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Epidemiology of congenital malaria in Nigeria: a multi-centre study

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Summary

OBJECTIVE To determine the burden of congenital malaria in newborns in Nigeria.
METHODS In a prospective multi-centre study, 1875 consecutive mother–baby pairs were enrolled over a continuous 12-month period. Blood smears were prepared from mothers, neonates, placental aspirates and cord blood within 4 h of delivery. Outcome variables were patent parasitaemia in the mother, placenta, cord and neonate in addition to maternal and neonatal haematocrit.
RESULTS Patent parasitaemia was detected in 95 neonates (5.1%). The occurrence varied between study centres, but was found year round in all sites. The mean parasite density among infected neonates was low (48 asexual forms per μl, range 8–200/μl). Maternal and placental parasitaemia were the most important risk factors for patent neonatal parasitaemia ($P < 0.0001$). Spontaneous clearance of parasitaemia occurred in 62.1% of neonates before day 2. 33.7% were symptomatic within 3 days of birth.
CONCLUSION Congenital malaria is often asymptomatic, clears spontaneously and may not warrant treatment. However, newborns with unexplained fever and refusal to feed in malaria endemic areas should be tested for malaria.

keywords epidemiology, congenital *falciparum* malaria, Nigeria

Introduction

Malaria is considered to be congenital in the neonate when asexual parasites are detected in the peripheral blood within the first week of life. Despite the high prevalence of maternal and placental parasitaemia in endemic areas, congenital malaria was reported to be rare in babies born to semi-immune mothers (Bruce-Chwatt 1952; McGregor *et al.* 1983; Lamikanra 1993). This was believed to be the result of an efficient placental barrier and transfer of maternally derived antibodies, which offer some protection to the newborn up to 6 months of age. Other factors potentially contributing to the rarity of congenital malaria are the presence of faetal haemoglobin, maternal IgG and breast-feeding practices (Bruce-Chwatt 1952; Maegraith *et al.* 1952; McGregor *et al.* 1983; Snow *et al.* 1998). However, recent reports in Nigeria and other malaria endemic regions indicate that this notion may no longer be valid (Ibhanesebor 1995; Sowunmi *et al.* 1996b; Fischer 1997; Olowu *et al.* 2000; Mukhtar *et al.* 2005). Sowunmi *et al.* (1996a) reported an incidence of 22.2% ($n = 16$) among 72 deliveries screened in Ibadan, Nigeria, while Olowu *et al.* (2000) also working in Ibadan, reported a prevalence rate of 14% (11/77) among groups of low socio-economic status and of 7% (2/27) among groups of middle to high socio-economic status. An African survey spanning seven sites in sub-Saharan Africa (Fischer 1997) showed a mean prevalence rate of 7% for congenital malaria (range 0–23%). Mukhtar *et al.* (2005) from Lagos, Nigeria, reported an incidence of 15.3% for congenital malaria, while Kamwendo *et al.* (2002) in Malawi reported malaria parasitaemia in cord blood of 6% by microscopy and of 20% by polymerase chain reaction (PCR).

All of these studies involved relatively small numbers of participants from similar socio-economic groups and from ecological systems in the immediate vicinity of the study site. None incorporated intensive and iterative quality assurance for standardized diagnostic reagents and

accurate blood smear microscopy among the study sites, with the result that the true burden and clinical features of congenital malaria is uncertain and anticipatory care compromised. Little is known of the response to therapy in this age group. The dearth of this information has precluded the formulation of an appropriate action programme to control malaria in newborns in Nigeria.

Our study was conducted to define the epidemiology and determine the disease burden of congenital malaria in Nigeria with a view to provide a framework for its control. We aimed to determine the incidence of congenital malaria in Nigeria, the incidence of anaemia in the newborn and other associated factors, such as prevalence of maternal, placental and cord parasitaemia at delivery and the peripartum factors associated with the occurrence of congenital malaria. The clinical and laboratory features of congenital malaria and its response to therapy as seen in three geopolitical zones in Nigeria are reported elsewhere (Orogade *et al.* 2006).

