Importance of Adequate Local Spatiotemporal Transmission Measures in Malaria Cohort Studies: Application to the Relation Between Placental Malaria and First Malaria Infection in Infants

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According to several studies, infants whose mothers had a malaria-infected placenta (MIP) at delivery are at increased risk of a first malaria infection. Immune tolerance caused by intrauterine contact with the parasite could explain this phenomenon, but it is also known that infants who are highly exposed to *Anopheles* mosquitoes infected with *Plasmodium* are at greater risk of contracting malaria. Consequently, local malaria transmission must be taken into account to demonstrate the immune tolerance hypothesis. From data collected between 2007 and 2010 on 545 infants followed from birth to age 18 months in southern Benin, we compared estimates of the effect of MIP on time to first malaria infection obtained through different Cox models. In these models, MIP was adjusted for either 1) "village-like" time-independent exposure variables or 2) spatiotemporal exposure prediction derived from local climatic, environmental, and behavioral factors. Only the use of exposure prediction improved the model’s goodness of fit (Bayesian Information Criterion) and led to clear conclusions regarding the effect of placental infection, whereas the models using the village-like variables were less successful than the univariate model. This demonstrated clearly the benefit of adequately taking transmission into account in cohort studies of malaria.

cohort studies; infant; malaria; malaria transmission; placenta

Abbreviations: BIC, Bayesian Information Criterion; IYCF, infant and young child feeding practices; MIP, malaria-infected placenta.

More than 215 million clinical cases of malaria occurred in 2010, with approximately 655,000 deaths, the vast majority among young children and pregnant women in Africa (1). *Plasmodium falciparum* is the most common species of *Plasmodium* in Africa and the main species responsible for the most severe forms of malaria, as well as for placental infection. During infection, host and parasite interactions result in a variable clinical presentation. Environmental and individual factors are involved in this variability (2).

Placental malaria infection could play an important role in this variability, at least during the first year of life when immunity develops (3). The consequences of having a *P. falciparum* malaria-infected placenta (MIP) were established in 4 studies (4–7). Despite different follow-up protocols, these studies all led to the conclusion that offspring of mothers with MIP at delivery experience their first *P. falciparum* parasitemia at a younger age. The authors concluded that these observations were the direct consequence of immune tolerance stemming from placental infection. However, babies with greater exposure to *Plasmodium* should also be at greater risk of contracting malaria rapidly after birth. Consequently, to demonstrate immune tolerance and to quantify its role, local variations in malaria transmission must be taken into account.
In Africa, transmission levels vary enormously and may be either seasonal or perennial (8). Differences exist not only between regions but also at a very local level (8–11). Therefore, localized variations ought to be taken into account when considering the risk of infection in a population and identifying the determinants of individual variability (12, 13).

The spatiotemporal variability in malaria transmission was insufficiently taken into account in the 4 previous studies on the consequences of MIP, since no entomological data had been collected. In a recent intermediate analysis of data from the first 12 months of follow-up (14), we attempted to better apprehend children’s spatiotemporal exposure to malaria using entomological data in the analysis, confirming its great importance.

We recently proposed a novel approach for predicting malaria transmission in houses within a limited area based on ecological and environmental characteristics (15). We demonstrated that a regression model including spatial and time-dependent variables at the village and house levels yields a spatiotemporal prediction of malaria transmission comparable to that obtained on the basis of entomological data (15).

In the present study we aimed to demonstrate, through the statistical comparison of different models applied to our complete data set, the benefit of using a spatiotemporal prediction variable and the drawbacks that could result from using a classical “village-like” exposure variable to evaluate the effect of MIP on time to first malaria infection after birth.

MATERIALS AND METHODS

The study, Survenue des Premières Infections Palustres chez le Nouveau-né: Determinants Génétiques, Biologiques, et Environnementaux (Genetic, Biologic, and Environmental Determinants of First Malaria Infection in Newborns), was initiated in 2007. The study protocol has been described in detail previously (16) and will be briefly described herein.

