First malaria infections in a cohort of infants in Benin: biological, environmental and genetic determinants. Description of the study site, population methods and preliminary results

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ABSTRACT
Objectives: Malaria infection of the placenta during pregnancy was found to be associated with infant susceptibility to malaria. Other factors such as the intensity of malaria transmission and the nutritional status of the child might also play a role, which has not been adequately taken into account in previous studies. The aim of this study was to assess precisely the parts played by environmental, nutritional and biological determinants in first malaria infections, with a special interest in the role of placental infection. The objective of this paper is not to present final results but to outline the rationale of the study, to describe the methods used and to report baseline data.

Design: A cohort of infants followed with a parasitological (symptomatic and asymptomatic parasitaemia) and nutritional follow-up from birth to 18 months. Ecological, entomological and behavioural data were collected along the duration of the study.

Setting: A rural area in Benin with two seasonal peaks in malaria transmission.

Participants: 656 infants of women willing to participate in the study, giving birth in one of the three maternity clinics and living in one of the nine villages of the study area.

Primary Outcome Measures: The time and frequency of first malaria parasitaemias in infants, according to Plasmodium falciparum infection of the placenta.

Results: 11% of mothers had a malaria-infected placenta at delivery. Mosquito catches made every 6 weeks in the area showed an average annual P. falciparum entomological inoculation rate of 15.5, with important time and space variations depending on villages. Similarly, the distribution of rainfalls, maximal during the two rainy seasons, was heterogeneous over the area.

Conclusions: Considering the multidisciplinary approach of all factors potentially influencing the malaria status of newborn babies, this study should bring evidence on the implication of placental malaria in the occurrence of first malaria infections in infants.

ARTICLE SUMMARY

Article focus
- The article presents a study on the factors influencing first malaria infections in infants, performed in a rural area of Benin.
- The aim of the study was to confirm the existence of an increased risk to present first malaria infection in infants born from a mother with malaria-infected placenta, while taking into account environmental and behavioural factors.
- The article presents in detail the methods used in the follow-up of the cohort and reports baseline data.

Key messages
- Malaria during pregnancy could have an impact on infants’ susceptibility to first malaria infections.
- An alternative explanation is a high exposure to anophelines in a subset of pregnant women and their offspring, acting as a confounding factor.
- This study will be able to discriminate between environmental and individual (ie, immune tolerance) risk factors of first malaria infection by studying the association between infected placenta and first malaria infections. Specific recommendations will be given to protect pregnant women adequately.

Every year, 200 000 newborns die for reasons directly related to maternal malaria during pregnancy, ie, stillbirth, fetal growth restriction, preterm delivery or low birth weight. Another 500 000 deaths occur among infants
In areas where malaria is holo or hyperendemic, the major risk group is represented by children under 5 years. While infants under 6 months of age appear to be relatively protected against clinical malaria and high density parasitaemias, older infants present an increased susceptibility to clinical and parasitological outcomes. Several reasons involving the child’s immune development have been put forward to explain this early protection against malaria. Among them, the protective role of passively transferred antibodies from mother to child and the presence of fetal haemoglobin in the blood of the newborn may prevent parasite multiplication during the first year of life. Later, the susceptibility of children increases and first malaria attacks rise in frequency and intensity, threatening life until approximately the age of 5 years, before the immunity to P. falciparum blood stages develops as a result of both repeated infections and age-related maturation of the immune system.