Subjects and methods

Study sites

The study was conducted in tertiary and secondary institutions in four geopolitical zones of Nigeria: University College Hospital and St Mary's Catholic Hospital in Ibadan, Oyo state, in the southwestern zone; University of Ilorin Teaching Hospital and the Children's Specialist Hospital, both in Ilorin, Kwara state, in the north central zone; University of Nigeria Teaching Hospital in Enugu in the southeast; and Ahmadu Bello University Teaching Hospital in Kaduna, Kaduna state, northwestern zone of Nigeria. At the University of Nigeria Teaching Hospital in Enugu, substitution of the multi-centre study's standardized staining reagents with lower-quality Giemsa stain and deviations in protocols for buffering the stain and appropriate storage of slides together precluded extramural confirmation of blood smears by the four senior microscopists at each centre and the reference malaria microscopist (Michael V. Callahan). The inability to validate the malaria blood films from this study site prevented the inclusion of their data (see data analysis for additional details).

Malaria transmission in Nigeria occurs all year round with a major peak during the rainy season. The rains are longer in the south and shorter in the drier northern parts of the country. Ibadan is located in the rain forest belt of southern Nigeria, where transmission of malaria peaks from May to September and has a lower peak from November to March (Salako *et al.* 1990). The temperature is 23–32 °C and the annual rainfall is 1530–2050 mm (Nigerian Metrological Services). Ilorin is typified by rolling terrain and savannah vegetation. The peak rainy season is between June and September and annual rainfall is 1200–1800 mm, with temperatures of 20–33 °C. Kaduna has a typical guinea savannah vegetation and a peak rainy season between June and September. The temperature is 16–35 °C and the annual rainfall is 1000—1500 mm. Malaria transmission is hyperendemic in Ibadan and Ilorin (Salako *et al.* 1990) and mesoendemic in Kaduna (Orogade 2004).

Study design and study population

Convenience sampling was used in a prospective, descriptive study design. Every consecutive delivery that fulfilled the inclusion criteria was enrolled. An average of 52 subjects were enrolled monthly per centre. Monthly enrolment ensured that the mother–neonate pairs were enrolled during both high and low malaria-transmission periods. The enrolment criteria included the delivery of a single, live neonate at the study centre, residing within the catchment area of the study centres for at least 2 years before enrollment and willingness to participate in the study. Mothers whose babies were stillborn or had gross congenital abnormalities were excluded from the study.

We set out to establish the incidence of congenital malaria in Nigeria. In determining the sample size, we used the prevalence rate of 7% reported in the African survey on congenital malaria by Fischer (1997) as our reference. Using a prevalence of 7%, the sample size needed to achieve a precision of 1% at 95% confidence level was obtained from the equation:

$$N = \frac{P(1-P)}{(d/Z\alpha/2)^2}, \text{ where } d = 0.01, Z\alpha = 1.96 \text{ and } P = 0.07$$

The calculated sample size was 2503 babies or 625 per centre.

Ethical issues

All centres involved in the study have Federal Wide Assurance (FWA) certification. Ethical approval was provided by the Joint University of Ibadan/University College Hospital Ethical Review Board (Ibadan), the University of Ilorin Ethical Review Committee (Ilorin), Ahmadu Bello University Ethical Review Board (Kaduna) and University of Nigeria Teaching Hospital Enugu Institutional Review Committee. In addition, ethics approval was obtained from the Boston University Institutional Review Board. Written informed consent was obtained from each study volunteer or from the study participant's mother or legal guardian (spouse or grandmother) for study volunteers under 18 years of age. Continuous ethical review was carried out

for each year the study was active. The study was done according to 'Good Clinical Practice' standard and followed the principles of the Declaration of Helsinki.