Study population

The study area included 9 villages and 3 health centers in southern Benin. A birth cohort was set up in June 2007, and subjects were recruited until July 2008. The study objectives and protocol were explained by midwives to all women who visited the health center for prenatal care from the seventh month of pregnancy onward. At enrollment, midwives obtained signed informed consent, in both French and the local language, before delivery. The approval of husbands was systematically sought by the pregnant women themselves, and husbands were invited to sign the informed consent form. Stillborn babies were excluded from the study.

Baseline data

At delivery, a questionnaire was administered to gather information on the women’s sociodemographic and obstetric backgrounds and on the course of the current pregnancy. Maternal blood and umbilical cord blood samples were taken to search for malaria infection and anemia. Thick and thin placental smears were taken and examined for evidence of MIP.

Surveillance

Every child was visited weekly by health-care agents and nurses. Every month, a thick blood smear was taken to detect asymptomatic malaria infection. The child’s weekly axillary temperature was taken and, in case of a temperature higher than 37.5°C, both a rapid diagnostic test and a thick blood smear were performed. Symptomatic malaria infection was defined as fever and a positive thick blood smear and/or rapid diagnostic test and was treated with artemisinin-based combination therapy, as recommended by the National Malaria Control Program. Mothers were also invited to bring their infants to the health center at any time in case of fever (suspected by the mother) or clinical signs, and the same protocol was applied. Thick blood smears, stained with Giemsa stain, were read twice by 2 technicians. In case of discordance, a third reading was performed by a senior parasitologist on the team.

Entomological and environmental follow-up

Mosquito collection and identification. Entomological surveys based on human landing catches were conducted in the villages every 6 weeks for 2 years (July 2007–July 2009). Insects were collected at 4 capture points (houses) in each village over 3 successive nights (4 indoors and 4 outdoors; that is, a total of 216 nights every 6 weeks in the 9 villages). Sites for the entomological collections were selected according to classical entomological methods: 1) identification of the village limits and 2) choice of 4 houses separated by 10 m from the vegetation surrounding the village and composed of 2 rooms to avoid families’ sleeping in the same room as the collector. The distance between infants and the mosquito-catching houses ranged from 0 m to 3,000 m, with a median of 373 m. Each collector caught all mosquitoes that landed on the lower legs and feet between 10 PM and 6 AM. The following morning, mosquitoes were identified on the basis of morphological criteria (17, 18). All Anopheles gambiae complex and Anopheles funestus mosquitoes were stored in individual tubes with silica gel and preserved at −20°C. Rates of P. falciparum infection were then determined on the head and thorax of individual anopheline specimens using circumsporozoite protein enzyme-linked immunosorbent assay (19).

Environmental and behavioral data. Rainfall was recorded twice a day with a pluviometer in each village. In and around each catch site, the following information was collected: 1) type of soil (dry lateritic or humid hydromorphic), assessed using a soil map of Benin at the 1/200,000 scale (maps: NB-31-XIV and NB-31-XV; Institut National Geographique, Saint Mandé, France, 1968), further georeferenced and entered into a geographic information system; 2) the presence of areas where construction in progress involved the presence of tools or holes, which are potential breeding habitats for Anopheles; 3) the presence of
abandoned objects (e.g., utensils) that could be used as ovi-
position sites for female mosquitoes; 4) a watercourse 
neary; 5) number of windows and doors; 6) type of roof 
(straw or metal); 7) number of inhabitants; 8) ownership of a 
bed net; 9) use of insect repellent; and 10) Normalized Dif-
ference Vegetation Index, estimated for a 100-m area around 
the catch site with a SPOT5 high-resolution (10-m colors) 
satellite image (image: SPOT5, Centre National d’Etudes 
Spatiales, Toulouse, France, 2003; distribution: Spot Image 
SA, Toulouse, France), with assessment of the chlorophyll 
density of each pixel of the image.