After the first 6 months, the time to first malaria infections and the intensity of symptoms seem to vary from one child to another. It is important to identify the factors involved in such differences and to explore whether the characteristics of first infections can inform on subsequent children’s response to malaria infections. Le Hesran et al. first identified P. falciparum infection of the placenta in 1997 as an important factor likely to influence the time to first malaria infections. Two recent studies confirmed this first result, and consistently showed that infants born to a mother with a malaria-infected placenta were more likely to present a first parasitaemia earlier than infants with no history of placental malaria infection. As an explanation, it was suggested that infant susceptibility to malaria may be influenced by the contact with parasite antigens during in-utero life, probably inducing an immune tolerance. A fourth study confirmed this hypothesis. However, the role of other factors, such as nutrition and environment, has to be clearly established in order to ascertain the role of placental malaria. As it is necessary to take into account simultaneously the different factors involved, a multidisciplinary approach was set up in Benin. It aimed at determining the parts played by environmental (entomological, ecological, behavioural), biological (parasitological, immunological, nutritional) and genetic determinants, respectively, in first malaria infections, giving priority to the role of placental infection and entomological factors influencing transmission. The objective of this paper is to present the study site and population and to provide environmental preliminary results. Special attention has been given to the description of the methods used in this multidisciplinary study and particularly to the follow-up of the cohort. This paper will not present final results but is expected to serve as a methodological reference for forthcoming papers.
Sample size
In the study carried out in Cameroon by Le Hesran and collaborators, 65% of the placental malaria-infected infants had a parasitaemia during the first year of life versus 50% in placental malaria-negative infants. Transmission being lower in the south of Benin and in the absence of pre-existing data concerning malaria transmission in the area, it was difficult to predict the relative proportions of parasitaemic children in the two groups during the 12-month follow-up. However, assuming 30% of the children would present with a first peripheral infection in the placental malaria-infected group and 15% in the placental malaria-negative group, with a ratio of 5, a 80% power and a 5% risk, 450 newborns had to be included (75 in the placental malaria-infected group and 375 in the placental malaria-negative group) (sampsi command, Stata V.8). At study initiation, having no better estimation for the final proportions, to be conservative and to account for loss to follow-up, we decided to include 600 newborn babies.

Enrolment and follow-up
Recruitment was performed in three health centres providing deliveries and postnatal care (Tori Avame, Tori Cada and Tori Gare). The study objectives and study protocol were explained by midwives to all women attending the health centre for antenatal care from the seventh month of pregnancy. Inclusion criteria were to live in one of the nine villages of the study area, to have no intention to move in the next month and to plan to deliver in the health centre. At enrolment, ie, before delivery, midwives again gave information about the study and collected signed informed consents. The approval of husbands was systematically looked for by pregnant women themselves. Consequently, husbands were invited to come and sign the informed consent form. After delivery, newborns were listed and received an identification card, giving them an access to free treatment in health centres during the 18 months of the follow-up.

The general follow-up included a weekly home visit by health workers to detect fever and the general health status of infants, and a monthly home visit by supervisors and field workers to search for malaria and to collect nutritional data. In addition, women were told to come with their offspring to the health centre for blood sampling once a trimester. At least three attempts were made before a scheduled visit was considered missed and infants were considered lost to follow-up after having missed more than four consecutive monthly visits.

Malaria follow-up
According to a recent entomological survey, *P. falciparum* is the commonest species in the study area (95%),
Plasmodium malariae and Plasmodium ovale representing 3% and 2%, respectively. To investigate the relation between malaria infection of the placenta and infant parasitaemias and to be consistent with previous studies, we considered only P. falciparum infections for the data analysis.

At delivery, maternal blood and umbilical cord blood samples were drawn to search for malaria infection and anaemia. Thick and thin placental smears were made by midwives to look for placental malaria infection.

During weekly home visits, the axillary temperature was measured by health workers with a digital thermometer to detect P. falciparum symptomatic infections. In the case of temperature higher than 37.5°C, mothers were told to bring their children to the health centre. On arrival at the health centre, a questionnaire was filled in and a Parascreen rapid diagnostic test (RDT) was made, to obtain an immediate diagnosis of symptomatic malaria infection (ie, the presence of parasites and temperature higher than 37.5°C). A thick blood smear (TBS) was made to provide a later confirmation of the RDT result. When RDT was positive, infants were treated by an artemisinin-based combination (artemeter and lumefantrin) as recommended by the National Malaria Control Program of Benin. If the mother did not bring her child to the health centre the following day, a field supervisor visited the family to get information on the infant’s clinical status and to invite the mother to consult. Mothers were also invited to bring their infants to the health centre at any time, for free attendance in the case of fever (suspected by the mother) or any clinical signs, related to malaria or not, and the same protocol (ie, questionnaire and RDT) was applied.

Once a month, a TBS and a sample of blood were systematically collected to assess asymptomatic P. falciparum infection (figure 2). The presence of insecticide-treated nets in the house was checked during the visit.

At 3, 6, 9, 12, 15 and 18 months, women were asked to attend the health centre with their offspring. In addition, venous blood was sampled (3 ml with EDTA). The level of haemoglobin was determined, blood was centrifuged and both plasma and buffy coat were kept frozen for further immunological and genetic studies.

Nutritional follow-up

At birth anthropometric measurements were performed by midwives (weight, length, head circumference and mid-upper arm circumference; MUAC), according to WHO recommendations. Estimation of the gestational age of the newborn was carried out by field supervisors within 72 h after delivery by the neurological and morphological Ballard score.

