Current Status of Malaria in Pregnant Women in Cameroon

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Abstract Studies in Cameroon have contributed to our knowledge of malaria in pregnant women, but much remains to be learned. As demonstrated in other African countries, the prevalence of *P. falciparum* in rural villages is higher in primigravidae than multigravidae. In Yaounde, however, age as well as parity is a significant risk factor for infection. The true prevalence of *P. falciparum* infections in pregnant women is difficult to assess, since parasites often sequester in the placenta but are not detected in the peripheral blood. At term, one out of every five Cameroonian women who had parasites sequestered in the placenta was found to be blood-smear negative. Thus, prevalence of malaria is under reported. Immunological studies in Cameroon have investigated the down-regulation of T cell responses, cytokine imbalance in the placenta, antibodies to various malarial antigens (age-dependent immunity), and antibodies that inhibit the sequestration of parasitized erythrocytes in the placenta (pregnancy-associated immunity). Results from these studies may aid in the development of a vaccine of use in women of children-bearing age. Due to parasite-drug resistance to chloroquine, Cameroon recently adopted intermittent preventive therapy (IPT) using sulfadoxine-pyrimethamine, but the efficacy of IPT needs to be evaluated.

Resume La recherche au Cameroun a contribué à une meilleure compréhension du paludisme chez la femme enceinte, cependant beaucoup reste encore à étudier. Comme démontrée dans d'autres pays africains, la prévalence de *P. falciparum* est plus élevée chez les primigestes que chez les multigestes. Toute fois à Yaoundé l'âge aussi bien que la parité constitue un facteur de risque significatif pour l'infection. La prévalence exacte de l'infection à *P. falciparum* chez les femmes enceintes est difficilement appréciable puisque le parasite souvent séquestré dans le placenta n'est pas détectable dans le sang périphérique. A terme, une femme camerounaise sur cinq ayant présenté une parasitémie placentaire a montré un frottis sanguin négatif. Ainsi donc la prévalence du paludisme est sous rapportée. Les études immunologiques menées au Cameroun ont examinées la rétro-régulation des réponses des cellules T, le rapport des cytokines dans le placenta, les anticorps aux différents antigènes malariques (immunité dépendant de l'âge) les anticorps inhibant la séquestration des érythrocytes parasités dans le placenta (immunité associée à la grossesse). Les résultats de ces études peuvent aider dans le développement d'un vaccin qui sera utilisé chez les femmes en âge de procréer. Suite à la résistance du parasite à la chloroquine, le Cameroun a récemment adopté une thérapie préventive intermittente (TPI) utilisant la Sulfadoxine-pyrimethamine mais l'efficacité de TPI a besoin d'être évaluée.

Introduction

During pregnancy, *Plasmodium falciparum* infections cause major health problems for Cameroonian women and their newborns. Problems encountered during pregnancy are similar to those in other sub-Saharan countries where transmission is perennial. Malaria increases the risk of maternal anemia and low birth weight (LBW) babies due to intrauterine growth reduction (IUGR) and preterm delivery (PTD) in woman (Tako, et al. 2005). Although the level of transmission differs during the two rainy and two dry seasons, Cameroonian women are exposed to malaria throughout pregnancy. It is estimated that in Yaoundé, the capital of Cameroon, individuals receive ~ 13 infectious bites per year (Manga, et al. 1992; Fondjo, et al. 1992), whereas in many rural villages individuals may receive over 600 infectious bites annually (Carnevale, et al. 1992; Robert, Le Goff et al. 1993; Manga, et al. 1995; Meunier, et al. 1999). Despite the severity of the problem, only recently has research on malaria in pregnant women been intensified in Cameroon.