Quality assurance

The methodology for the quantification of malarial parasites employed a standardized quality assurance training programme for malaria microscopists and investigators at each of the research centres. A mandatory 6-day training workshop was performed at one of the study centres (Ibadan). Each team was provided with identical protocols, and identical standardized reagents, including Wright Giemsa stains, unsilicated microscopy slides and buffer concentrates. After the completion of the workshop, monthly quality control was conducted at each study centre, whereby slides were given an intercode (blinded) and reviewed by a second microscopist at each respective centre. Refresher courses and quality assurance workshops took place 3 months after the study started, midway and at the end of the study period.

Study procedures

Maternal demographic and obstetric data were obtained for each mother–baby pair. The newborn was weighed to the nearest gram on a digital scale. Crown-heel length was measured with an infantogram and occipito-frontal circumference with a tape measure. The gestational age was determined using the Ballard score (Ballard *et al.* 1991). Anthropometric parameters and gestational age were used to classify the babies using a Lubchenco chart as pre-term, term and post-date. Babies <37 weeks were classified as pre-term, those between 37 and 40 weeks as term, and over 40 weeks as post-mature. The rectal temperature of the babies was taken using an electronic thermometer.

Laboratory procedures

Thick and thin blood smears were prepared from placental aspirates (Sowunmi *et al.* 1996a) and the cord blood was obtained within 1 h of delivery. Thick and thin blood smears were also prepared within 4 h of delivery after initial cardiopulmonary stabilization, from each mother and baby from finger and heel pricks, respectively. Blood smears were air dried without convection, and stained with 10% freshly prepared Giemsa stain maintained at a PH of 7.2. Thin blood smears were fixed with 100% methanol prior to staining. The stained blood smears were viewed under a light microscope at ×1000 magnification. The diagnosis of malaria was based on the identification of asexual stages of *Plasmodium* on the thick blood smears, while thin blood smears were used to identify species of *Plasmodium* or other blood-borne pathogens. Plasmodium parasite density was determined by counting the number of asexual parasites against 200 leucocytes on the thick blood film and converted to parasites per μl using an assumed total white blood cell (WBC) count of 8000/μl (Trape 1985). Blood films were declared negative if no parasite was seen after viewing 500 WBC. Capillary blood was also collected from finger and heel pricks for the determination of maternal and neonatal haematocrit. Maternal and neonatal blood samples were spun for 10 min in a Hawksley® micro-haematocrit centrifuge, after which the haematocrit was determined using Hawksley® reader. The mean haematocrit was determined from the average of two readings of capillary blood samples.

Treatment and follow-up

Smear-positive (cord or peripheral) neonates were admitted for observation to the respective neonatal unit of each centre. Neonates were monitored for clinical features attributable to malaria, such as fever, vomiting, diarrhea, jaundice and altered consciousness. Follow-up blood smears were made on day 2. Neonates who were symptomatic before day 3 or whose parasitaemia persisted were treated with oral chloroquine (Nivaquine™; May & Baker, Lagos, Nigeria) at the WHO recommended dosage. All patients underwent follow-up with clinical and parasitological review on days 3, 7 and 14. Babies whose infection failed to respond to chloroquine by day 3 received sulfadoxine-pyrimethamine (SP) (Fansidar™; Swipha, Lagos, Nigeria) as a single dose on day 3 whether or not they were symptomatic. Mothers who had patent parasitaemia at enrolment were also treated with oral chloroquine or SP at the WHO recommended dosage if they were allergic to chloroquine or if the infection failed to respond to chloroquine. Chloroquine and SP were the drugs of first and second choice for the treatment of uncomplicated malaria in Nigeria at the time of the study. The 14-day modification of the WHO extended field test was used to evaluate the response to antimalarial therapy (WHO 1996).

Data analysis

Data were recorded in case record forms specifically designed for the study, cross-checked and double entered using EPI-INFO 6.04d-software packages, which had been programmed to automatically check errors. Each of the independently entered data sets was validated. Data were further cleaned after entry using range and consistency checks, and analysed using both EPI-INFO 6.04 and SPSS version 11.