On monthly home visits a nutrition questionnaire was administered by supervisors to collect information about breastfeeding and to assess the quality of feeding practices through a qualitative dietary 24-h recall. Dietary diversity was assessed through the means of a list of 24 food groups that were further collapsed into 14 groups to create an individual dietary diversity score according to Food and Agriculture Organization recommendations. Other indicators of infant and young child practices were constructed as recommended by WHO. Anthropometric measurements (weight, length and MUAC) were performed every month from birth to 6 months, then at 9, 12, 15 and 18 months. Weight was recorded to the nearest 10 g using mechanical baby scales (SECA type 745, PHI Medical S.A., France). Length was measured to the nearest millimetre with a locally made wooden board equipped with two metal measuring tapes. MUAC was measured to the nearest millimetre using non-stretchable tapes (SECA 200). To...
ensure good quality anthropometric data, all measurements were performed twice, by two different operators.

**Environmental and entomological follow-up**

Entomological measures were made during 2 years in 36 sampling houses (simultaneously indoor and outdoor in four houses per village). Human landing catches were performed every 6 weeks, during three nights, simultaneously in three villages. Collections were made inside and outside the houses simultaneously, from 22:00 to 06:00 hours the following morning. At daybreak, morphological identification was made and *Anopheles gambiae* and *Anopheles funestus* were sent to Cotonou laboratory where they were kept and frozen at −20°C.

Thereafter, *P. falciparum* infected anophelines were identified by ELISA to determine sporozoite rates. The entomological inoculation rate (EIR), expressed in the number of infected anophelines per night and per man, was calculated for each village. To account for environmental factors that might influence transmission, climatic measures (temperature, humidity rate) were recorded every hour by electronic microchips placed in each of the nine villages. Rain levels were collected twice a day by means of pluviometers.

At the end of the study, these data will be entered into a geographical information system, based on high resolution imagery (Spot 5, 10m colour, 2003), as well as data from mosquito catches, climate data, data related to the soil occupation of the area, vegetation favourable to breeding sites, water collections, information concerning human behaviour (use of bed nets, etc) and way of life (housing characteristics, habitat conditions, water supply, use of pesticides, number of persons sleeping in the house, etc) to characterise the spatial and temporal variability of malaria transmission and to build up an individual indicator of the risk of infection.

**Biological and laboratory procedures**

All blood samples were processed in the Tori Bossito laboratory. Haemoglobin rates were measured at birth and quarterly on blood samples with a Hemocue analyser (Hemocue@AB, Sweden). TBS obtained monthly or during consultations were stained with Giemsa. Leucocytes and parasites were counted simultaneously until leucocyte or parasite numbers reached 500. A TBS was declared negative if no parasite was found after counting 500 leucocytes. Blood samples (maternal peripheral blood, cord blood or infant 3-monthly peripheral blood) were centrifuged at 1500 rpm for 10 min. Plasma anduffy coat were sent to Cotonou to be frozen (at −80°C and −20°C, respectively) for subsequent immunological and genetic analyses. Collections of whole blood or plasma were deposited on filter papers for further parasite genotyping and immunological analyses.

**Data management and analysis**

Questionnaires were transferred to the Cotonou laboratory to be entered using Epi Data software V.3.1. In case of discordan after double entry control, data were sent back to the field to be corrected. The first phase of the analysis, which is ongoing, consists of a semi-parametric model to study the association between the first occurrence of parasitaemia and the existence of an infected placenta at delivery. A Cox proportional hazards model was used to identify potential risk factors from fixed covariates, collected at the beginning of the study, and time-dependent covariates collected during the follow-up. Entomological and nutritional factors have been integrated in the model as time-dependent covariates. As there were few missing data, and as they were randomly distributed, no specific procedure was used to deal with them. In particular, we checked that there was no problem of informative censure (malaria infections not related to loss to follow-up).

A second phase of the analysis will focus on the successive of recurrent malaria events in the same individual. The first parasitaemia (or the first malaria attack) and the following ones will be analysed with appropriate models based on an extension of the Cox model. This analysis will allow us to assess if the risk factors identified for the first malaria episodes persist in the following episodes.

Data are analysed using Stata V.8.0 and SAS V 9.0 software.

**Ethics clearance**

The protocol was approved by both the Ethical Committee of the Faculté des Sciences de la Santé (FSS) in Benin and the IRD Consultative Committee on Professional Conduct and Ethics (CCDE).