Prevalence of Malaria in Pregnant Women in Cameroon

Based on detection of parasites in peripheral blood smears, the prevalence of asymptomatic malaria is higher in pregnant compared to non-pregnant Cameroonian women. For example in 1992 in Yaoundé, 45% of pregnant

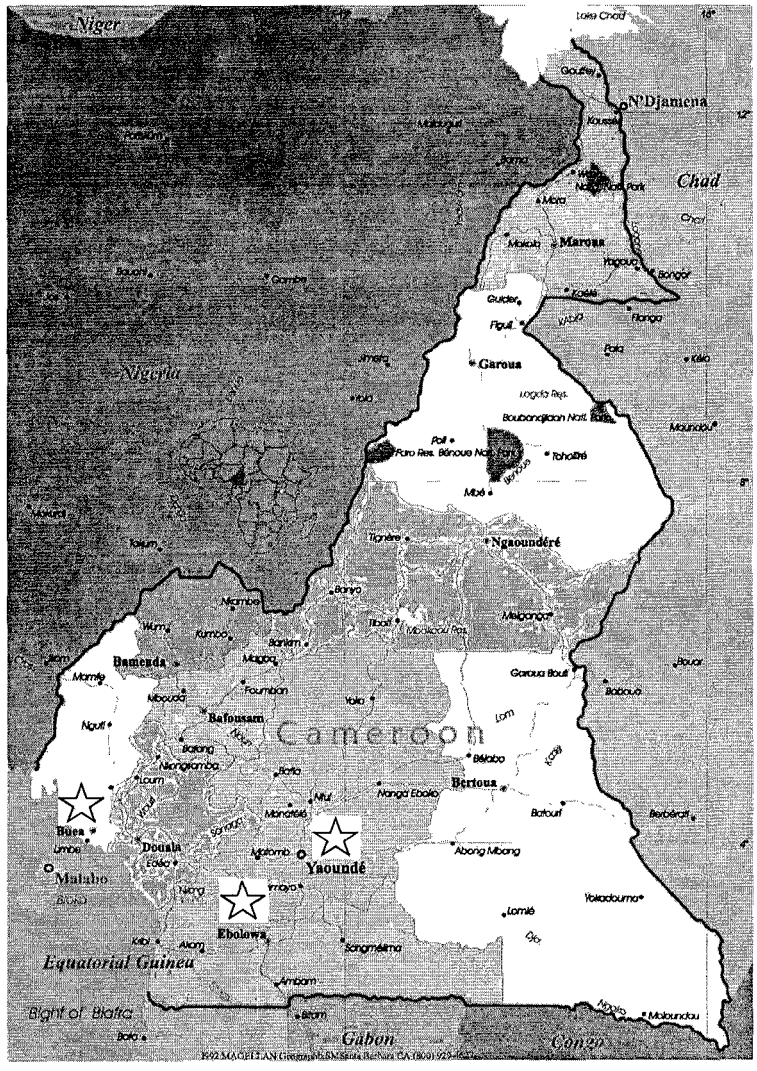

Figure 1: Map of Cameroon showing where studies on malaria in pregnant women have been conducted

women compared to 34% of non-pregnant were slide-positive for *P. falciparum* (Mvondo, et al. 1992) and in 1995 in the village of Ebolowa in southern Cameroon a higher prevalence of malaria infection in pregnant women was reported (Fievet, et al. 1995). More recently, Achidi *et al.* (2005) reported a prevalence of 44.5% in pregnant women in SW Cameroon, with women in Mutenguene more likely to be infected than those living in Buea which is located at a higher altitude (Achidi, et al. 2005).

As expected, the prevalence of malaria in primigravidae is higher than in multigravidae, but age is also a major factor in Cameroon. Based on longitudinal studies in the peri-urban village of Etoa, malaria prevalence decreased with gravidity from 76% in primigravidae to 40% in women with ≥3 pregnancies (multigravidae). However, this decrease was only seen in women <20 years of age and a similar decline with gravidity was not seen in the city of Yaoundé. At both sites, women ≤20 years of age were at a higher risk of malaria infection regardless of stage of pregnancy and parity (Zhou, et al. 2002). In subsequent studies conducted at term between 1996-2001 in Yaoundé, the presence of both peripheral blood and placental parasites were found to decrease with age (Fig. 3). Thus, both age and gravidity are important risk factors in Cameroon. As reported in other African countries, the prevalence of asymptomatic malaria is highest during the fourth month of pregnancy (Fig. 2) (Zhou, et al. 2002). At term, 19.9% of women in Yaoundé (1998-2001) (Tako, et al. 2005) and 33.7% in SW Cameroon (1999-2001) (Achidi, et al. 2005) had placental infections, with the prevalence being higher in primigravidae.