The mother–baby pairs enrolled in Enugu had to be excluded from analysis owing to the substitution of standardized staining reagents and subsequent deterioration of slides. The remaining three sites maintained staining and slide storage protocols without incident. Therefore, the final sample size analysed in this report was 1875 mother–baby pairs instead of 2500 as originally planned. The outcome variables were patent parasitaemia in the mother, placenta, cord and neonate in addition to maternal and neonatal haematocrit. All outcome variables were determined using univariate analysis to determine their means and standard deviations. The effect of continuous independent variables (e.g. age, anthropometric measurements, haematocrit) and the outcome variables were tested using Student's *t*-test or ANOVA, for more than two groups. To test the relationship of the outcome variables with categorical variables (e.g. level of education, clinical features), the outcome variables were transformed into categorical variables and thereafter the significance of their association was tested using the chi-square test or Fisher's exact test where applicable. For significant associations, the relative risk and 95% confidence interval were computed to determine the strength of association between a risk factor and particular categorical outcome variable. A *P*-value less than 0.05 was considered significant.

Results

Between 1 April 2003 and 30 March 2004, 1875 mother–baby pairs enrolled in the three centres (625 per centre) were included in the analysis (Table 1). Ninety-eight per cent claimed to have used some form of anti-vector measure during pregnancy, 84.4% said they took anti-malarial chemoprophylaxis or intermittent preventive treatment (IPT) with SP during the index pregnancy. The effect of anti-malarial drug use and anti-vector usage on the epidemiology of congenital malaria is reported elsewhere (Okafor *et al.* 2006). 13.6% gave a history of a febrile illness within 2 weeks of parturition. 71.8% of these were treated for malaria; 71.6% (131) were treated with chloroquine; 15.9% with SP; 7.1% with herbal remedies; and 2.7% with amodiaquine or halofantrine.

Table 1 Demographic characteristics of mother–baby pairs

Characteristic	Ibadan (*N* = 625)	Ilorin (*N* = 625)	Kaduna (*N* = 625)	Total (*N* = 1875)
Age (years)				
Mean ± SD	29.2 ± 5.2	28.8 ± 4.9	29.1 ± 5.2	29.0 ± 5.1
Range	17–43	14–46	16–50	14–50
Parity				
Mean ± SD	1.58 ± 1.5	1.52 ± 1.5	2.77 ± 1.93	1.96 ± 1.76
Range	0–8	0–7	0–9	0–10
Parity grouping				
Parity 0	189 (30.3%)	217 (32.4%)	198 (29.6%)	604 (32.2%)
Parity 1	159 (25.5%)	149 (22.6%)	149 (22.6%)	457 (24.4%)
Parity ≥2	277 (44.2%)	259 (41.6%)	278 (23.7%)	824 (43.4%)
Gestational age group*				
Pre-term (<37 weeks)	119 (19.1%)	45 (7.2%)	23 (3.2%)	187 (10%)
Term (37–40 weeks)	463 (63.9%)	550 (88.0%)	601 (96.2%)	1614 (86.5%)
Post-term (>40 weeks)	43 (5.9%)	21 (3.4%)	1 (0.2%)	65 (3.5%)
Birth weight (g)				
Mean	3157.63	3089.13	3228.43	3158.4
SD	469.2	499.176	481.307	486.5
Range	1700–4600	1200–4600	1000–4950	1000–4950
Babies' weight group (g)				
<2500	33 (5.3%)	56 (9.0%)	39 (6.2%)	128 (6.8%)
2500–3999	557 (89.3%)	549 (87%)	554 (88.6%)	1660 (88.6%)
≥4000	34 (5.4%)	19 (3.0%)	32 (5.1%)	85 (4.5%)
Mother's haematocrit (%)				
Mean ± SD	36.3 ± 5.4	38.9 ± 5.4	33.16 ± 3.7	36.1 ± 5.4
Range	10–49	7–54	20–50	7–54
Baby's haematocrit (%)				
Mean ± SD	57.3 ± 7.8	49.9 ± 8.4	50.4 ± 6.2	52.5 ± 8.2
Range	31–79	12–72	25–70	12–79

*N for Ilorin = 616.