Statistical analysis

We fitted different Cox models to analyze the association 
between the onset of the first malaria infection up to age 18 
months and placental infection, adjusted for the children’s 
exposure and covariates. The first malaria infection (sympto-
matic or asymptomatic) was de
ned as the first positive 
thick blood smear, performed either at monthly home visits 
or when the child was brought to the health center because 
of fever.

The exposure variables. Prediction of the entomological 
risk. We fitted a prediction model in which the total number 
of Anopheles mosquitoes was predicted by the cli-
matic, environmental, and behavioral data at the village and 
household levels. We used the total number of anophelines 
because no convergence was obtained with the number of 
infected anophelines. However, we have shown that the total 
number of anophelines is almost linearly correlated with the 
number of infected anophelines (15). The best subset of pre-
dictive variables was selected using a leave-one-out method 
iminizing the prediction error, and it contained the follow-
ing variables: mean rainfall between 2 catches (classi-
ded according to quartile), number of rainy days in the 10 
days preceding the catch (3 groups: 0–1 days, 2–4 days, or >4 
days), season (4 groups: end of the dry season (February– 
April), beginning of the rainy season (May–July), end of the 
rainy season (August–October), or beginning of the dry 
season (November–January)), village, Normalized Differ-
ence Vegetation Index (classi
ded according to quartile), and 
use of insect repellent in the house. This model has been 
extensively described elsewhere (15).

Spatiotemporal prediction of the entomological risk \( \hat{Y} \) was 
then given by \( \hat{Y} = X_\beta \), where \( X_\beta \) is the time-dependent 
vector of covariates of the best predictive model and \( \beta \) is the 
vector of estimates corresponding to the covariates. This 
model was applied in each month of life for each child (i.e., 
the estimated prediction was computed for each child at \( t = 1 \) 
month, \( t = 2 \) months, . . . , \( t = 18 \) months), leading to spatio-
temporal predictions of the entomological risk for all chil-
dren and for every month from 1 month to 18 months. 
Below, we call these predictions the “prediction variable.”

Other exposure variables. We considered as possible 
exposure variables the village of residence and the season, 
defined as rainy or dry according to the beginning and end 
of the rains. Season was a time-dependent variable.

Covariates. We considered the following maternal 
covariates: age, anemia at delivery, gravidity status, possess-
ion of a bed net, intermittent preventive treatment during 
pregnancy, antenatal visits, and educational level. For the 
children, gender and birth weight were taken into account as 
covariates.

We used an infant and young child feeding practices 
(IYCF) score as a time-dependent covariate, created as 
follows. For the first 6 months of life, IYCF score was coded 
as 0 if infants were not breastfed, 0.5 if infants were not 
exclusively breastfed, and 1 if infants were exclusively 
breastfed. Afterwards, the presence of a minimum accept-
able diet, a monthly indicator based on qualitative dietary 
24-hour recall, between 6 and 18 months of life was deter-
mined. A minimum acceptable diet is made up of the combi-
nation of 2 indicators: the minimum dietary diversity (at 
least 4 food groups consumed on the day preceding the inter-
view, out of 7) and the minimum meal frequency (3 meals 
per day for breastfed infants and 4 meals per day for infants 
who are not breastfed) (20). Infants had an overall IYCF 
score of 1 if they met the criteria for a minimum acceptable 
diet and 0 if they did not.

