation of the data collection tools.

The day of the survey, according to WHO recommendations, EBF mothers had been identified by the 24 h recall questionnaire that collects information about the feed, liquids and solids received by the child the day before the survey. The responses provided by breastfeeding mothers classified the types of breastfeeding [10] into three categories: exclusive breastfeeding (EBF)=breast milk alone, predominant breastfeeding=breast milk plus other liquids such as water, herbal tea, fruit juice, oral rehydration solution, drops and vitamins, mineral salts and medicines and partial breastfeeding=other

food or artificial milk in addition to breast milk. Predominant and partial breastfeedings constitute non-exclusive breastfeeding.

The informed mothers who gave their consent that met the inclusion criteria participated in the study. Each MCP responded to a questionnaire and benefited from a measurement of the anthropometric. Each child received an anthropometric measurement, a clinical examination with temperature and a biological check-up (thick drop, blood smear, hemoglobin test, and qPCR).

Variables studied were demographic (age and sex), nutritional (weight, height, body mass index), epidemio-clinics (a fever with a temperature above 37.5°C, taking at least one dose of sulphadoxine-pyrimethamine or SP, the use of the long-lasting insecticidal nets or LLINs, presence of trophozoites and gametocytes).

The SP use for malaria prevention during pregnancy as intermittent preventive treatment in pregnancy (IPTp) and child HIV status, child's birthweight were recorded in the child's health record and in the maternity register of the health centers. Taking the temperature and looking for an abnormality of the physical examination was carried out respectively by nurses and a pediatrician. The anthropometric measurements (weight, height) of the mother-child pairs were carried out by nutrition specialists trained according to the recommended operational procedures (SOPs) [17-19].

Laboratory Examinations

Capillary blood sampling

Sample of six drops of blood in the heel of the child is prelevated: one drop for the realization of a thick drop, one for a blood smear, one for a hemoglobin test, and the remaining three drops of blood (50 $\mu\text{L} \times 3$) on Whatman 3 MM filter paper. The dried blood impregnated filter papers were stored at -20°C (under conditions of good quality) in a sealed film with silica after complete air drying until DNA extraction and PCR (Polymerase Chain Reaction) in real time was achieved.

Realization of the thick drop and the blood smear

The thick drop and blood smears produced were stained with Giemsa diluted 10% in the laboratory of parasitology of the Hubert Koutoukou Maga National Hospital and University Center (CNHU-HKM) according to the recommendations of WHO [20]. The identification of *P. falciparum* species was carried out on the blood smear and the parasitic density (DP) was counted in a blood volume occupied by 200 leucocytes and parasites. When the number of parasites counted for 200 leucocytes is less than 10, the reading is then made for 500 leucocytes. We calculated DP by microliters of blood using the formula: $\text{DP}/\mu\text{L} = \text{numbers of trophozoites counted}/\mu\text{L} \times 8000$ leucocytes/200 leukocytes. The presence of gametocytes has been reported. The parasitaemia is the presence of *P. falciparum* asexual blood forms or gametocytes.

Real-time quantitative PCR

DNA extraction was performed using Chelex 100 technique from the filter papers [21]. Real-time duplex PCR was performed using primers and probes specific to *Plasmodium spp/P. falciparum* for the gene encoding the small (18S) subunit of *Plasmodium* rRNA. This technique was used to test the 106 samples of children exclusively breastfed, as described by Diallo et al. [22] The samples were subjected to 40 cycles of amplification in the real-time PCR system ViiA™ 7

(Applied Biosystem) at the Molecular Biology Laboratory of the Integrated Malaria Control Center (CLIP) in our faculty of health Science at Cotonou.

A positive control signal for the human GAPDH (human glyceraldehyde-3-phosphate dehydrogenase) gene was amplified for all samples (control of good performance of DNA extraction from the filter paper). To declared that a sample was positive: the Ct should be: Plasmoprobe<40, *P. falciparum* <37, Ct GAPDH<40, the Ct of *P. falciparum* 3D7<23-25 and the Négative Control must be undetectable.

The parasites were quantified using a standard range of DNA made from an in vitro culture suspension of a *P. falciparum* 3D7 line offered by IRD/NIH: (*P. falciparum* 3D7 GL, MRA-1001) filed by Megan Dowler, Walter Reed Army Institute of Research).