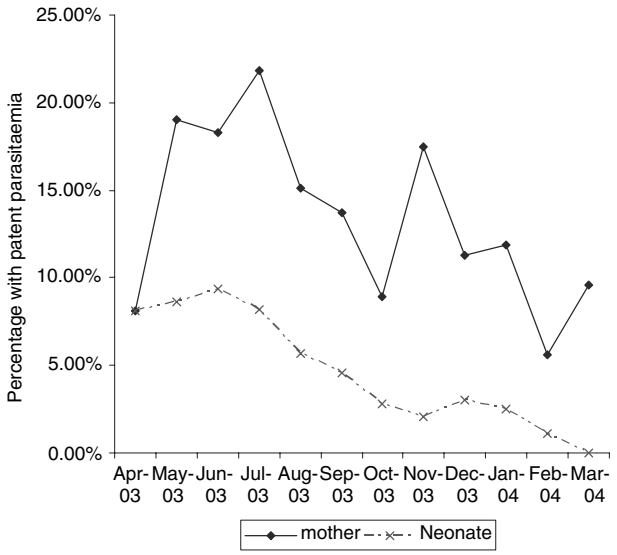

Figure 1 Monthly prevalence of peripartum maternal and neonatal parasitaemia among pregnant women in Nigeria.

Malaria parasitaemia in mother, placenta, cord and neonate

Malarial parasites were detected in maternal, placental, cord and neonatal blood smears year round at each study centre. The overall prevalence of patent parasitaemia in placental, cord and neonatal blood smears peaked during the rainy season months of May to August in all centres (Figure 1). The same was true for the overall prevalence of maternal parasitaemia, although there was an outlier in November 2004 (Table 2).

Malaria parasitaemia in the neonates

The overall incidence of congenital malaria in Nigeria was 5.1% (95/1875). Neonates were closely monitored at all study centres. Only 33.7% neonates (32/95) who had patent parasitaemia on day 0 were symptomatic. Symptomatic neonates were investigated to rule out neonatal sepsis and meningitis. The occurrence of symptomatic congenital malaria varied across study centres; Ibadan and Ilorin recorded no case of symptomatic congenital malaria while Kaduna had 32 (44.4%). Four neonates (4.2%) (Ibadan 1, Ilorin 3) had persistent patent parasitaemia up to day 2 without developing any observable symptoms. Parasitaemia in the four neonates with persistent parasitaemia cleared promptly following administration of oral chloroquine. Parasitaemia among the remaining 59 (62.1%) neonates cleared spontaneously without the use of anti-malarial drugs before day 2. Fever (100%) and refusal to feed (10%) were the two most common signs of infection among symptomatic neonates. The response of malarial infection to chloroquine among both symptomatic and asymptomatic babies was good with prompt clinical improvement and clearance of patent parasitaemia. Parasitological cure rate at day 14 was 88.4% among the neonates. Four (11.4%) of the symptomatic babies who did not respond to oral chloroquine were successfully treated with oral SP. None of the babies required parenteral

Table 2 Prevalence and malarial-parasite densities in maternal, placental, cord and neonatal blood smears during the peripartum period in Nigeria