Survival analysis. To assess the effect of MIP on time to 
first infection, we constructed Kaplan-Meier curves and per-
formed a log-rank test. We conducted univariate Cox analy-
lysis to study the association between all covariates and the 
first malaria infection. In the analyses, the prediction vari-
able was classi
ded according to quartile for ease of interpre-
tation of results between the different classes and to avoid 
the requirement to assume a linear relationship between time 
to first infection and exposure. The right-censoring date 
was the date of first malaria infection or the last available date of 
follow-up. Then different multivariate models were used to 
study the association between first malaria infection and pla-
cental infection. Individual covariates which were found to 
be significant during univariate analysis were included in the 
models, together with different subsets of the exposure vari-
ables, in order to determine the best way to take into account 
the children’s exposure to malaria. Models were compared 
using the Bayesian Information Criterion (BIC). The propor-
tional hazards assumption was assessed for all covariates 
using graphical methods (–\( \ln(\ln(S)) \) function of time) and 
by testing the interaction with time.

The data were analyzed with Stata, version 8.0 (StataCorp 
LP, College Station, Texas), and SAS, version 9.0 (SAS 
Institute, Inc., Cary, North Carolina).

Ethics

The study proposal was approved by the Ethics Committee 
of the University of Abomey-Calavi, the Faculté des Sciences 
de la Santé of Benin, and the Consultative Ethics Committee 
of the Institut de Recherche pour le Développement.

RESULTS

Study population

A total of 620 infants in the cohort were followed for 
more than 28 days. After exclusion of 45 twins (22 pairs and 
1 stillbirth) and 1 set of triplets, 572 infants were kept for the 
analysis. Additionally, 23 infants out of 572 were excluded 
because of missing or inadequate data at the 18-month
follow-up (more visits missed than performed or doubtful identification). Exposure prediction was not available for 4 infants because the environmental and behavioral data were missing (Figure 1). Finally, 545 liveborn singletons were analyzed in the survival analysis. Among them, 62 were born to a mother who had an infected placenta at delivery and 477 were born to a mother with no infection of the placenta; 6 results on placental infection were missing.

Kaplan-Meier curves

The Kaplan-Meier curves (Figure 2) showed that infants born to a mother with MIP had a higher probability of contracting a first malaria infection than infants born to a mother with an uninfected placenta throughout the follow-up period (log-rank test: $P < 0.001$). The median ages of first infection were 8.62 months (range, 0.33–18.33) and 11.04 months (range, 0.33–18.49) for the infected-placenta and uninfected-placenta infants, respectively.

Univariate Cox analysis

The univariate analysis showed that placental infection was associated with a higher risk of earlier first infection, confirming the results shown by the Kaplan-Meier curves (Table 1). The village variable and the prediction variable were also highly related to the occurrence of first infection, as was the mother’s age and bed-net ownership. There was no association between nutritional status and first malaria infection ($P = 0.40$).

Comparison of models in multivariate analysis

Maternal age and ownership of a bed net were no longer significant in the multivariate models. The multivariate analysis showed that the prediction variable (Table 2, model d) was the only one that substantively improved the BIC in comparison with the univariate model (model a). The best-fitting model, model d (based on the BIC), confirmed the association between both placental infection and exposure and the probability of contracting a first malaria infection during the first 18 months of life (Table 3).

On the other hand, the other exposure variables (village (model b) or village combined with season (model c)) showed a critical drawback: They failed to improve the model’s goodness of fit (BIC) and led to misleading conclusions (only marginal significance of the placental infection ($P = 0.06$); that is, stricto sensu no rejection of the null hypothesis). We observed that adding the village and season variables to the prediction variable not only did not improve the results but led to the same drawbacks as those mentioned above (model e vs. model d).

DISCUSSION

These results show the utmost importance of adequately taking into account malarial exposure to assess and quantify the association between placental infection and the onset of first malaria infection during the first months of life. Indeed, the models that did not contain the prediction variable failed to improve the quality of the model in terms of the BIC, finally performed less well than the univariate model in quantifying the effect of placental infection, and led to nonsignificant results, precluding a clear interpretation. Moreover, the prediction variable was by itself sufficient to properly take malarial exposure into account. All of these results clearly demonstrate the drawbacks of using overly simple variables as markers of complex spatiotemporal malarial exposure and the benefit of using a more refined variable.