**RESULTS**

Data presented in this paper are general information collected from women enrolled from 4 June 2007 to 31 July 2008. In addition, environmental data (climatic and entomological data) collected during the same period (first year of follow-up) are also presented. Demographic characteristics of the study population in 2006 and the number of infants per village followed more than 28 days are presented in table 1.

**Enrolment and follow-up**

As presented in the flowchart diagram in figure 3, 660 women were considered for inclusion in the study, among them 629 fitted with the inclusion criteria. Six hundred and seventeen women among the 629 included had a questionnaire filled at enrolment (missing questionnaires were mostly related to women whose pregnancy ended with a stillbirth); 32.6% (201/617) of the women were enrolled in the health centre of Tori Avamé, 48.1% (297/617) in Tori Cada and 19.3% (119/617) in Tori Gare.

The mean age of the women at enrolment was 27.5 years (SD 5.6; (16−49)); 14.8% (91/613) were primigravidae, 12.5% (77/613) secundigravidae, 72.7% (446/613) multigravidae. Women had experienced on average 3.1 previous pregnancies, ranging from none to nine. Overall, the women included had given birth
(excluding the present pregnancy) to 1690 alive children, of whom 1387 (83%) were still alive at the time of enrolment. Forty-two per cent (220/523) of the women had experienced the death of at least one child, among whom 70% had lost one child, 22.3% two children, 7.7% more than two children. In the present study, these women gave birth to 656 newborns (603 singletons, 25 twins and one triplet). After enrolment, 10 stillbirths, 11 early neonatal deaths, five late neonatal deaths and 10 early losses to follow-up occurred, from which only 620 were followed more than 28 days (figure 3).

Placental infection
Mother and infant baseline characteristics according to placental malaria infection are presented in table 2. Placental malaria was statistically associated, in univariate analysis, with mother’s age, gravidity status, low birthweight, village of residence and maternity clinic.

Housing, education and socioeconomic indicators
Most houses were built with traditional materials; 74.8% of the houses had mud walls, 18.1% mud covering cement walls and 7.1% cement walls. Floors were made of clay for 56% and cement for 44%; 59.2% of the houses had a sheet roof, the rest having a straw roof; 84.8% (520/613) of the women reported not having received any formal education, 10.5% had partly attended primary school (<5 years), 3.1% had attended primary school for at least 5 years and 1.6% had reached secondary school or more.

Hygrometry and temperature
Figure 4 displays the temperature per village and per month. The variability of temperatures observed is related to the alternation of dry and rainy seasons. The lowest temperature (24°C) caused by the arrival of the dry Sahelian wind (harmattan) in December 2007 occurred in the dry season in January. Then, temperatures increased until approximately 29°C from February to April, and decreased slowly during the wet season from April to July 2008 (approximately 26°C). As shown on the figure, the variations were homogeneous between villages. The average hygrometry per month followed a similar trend (data not shown).

Rainfall
In a typical year in the south of Benin, there are two rainy seasons, the long rains from April to July and the short rains from October to November (figure 5). During the first year of the study, rainfall had globally followed this schedule. However, it can be noted that the year 2008 seemed a little rainier than the trend over the past 30 years. Rainfall was collected every day in pluviometers by the village inhabitants. Daily collection of information on the occurrence of rains (yes/no) in each village in June 2008 showed a spatial heterogeneity between villages (figure 6), with possible consequences on the local conditions, which may favour or prevent the development of anopheles at a given time.

Entomological inoculation rates
During the first year of the study, 10 3-day mosquito catches were conducted in all villages of the area. Figure 7 shows the EIR (limited to five villages for reasons of clarity). The annual *P. falciparum* EIR calculated on all nine villages indicates the existence of a slight but constant transmission in the area, with an average of
15.48 infected anopheles/man per year and two peaks during the rainy seasons, consistent with previous studies made in different areas of Benin (ranging from 11 in the region of Nokoué Lake to 58 in urban Cotonou). The EIR rates in each village reveal a strong heterogeneity in local malaria transmission between villages, sometimes