	Parasitaemia		
Sampling site N = 625 per centre	Ibadan	Ilorin	Kaduna
Maternal			
Prevalence [no (%)]	89 (14.2)	87 (13.9)	142 (22.9)
Parasite density per μl (mean ± SD)	5969 ± 13 453	3644 ± 2288	70 ± 46
Range	8–81 600	16–187 200	40–320
Placenta			
Prevalence [no (%)]	88 (14.1)	73 (11.7)	106 (17.0)
Parasite density per μl (mean ± SD)	15 829 ± 52 092	8845 ± 5820	84 ± 56
Range	8–384 000	32–165 000	40–280
Cord			
Prevalence [no (%)]	15 (2.6)	21 (3.4)	53 (8.5)
Parasite density per μl (mean ± SD)	176 ± 306	48 ± 4	51 ± 21
Range	8–1149	40–80	40–120
Baby			
Parasite density per μl (mean ± SD)	7 (1.1)	16 (2.6)	72 (11.5)
Parasite density per μl (mean ± SD)	54 ± 75	48 ± 4	43 ± 13
Range	8–200	40–80	40–120

therapy. There were no maternal or neonatal deaths among the study participants.

Neonatal parasitaemia occurred in 19.4% (6/319) of mothers who had positive malaria smears, 21.3% (57/267) of malaria positive placental smears and 44.9% (40/89) of positive cord smears. In contrast, 2.1% (33/1555), 2.4% (38/1607) and 3.1% (55/1785) of neonatal smears of malaria parasite-negative maternal, placental and cord smears had patent parasitaemia, respectively. The concordance between neonatal parasitaemia and maternal, placental and cord parasitaemia was highly significant ($P < 0.0001$) in each case. Although concordance was very good between the occurrence of maternal, placental, cord and neonatal parasitaemia, there was no relationship between either maternal, placental or cord parasite density and neonatal parasite density. There were 13 instances (Ibadan, 2; Ilorin, 2; Kaduna, 9) of isolated malaria parasitaemia in neonates without associated maternal, placental or cord parasitaemia. Babies born with congenital malaria had significantly ($P < 0.0001$) lower haematocrits than those without (Table 3). There was no statistically significant association between patent parasitaemia in the neonate and gravidity, maternal age group and neonatal birth weight. However, babies born to mothers with patent parasitaemia had significantly lower birth weights ($P = 0.002$) than babies whose mothers were not parasitaemic (Table 3). In addition, the prevalence of maternal parasitaemia was significantly higher among primigravida and secondigravida women than those carrying third pregnancies and above ($P = 0.04$). Women less than 20 years of age had a higher prevalence of maternal and placental parasitaemia than those 20 years and above ($P = 0.034$ and 0.021, respectively). However, the influence of age on maternal parasitaemia was no longer statistically significant following multivariate analysis (Table 4). In a similar manner, the incidence of congenital malaria was less among patients who used SP-IPT as malaria preventive measure [$P < 0.0001$ odds ratio (OR) 3.22, 95% confidence interval (CI) = 1.29–8.04] compared with those who did not; however, the influence of this intervention was no longer statistically significant following multiple regression analysis. Low parity, maternal parasitaemia, and placental parasitaemia were associated with increased risk of congenital malaria (Table 4).

Discussion

Incidence of congenital malaria

The overall incidence of congenital malaria in Nigeria in this large, multi-centre study was 5.1%. The incidence varied with geographical location, with the lowest values being found in the southwestern zone (1.1%), followed by the north-central zone (2.2%) and highest in the northwestern zone (11.5%). These findings are consistent with the results of previous studies (Egwunyenga *et al.* 1995; Sowunmi *et al.* 1996a; Fischer 1997; Olowu *et al.* 2000; Sule-Odu *et al.* 2002). Fischer (1997) reported a similar variation in the incidence of congenital malaria from one city to another. Sowunmi *et al.* (1996a) and Olowu *et al.* (2000) in two studies in the same hospitals recorded incidence rates ranging from 7% to 23.7%. However, in contrast to our study, the total number of mother–baby pairs evaluated in the two studies was only 180.