In the present work, the BIC confirmed that the exposure prediction variable was selected in the best model. We believe that the results from a Cox model using such a time- and space-dependent exposure variable are more valid than those derived using a variable such as village and/or season,
which we think can lead to biased results and misleading conclusions.

The impact of MIP on first malaria infection in infants was established in 4 studies based on very different protocols. In these studies, the variability of transmission intensity was not taken into account or was accounted for only superficially, though one of the most important factors in the complex malaria pathogenic system lies in exposure to parasites. In Cameroon, Le Hesran et al. (4) did not use entomological information but used only children’s residence area.

Table 1. Continued

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season(^{cd})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Rainy</td>
<td>1.20</td>
<td>0.94, 1.55</td>
</tr>
<tr>
<td>Village</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avame</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Gbedjougo</td>
<td>0.89</td>
<td>0.58, 1.37</td>
</tr>
<tr>
<td>Houngo</td>
<td>1.10</td>
<td>0.70, 1.73</td>
</tr>
<tr>
<td>Anavrie</td>
<td>1.73</td>
<td>1.16, 2.59</td>
</tr>
<tr>
<td>Dohinoko</td>
<td>1.81</td>
<td>1.19, 2.76</td>
</tr>
<tr>
<td>Gbetaga</td>
<td>3.49</td>
<td>2.32, 5.24</td>
</tr>
<tr>
<td>Tori Cada</td>
<td>2.03</td>
<td>1.38, 2.98</td>
</tr>
<tr>
<td>Zebe</td>
<td>1.17</td>
<td>0.72, 1.91</td>
</tr>
<tr>
<td>Zoungoudo</td>
<td>4.03</td>
<td>2.60, 6.23</td>
</tr>
</tbody>
</table>

Abbreviation: IYCF, infant and young child feeding practices.
\(^{a}\) In quartiles.
\(^{b}\) Low: no education; intermediate: partial primary school attendance; high: primary school completion or more.
\(^{c}\) Time-dependent covariate.
\(^{d}\) Season was determined according to rainfall (same dates of starting and ending rainfall in all villages).

Table 2. Hazard Ratio for First Malaria Infection According to Maternal Placental Malaria Infection in Various Models Compared Using the Bayesian Information Criterion, Tori Bossito, Benin, 2007–2010

<table>
<thead>
<tr>
<th>Model</th>
<th>Bayesian Information Criterion</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model a: MIP</td>
<td>2,166.46</td>
<td>1.59</td>
<td>1.17, 2.16</td>
</tr>
<tr>
<td>Model b: MIP + village</td>
<td>2,168.59</td>
<td>1.36</td>
<td>1.00, 1.87</td>
</tr>
<tr>
<td>Model c: MIP + village + season</td>
<td>2,170.64</td>
<td>1.35</td>
<td>0.99, 1.86</td>
</tr>
<tr>
<td>Model d: MIP + EP</td>
<td>2,134.27</td>
<td>1.57</td>
<td>1.16, 2.14</td>
</tr>
<tr>
<td>Model e: MIP + EP + village + season</td>
<td>2,141.79</td>
<td>1.38</td>
<td>1.00, 1.90</td>
</tr>
</tbody>
</table>

Abbreviations: EP, exposure prediction; MIP, malaria-infected placenta.
to take into account the potentially confounding effect of exposure. In Tanzania, Mutabingwa et al. (5) used the same geographical information and added the child’s birth season. In the most recent study, Malhotra et al. (7) used the same strategy by introducing adjustments for location and season. Finally, the only study that used entomological information was conducted by Schwarz et al. in Gabon (6). The authors used village of residence and birth season as covariates, and they assumed that transmission was homogeneous in the area (21), despite the fact that entomological surveys had been performed several years prior to the follow-up. The statistical method used to take into account malaria exposure consisted, at best, of introducing a “village” variable into a Cox model. This type of variable, which is constant across time, with the same value for all children living in the same village, does not adequately reflect the spatiotemporal transmission pattern that each child undergoes during follow-up.