Haemoglobin measurement

Finger-pick blood sample was used to measure haemoglobin using a portable spectrophotometer (Hemo-Control, EKF-Diagnostic GmbH, Germany). The positive control microdish was used to control the device. From a drop of blood collected at the tip of the finger and deposited on the microcuvette (then introduced into the apparatus), the hemoglobin level is measured and displayed in 60 s [23]. Individuals found with *Plasmodium parasitaemia* or hemoglobin level less than 7 g/dl were referred to the pediatrician for evaluation and treatment.

Ethical considerations

This study was approved by the Ethics Committee of the Faculty of Health Sciences and the Health Authorities of OKT District. The written informed consent of all the mothers included in the study was obtained. Children with a positive thick drop were taken care of in the health center free of charge.

Statistical analysis

The statistical analysis of the data was carried out using the EPI data software. Descriptive analyses were done by determining means, medians, and standard deviations. The prevalence of parasitaemia was calculated as the proportion of the *P. falciparum* asexual blood forms in parasite positive blood thick films and or by qPCR of the total number of samples successfully analysed (106).

Results

Characteristics of study infants

Information about the children is summarized in Table 1. The average age was 2.2 months. 50% of the children were female. The average weight at the time of the study was 5.00 Kg, the average height was 56.54 cm, and 99.1% of them regularly slept under LLINs.

Information about the mothers

The characteristics of the mothers are presented in Table 2. The mean age of mothers is 26.8 years with outliers, ranging from 18 to 40. 29.2% of mothers were out of school. Their average weight was 61.5 kg and their average height was 161 cm. The average body mass index (BMI) is 24 Kgm⁻² between 18.5 and 25 within the normal range. 99.1% of mothers regularly slept under LLIN and had taken at least one dose of SP before the birth of the children.

Characteristics of children	Mean ± sd or n (%)
Age of the infant (months)	2.2 ± 1.6
Infant's birthweight (kg)	3.1 ± 0.5
Gender ratio (male/female)	53/53
LLINs net use	105 (99.1)
106 MCPs have been included in this study. n: Number; sd: Standard Deviation; 99.1% of them regularly slept LLINs.	

Table 1: Characteristics of study's children.

Characteristics of mothers	Mean ± sd or n (%)
Age of the mother (years)	26.8 ± 5.5
Mother's education	
Unschool	31 (29.2)
Primary	38 (35.8)
Secondary or more	37 (34.9)
Mother's weight (kg)	61.5 ± 13.9
Mother's height (cm)	156.0 ± 6.3
Mother's BMI (kg m²-1)	24.0 ± 4.9
LLINs	105 (99.1)
SP* one dose	105 (99.1)
*SP is still used for malaria prevention during pregnancy as intermittent. Preventive treatment in pregnancy (IPTp). The SP-IPTp regimen (at least two curative doses of SP starting in the second trimester of pregnancy (16 weeks of gestation) and ensuring a dose spacing interval of at least one month) has been recommended by WHO.	

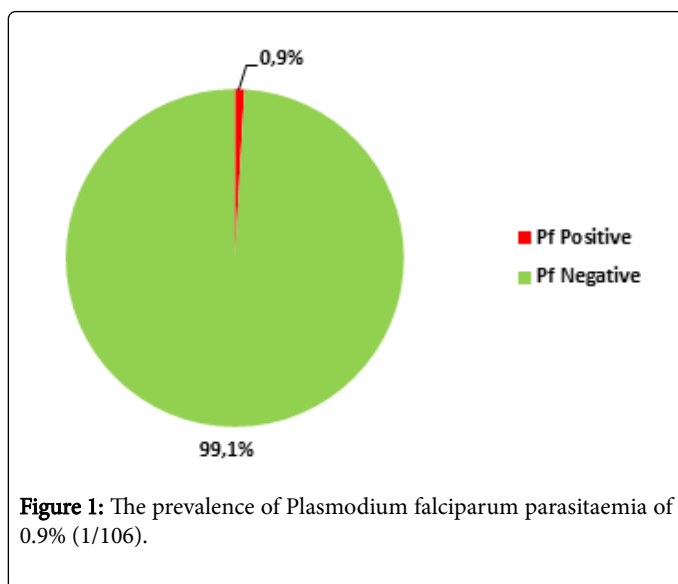
Table 2: Characteristics of mothers.