Table 3 Association between maternal, placental or cord blood parasitaemia and maternal or neonatal outcome measures

Sampling site								
Outcome measures	Maternal parasitaemia		Placental parasitaemia		Cord parasitemia		Neonatal parasitaemia	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Maternal haematocrit (%) ± SD	34.26 ± 5.1*	36.5 ± 5.4	34.4 ± 5.0*	36.2 ± 5.5	34.9 ± 4.9*	36.2 ± 5.5	34.3 ± 4.9*	36.2 ± 5.5
Neonatal parasitaemia (*N*)	62*	55	57*	38	40*	55	NA	NA
Neonatal haematocrit (%) ± SD	52.3 ± 7.9	52.6 ± 8.3	52.5 ± 7.8	52.5 ± 8.3	49.7 ± 7.1*	52.8 ± 8.3	49.7 ± 6.6*	52.8 ± 8.3
Birth weight (g) ± SD	3077.5 ± 523.2*	3176.7 ± 477.4	3114.4 ± 481.6	3167.4 ± 487.3	3093 ± 444.7	3163.1 ± 488.6	3182.6 ± 524	3158.6 ± 480

*$P < 0.001$.

Table 4 Regression table of independent variables associated with neonatal parasitaemia

Model constant	B	SE	Sig
1.46	-	0.78	0.000
Malaria chemoprophylaxis	3.701 E03	0.03	0.006
Parity	1.77 E03	0.004	0.027
Maternal parasitaemia	0.108	0.015	<0.0001
Placental parasitaemia	0.127	0.17	<0.0001
Maternal age	4.143 E03	0.36	0.9
Low birth weight	0.371	0.523	0.560
SP-IPT use	0.344	0.590	0.560
Study centre	1.544	0.319	<0.0001

SP-IPT, sulfadoxine-pyrimethamine-intermittent preventive treatment.

Egwunyenga *et al.* (1995) reported a rate of 2.8% (8/284) in Jos in north-central Nigeria, while Sule-Odu *et al.* (2002) working in Shagamu, south-western Nigeria, reported an incidence rate of 0.7% among (1/140) among parasitemic parturient women only. Runsewe-Abiodun *et al.* (2006), in a retrospective study, reported a congenital malaria prevalence rate of 17.4% (40/230) among sick neonates in a tertiary hospital also in Shagamu, south-western Nigeria, over a 2-year period. Most of the neonates in the study reported by Runsewe-Abiodun *et al.* were referred from various hospitals as a result of ill health. We believe that the results from this systematic 1-year prospective study with its much larger sample size (1875 mother–baby pairs) in three geographical zones of Nigeria and strict quality assurance are more generalizable than the earlier ones which did not have these advantages.

We believe that excluding the results of the fourth study centre has not adversely affected our results for several reasons. In previous studies, the prevalence of congenital malaria varied widely. We used the available prevalence data for calculating our sample size *ab initio*. In addition, we believe that the 1875 mother–baby pairs enrolled in the three study sites provided a sample size that is adequately powered to allow us to effectively evaluate the epidemiology of congenital malaria in Nigeria. Because of the problems with quality staining and storage of blood smears at the fourth site, we feel that their exclusion from this analysis is appropriate.

Our findings of a rather low incidence rate of 5.1% for congenital malaria is in agreement with those of previous workers on the effectiveness of the placental barrier in limiting transplacental transmission of malarial infection from mother to baby (Bruce-Chwatt 1952; Lamikanra 1993; Egwunyenga *et al.* 1995), particularly among the study populations of Ibadan and Ilorin. The reason for the higher incidence of congenital malaria in Kaduna is not clear. HIV enhances the risk of malaria in women of all gravidities (Steketee *et al.* 1996; van Eijk *et al.* 2003). The higher incidence of congenital malaria in Kaduna may be attributed to the high level of HIV seropositivity in the city of Kaduna, which ranks second highest among all adult age groups in Nigeria [Federal Ministry of Health (FMOH) 2004]. Unfortunately, determination of HIV status was not a part of the study protocol.