Investigators in all of these studies concluded that MIP could be associated with a higher risk of malaria infection during the first months of life. However, the strength of the association seemed limited, since both the Mutabingwa et al. study (5) and the Schwarz et al. study (6) found an effect of placental infection only for children born to a multigravida. In our study, as well as in the study by Malhotra et al. (7), parity was not associated with child susceptibility, and despite a low power of the interaction test (22), this interaction was not statistically significant in previous studies. Although it is difficult to evaluate, we cannot exclude the possibility that inadequate consideration of the children’s exposure in these studies could explain—at least partly—some of the discrepant results.

The differences observed between children born to mothers with MIP and those born to mothers without MIP are classically explained by the immune tolerance related to placental infection. The effects of placental infection on children’s immune responses have been explored for a long time. However, although the ability to construct a P. falciparum-specific immune response is acquired by the fetus very early in utero, to our knowledge no clear hypothesis has been put forward to clarify the underlying mechanisms that could lead to immune tolerance (23–25).

We used the BIC for selecting the variables in our models. Although other criteria exist (the Akaike Information Criterion, for example), this criterion is commonly used for variable selection in epidemiologic models and is included in most of the current statistical software programs, providing a good compromise between the model’s parsimony and likelihood maximization.

Compared with our previous intermediate analysis of data from a 12-month follow-up period (14), we used a more precise “exposure” variable with the advantage of being time- and space-dependent at the individual level. However, there is certainly a gap between this predicted number of anophelines based on basic environmental and climatic factors and a child’s “exact” exposure. The consequences of this discrepancy between our prediction and reality for the results are difficult to evaluate. However, we argue that a child’s “exact” exposure is almost impossible to capture, and we recently showed that our prediction was at least as effective as entomological data in characterizing children’s exposure (15).

Alternatively, characterizing individuals’ exposure in field studies could include an adequate biomarker of malarial infection. Several candidates have been identified, among which the specific human immunoglobulin G responses to a salivary peptide (gSG6-P1) seems to be the most promising. This biomarker could be, at the population and individual levels, a credible new alternative tool with which to accurately assess the heterogeneity of people’s level of exposure to Anopheles bites (26). It would then be very interesting to compare the cost and effectiveness of an “environmental” prediction of exposure and a “biomarker” prediction of exposure.

In summary, the results of the present study confirm the existence of a strong association between placental infection and the risk of infection early in life, as well as the expected strong effect of exposure, giving a better understanding of each factor’s importance. From a more general point of view, correct classification of exposed and unexposed children is important in interpreting the results of any kind of study on childhood malaria, especially in the assessment of interventions (27). In studies on malaria immunity, the absence of a febrile episode is commonly considered evidence of “protection.” However, apparent protection can be due to a lack of exposure to mosquito bites, and this type of misclassification may give rise to misleading results (28). Our approach could then mitigate the information bias, help to improve the quality of the results, and facilitate their interpretation, providing a possibly useful epidemiologic tool for data analyses in this type of birth cohort.

Table 3. Hazard Ratio for First Malaria Infection According to Maternal Placental Malaria Infection in the Best-Fitting Cox Model (Model d), Tori Bossito, Benin, 2007–2010

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria-infected placenta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>Reference</td>
</tr>
<tr>
<td>Yes</td>
<td>1.57</td>
<td>1.16, 2.14</td>
</tr>
<tr>
<td>Exposure prediction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very low</td>
<td>1</td>
<td>Reference</td>
</tr>
<tr>
<td>Low</td>
<td>1.17</td>
<td>0.85, 1.63</td>
</tr>
<tr>
<td>Medium</td>
<td>1.62</td>
<td>1.17, 2.23</td>
</tr>
<tr>
<td>High</td>
<td>2.64</td>
<td>1.95, 3.58</td>
</tr>
</tbody>
</table>

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REFERENCES