Plasmodium falciparum parasitaemia

The prevalence of *Plasmodium falciparum* parasitaemia 0.9% (1/106) is shown in Figure 1. A single 4.2-month-old male infant was parasitized and had a parasitic density of 3298 parasites per µL confirmed by Qpcr with 9074.102 copies/µL at 27 (Ct). He had a fever at 38.2°C. On the blood smear, gamétocytes of *P. falciparum* were presents. Her mother was 20 years old with a BMI of 19.9 Kgm⁻². From a primary school level, she was sleeping under LLINs.

Discussion

The average age of infants in our study was 2.2 months. This value is close to those found by Medoua in 2007 who did his study in infants of 1.15 to 4.5 months with an average age of 3 months [24]. In a study of Haisma the infants were up to 4 months old with an average age of 2.1 months (2003) [25]. In our study, 50% of the children were female. This result is different from Cameroonian research whose sample was 65.9% female [24].



The age of the mothers in our study ranged from 17 to 45 with an average age of 27 close to those of the Cameroonian mothers, who were aged between 19 and 42 with an average of 25 in the study of Medoua [24]. The average body mass index (BMI) of mothers is 24 Kg/m² and between 18.5 and 25 is within the normal BMI range. 34.9% of mothers possessed at least secondary level education. These educated mothers would be well informed and aware of the benefits of sleeping under an LLIN and breastfeeding their child. The majority of mothers would therefore be able to apply the advice they received during immunisation sessions. During pregnancy, they have taken at least one dose of SP, which would limit significant placental parasitism and low birth weight. SP continues to be the drug of choice for IPTp because a single treatment dose has long-lasting prophylactic effect (up to 60 days).

During our study, the rate of availability and use of LLINs was high. A study in southern Benin on the availability and use of impregnated mosquito nets showed protection against malaria and a significant reduction in malaria prevalence with an odds ratio of 0.76 (95% CI 0.59, 0.98) in one area resistant to pyrethroids [26]. Indeed, the average rates of availability and use of LLINs were 92% and 70% respectively in the OKT area of low resistance to pyrethroids. [9,27].

The prevalence of *P. falciparum* malaria in children aged 0-6 months in southern Benin is estimated at 10.2% [8]. At the site of our study, the results reported in 2008 by Georgia et al. revealed a prevalence of parasitaemia of 7.8% in children younger than 12 months. Age stratification during the analysis of Georgia's data may have revealed a lower prevalence than that found in this area in children less than one year. The OKT zone is a mesoendemic zone characterised by an entomological inoculation rate of 5.3 (95% CI, 1.1 to 25.9) infectious mosquito bites (*Anopheles gambiae* and *Anopheles funestus*) per person per year [9,27]. Our study in the same area showed a prevalence of 0.9% parasitaemia in children fed exclusively on breast milk. Here, the diagnostic technique used has a great importance. In fact, recent studies have revealed differences in sensitivity between the techniques used for the diagnosis of malaria, the PCR technique used, and targeting DNA in this study detected more cases than microscopy [28,29]. This is a very sensitive technique that can detect up to 0.5 parasites per microlitre of blood, which allows the detection of low parasitaemia. The only case of *P. falciparum* parasitaemia found in our

study with a parasitic density of 3298 trophozoites by thick blood film and blood smear was confirmed by qPCR at 27 Ct. We found no case of asymptomatic malaria in exclusively breastfed children. The infected child in our study had a fever and was treated according to the protocol of simple malaria management. This result is similar to Ceasay result in southern Benin, which also found cases of symptomatic malaria with the presence of gametocytes in children aged from 0 to 6 months [4]. There were no false positives in our study as we dealt with true cases of malaria confirmed by two techniques including real time qPCR, which is a very reliable tool. The low prevalence in our study suggests that the risk of contracting malaria may be very low or even absent in exclusively breastfed who slept under LLINs children in a mesoendemic area. Kassim et al. showed that lactoferrin in breast milk inhibited *P. falciparum* [16], which raises the question of whether this case of symptomatic malaria actually occurred in a child exclusively breastfed?

Our study has limitations like the bias associated with mothers' responses to the 24 h recall questionnaire, which is a qualitative method [30,31]. The assessment of the exclusivity of breastfeeding by a quantitative biological method would be more objective.

Conclusion

As the prevalence of *P. falciparum* parasitaemia in our study population was low in exclusively breastfed children, the practice of breastfeeding should be encouraged. Our study did not reveal asymptomatic parasitaemia. Subsequent studies with large sample and quantitative tests of the assessment of the exclusivity of breast milk of infants should be considered to confirm our results.

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Conflicts of Interest

The authors state that they have no conflict of interest.

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