<table>
<thead>
<tr>
<th>Table 2 Factors associated with placental malaria</th>
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<tbody>
<tr>
<td><strong>Malaria-infected placenta</strong></td>
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<tr>
<td><strong>Maternal factors</strong></td>
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<tr>
<td>Age class, years</td>
</tr>
<tr>
<td>≥20</td>
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<tr>
<td>21–25</td>
</tr>
<tr>
<td>26–30</td>
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<tr>
<td>&gt;30</td>
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<tr>
<td>Gravity status</td>
</tr>
<tr>
<td>Multigravidity</td>
</tr>
<tr>
<td>Primigravidity</td>
</tr>
<tr>
<td>Bed net possession</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>IPTp use†</td>
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<tr>
<td>No</td>
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<tr>
<td>Yes</td>
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<tr>
<td>Chloroquine use</td>
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<tr>
<td>No</td>
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<tr>
<td>Yes</td>
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<tr>
<td>No of ANVs</td>
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<tr>
<td>≤3 ANVs</td>
</tr>
<tr>
<td>&gt;3 ANVs</td>
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<tr>
<td>Education of women</td>
</tr>
<tr>
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<tr>
<td>Partial primary</td>
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<td>Severe maternal anaemia (&lt;7 g/dl)</td>
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<td>No</td>
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<tr>
<td>Yes</td>
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<tr>
<td>Infant factors‡</td>
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<tr>
<td>Gender</td>
</tr>
<tr>
<td>Female</td>
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<tr>
<td>Male</td>
</tr>
<tr>
<td>Low birthweight§</td>
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<tr>
<td>No</td>
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<tr>
<td>Yes</td>
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<tr>
<td>Geographical factors</td>
</tr>
<tr>
<td>Village of residence</td>
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<tr>
<td>Avame centre</td>
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<tr>
<td>Gbedjougo</td>
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<td>Houno</td>
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<tr>
<td>Ananvie</td>
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<tr>
<td>Dohinoko</td>
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<tr>
<td>Gbétaga</td>
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<tr>
<td>Cada centre</td>
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<tr>
<td>Zebe</td>
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<tr>
<td>Zoungoudo</td>
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<tr>
<td>Maternity clinic</td>
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<tr>
<td>Tori Avame</td>
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<td>Tori Cada</td>
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<tr>
<td>Tori Gare</td>
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</tbody>
</table>

* Nine placental smears were missing on 617 total deliveries.
† Intermittent preventive treatment during pregnancy (IPTp) with sulfadoxine pyrimethamine.
‡ Excluding twins and triplets, analysis were performed on 583 singletons.
§ Low birthweight defined as birth weight <2500 g.
¶ Fisher’s exact test.

ANVs, antenatal visits.
independent of the season (e.g., the village of Gbedjougo in January, where EIR was very high, whereas there was no anopheline transmission detected in other villages).

**Vector control**

In response to questions on the use of vector control measures, 66.2% (401/606) of the women reported having a bed net but only 22% of them (88/400) declared having used it the night preceding the interview; 76.1% (302/397) were impregnated bed nets, 11.1% were not and for 12.8% it was unspecified. Thirty-two per cent of the women (182/606) used other vector control measures (172 used mosquito serpentines and 10 an insecticide spray).

**DISCUSSION**

Three studies concerning the influence of placental malaria on the delay to first infections have been conducted in different parts of Africa, in Cameroon (1997), Tanzania (2005) and Gabon (2008). The main finding was that infants born to mothers with an infected placenta developed a first parasitaemia earlier than those born to mothers whose placenta was not infected. To explain this result further, a recent study carried out in Kenya (2009) investigated the influence of in-utero exposure on the acquisition of cellular and humoral immune responses to malaria blood stage antigens. It showed that children defined as exposed but non-sensitised to malaria (born to mothers with an infected placenta and a negative cord T-cell response), had a 61% greater risk of infection compared with non-exposed children (negative placenta and negative cord T-cell response) and a 41% greater risk compared with sensitised children (positive placenta and cord T-cell response). The conclusion was that a subset of children exposed to malaria in utero acquired a tolerant phenotype to blood-stage antigens that could persist during childhood.

The Tori Bossito study was designed specifically with the purpose of investigating the relation between placental malaria infection and peripheral parasitaemias in infants, while taking into account environment-related and nutritional variables, thus decreasing the possibility of biases. Consequently, the sample size was adapted and potential confounding factors related to placental malaria infection or parasitaemias in infants as well as in mothers were systematically collected. In particular, our study collected longitudinal data on nutrition status, which is likely to influence the risk of malaria infection and the development of the immune system. Almost any nutrient deficiency can modulate the resistance to infections, as most nutrients are involved in the immune response. In our study, the infants’ nutritional status was assessed through anthropometric measures taken regularly, and dietary diversity was estimated monthly for all infants, to identify micronutrient deficiencies. Climatic data (hygrometry and temperature) were also collected all the way through the study. They showed a strong homogeneity between villages but also a high seasonality. On the contrary, within each village, there was heterogeneity in the number of rainy days, as well as in the spatial distribution of the results of entomological captures. Altogether, these results suggest that the conditions influencing the multiplication of anophelines vary considerably from one village to another and probably within villages. Such variability will be taken into account to assess an environmental risk of infection per child, which may depend on both the date of birth and the place of residence of the followed children. Information concerning environmental factors was collected during all the follow-up. The close
surroundings of the children (housing characteristics, presence of vegetation favourable to breeding sites, or water collections) as well as the family behaviour (use of impregnated bed nets, pesticides, number of persons sleeping in the house) were also noted, as possibly influencing the risk of exposure to the vectors.