Distribution of maternal and placental parasitaemia and concordance with neonatal parasitaemia

The incidence of congenital malaria showed a highly significant concordance with maternal, placental and cord parasitaemia ($P < 0.0001$ for each sampling site). The significant difference in the incidence of congenital malaria between study centres is probably not unrelated to the marked difference in the prevalence of maternal and placental malaria parasitaemia at the different study centres. The Ilorin and Ibadan study centres recorded lower prevalence of parasitaemia in both mothers (14.2% and 13.9%) and placentae (14.1% and 11.7%) when compared with those of Kaduna, which recorded values of 22.7% and 17.9% for mothers and placentae, respectively. The observed variations are in agreement with the findings in previous studies from Nigeria (Egwunyenga *et al.* 1995; Sowunmi *et al.* 1996b; Olowu *et al.* 2000; Sule-Odu *et al.* 2002). Factors that may be responsible for these observed differences in the prevalence of parasitaemia in different geographical zones of Nigeria include difference in transmission intensity, use of different forms of malaria preventive measures and prevalence of HIV seropositivity. Malaria transmission is hyperendemic in both Ibadan and Ilorin while transmission is mesoendemic in Kaduna. It is noteworthy that SP-IPT was introduced to the study centre in southwestern Nigeria. SP-IPT was found to be highly effective in preventing placental malaria among Kenyan (Parise *et al.* 1998) and Mozambican women (Challis *et al.* 2004). Genetic differences in predisposition to malaria may also have had some influence as the residents of Kaduna are predominantly Hausa/Fulani, in contrast to the predominantly Yoruba residents of Ibadan/Ilorin. Differences in clinical malaria presentation and immune responses to malaria among different tribes in the West African sub region have been reported by Oomen *et al.* (1979), Modiano *et al.* (2001) and Paganotti *et al.* (2004).

Despite the highly significant concordance between neonatal parasitaemia and maternal, placental and cord parasitaemia, isolated malaria parasitaemia was seen in 13 neonates, i.e. without associated maternal, placental or

cord parasitaemia. The detection of patent parasitemia in neonates without associated maternal, placental or cord parasitemia could have resulted from transplacental transmission of infection earlier in pregnancy. Rubio *et al.* (2000) reported congenital malaria occurring in a set of twins in which genotyping by PCR for merozoite surface proteins 1 and 2 showed that mothers and newborns had different *Plasmodium falciparum* strains, thus suggesting transplacental transmission earlier in pregnancy. It is possible that this scenario occurred in the cases reported in our study. However, this may have resulted from the insensitivity of microscopy for detecting malarial parasites in maternal peripheral blood or the placenta, especially at low levels of parasitaemia. Despite the implementation of quality assurance for slide microscopy quantification throughout the study period, the lack of PCR analysis is a limitation in this study.

Pattern of parasitaemia in the newborn

The parasite densities of infection were remarkably low among neonates with patent parasitaemia in all study centres compared with maternal placental parasite densities (Table 2). Although patent parasitaemia was detected all year round, the prevalence of maternal, placental, cord and neonatal malaria was highest during the rainy season when transmission is most intense. This was followed by a sharp drop during the dry season (Figure 1). Babies born to mothers with patent maternal parasitaemia weighed significantly less than those whose mothers were free of maternal and placental parasitaemia in this study. Maternal and placental parasitaemia were major risk factors for neonatal parasitaemia (Table 3).

Conclusions

The epidemiology of congenital malaria in Nigeria varied from one location to another with an overall incidence rate of 5.1% of live births. In addition, parasite density was remarkably low in the neonates with patent parasitemia and not related to maternal and placental parasite density. Congenital malaria was asymptomatic in 62.1% neonates with patent parasitaemia clearing spontaneously within 72 h in most. Asymptomatic malaria parasitaemia in the newborn therefore does not warrant any treatment in the first 72 h of life. Fever and refusal to feed were the observed signs of congenital malaria among babies who were symptomatic. We recommend that all neonates with unexplained fever and refusal to feed in malaria endemic areas should be evaluated for congenital malaria and treated with effective anti-malarial drugs.

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