The population follow-up started with delivering women who were all included from the three main maternity clinics of the area. A first analysis of potential risk factors for placental infection showed the validity of our sample selection and the quality of the data collected, by confirming the role of the mother's

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**Figure 6** Number of rainy days per village in Tori Bossito (example of June 2008). Note: Black squares symbolise rainy days and white squares days with no rainfall.

**Figure 7** Entomological inoculation rate (EIR; number of infected anopheles/man per night) during the capture sessions in five villages of the Tori Bossito area, Benin. Average EIR calculated in nine villages.

**Anophele transmission**

1st year of study in Tori Bossito

![Graph showing Anophele transmission over time](image-url)
First malaria infections in infants in Benin

gravidity and age, and of geographical factors known to influence malaria transmission. In addition, a significant association was found between placental malaria and low birthweight of the baby.

A close follow-up was then started on newborn babies. Visits at infants’ homes for the detection of fever were made once a week. If feverish, infants were sent to the clinic where a RDT was performed, indicating a symptomatic malaria infection if positive. A monthly TBS was performed on all children during the 18 months of the follow-up to detect asymptomatic infections. With this protocol of surveillance, we assume that very few infections could escape our vigilance.

A limitation to the study may be the approximation of individual exposure to malaria vectors by the use of anophelines landing catches in nearby houses, but different from the infants’ homes, at 6-week intervals, in only 36 sampling houses of the study area. Moreover, anophelines catches were performed on adults, less attractive for anopheles than infants. However, we cannot figure how more precise estimations of malaria transmission at the infant level could have been made within the framework of our study, given the ethical, financial and logistical issues raised. An integration of seasonality and immunological data on anti-sporozoite antibodies and antibodies against anopheles salivary proteins is planned for the final analysis of the data, thus providing a reliable estimation of the overall exposure of our population to malaria vectors.

In conclusion, our approach combines demographic, socioeconomic, behavioural and environmental data obtained from a diversity of sources, from simple questionnaires to ground maps and satellite data. These data will be entered in a geographical information system and will allow us to determine a time and space-dependent index of vector exposure, which can be applied to each individual. This type of time-dependent variable will be integrated in a Cox model as a factor of adjustment to influence malaria transmission. In addition, a significant association was found between placental malaria and low birthweight of the baby.

Acknowledgements
The authors are grateful to all women and infants of Tori Bossito who agreed to participate in this project, to field supervisors (Edgard Godonou, Stéphane Gehou, Sylvestre Zehounkpe, Pierre Adanchoed and Patrick Pomealegn) and to the 18 community health workers of villages who worked on the project, to midwives, nurses and health helpers of Tori Avamé, Tori Cada and Tori Gare health centres as well as to laboratory technicians of Tori Bossito for their collaboration. The authors would like to thank Laurence Watier, Fabrice Chandre, Célia Dechavanne, Charlotte Pierrat, Aziz Bouraima, Achille Massougobdji and Benjamin Fayomi who participated actively in the study conception and supervision, and to the Faculté des Sciences de la Santé (FSS), the Institut des Sciences Biomédicales Appliquées de Cotonou (ISBA), the Programme National de Lutte contre le Paludisme (PNLP) for their institutional support.

Contributors
ALP and GC supervised the study in the field, AG and MC planned the study and AG was the coordinator of the study. YM supervised nutritional data collection, FMN supervised biological data collection. ALP, YM, GC, FMN, AG and MC participated in the interpretation of findings and wrote the paper.

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Competing interests
None.

Ethics approval
The protocol was approved by both the Ethical Committee of the Faculté des Sciences de la Santé (FSS) in Benin and the IRD Consultative Committee on Professional Conduct and Ethics (CCDE).

Provenance and peer review
Not commissioned; externally peer reviewed.

Data sharing statement
Environmental data presented in the article will be available by request to Dr André Garcia (André.Garcia@ird.fr).

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