Data based on blood smears is most likely an underestimate of prevalence, because parasites often sequester in the placenta but remain below detectable levels in the peripheral blood. For example, Leke et al. (1999) showed that at delivery 20.1% of women with placental malaria were blood smear negative. Thus, it is likely that one out of every 5 Cameroonian women with placental parasites is misdiagnosed as malaria negative. Studies showed that among women who were peripheral blood smear negative but had pla-

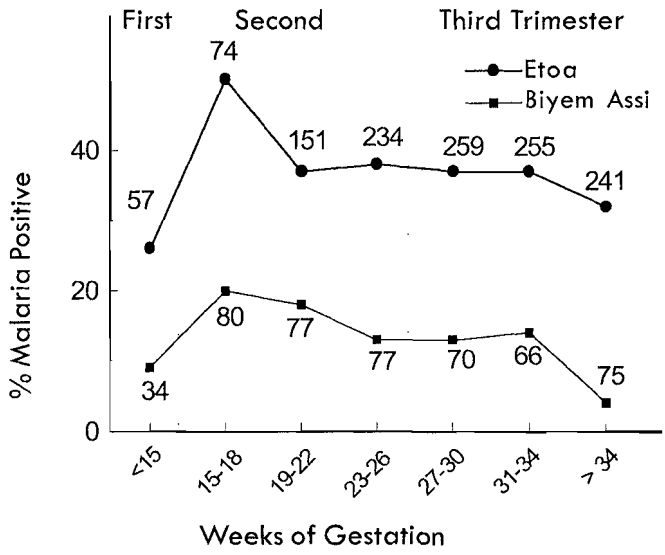

Figure 2: Prevalence of malaria in pregnant women in the city of Yaounde and the peri-urban village of Etoa. The prevalence of malaria peaks around the 4th month of gestation. Numbers next to the data points represent the number of women examined. Reprinted with permission from the

cental parasites, 88% had detectable levels of PfHRP-2 in their peripheral circulation. Thus, commercially chromatographic strip tests improve diagnosis during pregnancy (Leke, et al. 1999).

Using a PCR-detection of the gene for ribosomal RNA, Walker-Abbey et al. (2005) reported that 82.4% of women in Yaoundé were infected with *P. falciparum* at term; 7.5% had *P. malariae* and 2.5% *P. ovale*. Based on genotyping for polymorphisms in *msp1* and *msp2*, women in Yaoundé have an average of 3.4 parasite genotypes in their peripheral (range 0-9) and placental (range 0-8) blood at delivery. Thus, even under low transmission conditions, most pregnant Cameroonian women become infected with malaria.

Malaria, Anemia and Pregnancy Outcome

Throughout sub-Saharan Africa, P. *falciparum* is a significant risk factor for maternal anemia and LBW babies. In a study conducted in Yaoundé between 1996 and 2001, 47.3% of primigravidae were found to be anemic at delivery and 16.4% of women had LBW babies (Tako, et al. 2005). The highest prevalence of LBW babies was in primigravidae and those <20 years of age with placental malaria. The major risk factor for LBW babies was first and second pregnancies, whereas the major risk for PTD was anemia (Tako, et al. 2005). Overall, 69.6% of pregnant women in Buea were anemic at delivery with 4.3% having severe anemia (Achidi, et al. 2005). Surprisingly, hemoglobin levels were higher in primigravidae than multigravidae and anemia was more common in primigravidae who were malaria negative.

Antibodies to Malaria in Pregnant Women

Since antibodies (Ab) play an important role in immunity to malaria, several studies have measured changes in Ab titers in pregnant women. In early 1990's, Mvondo *et al.* monitored IgG to the circumsporozoite protein (CSP) and the ring-infected erythrocyte surface antigen (RESA) in pregnant and

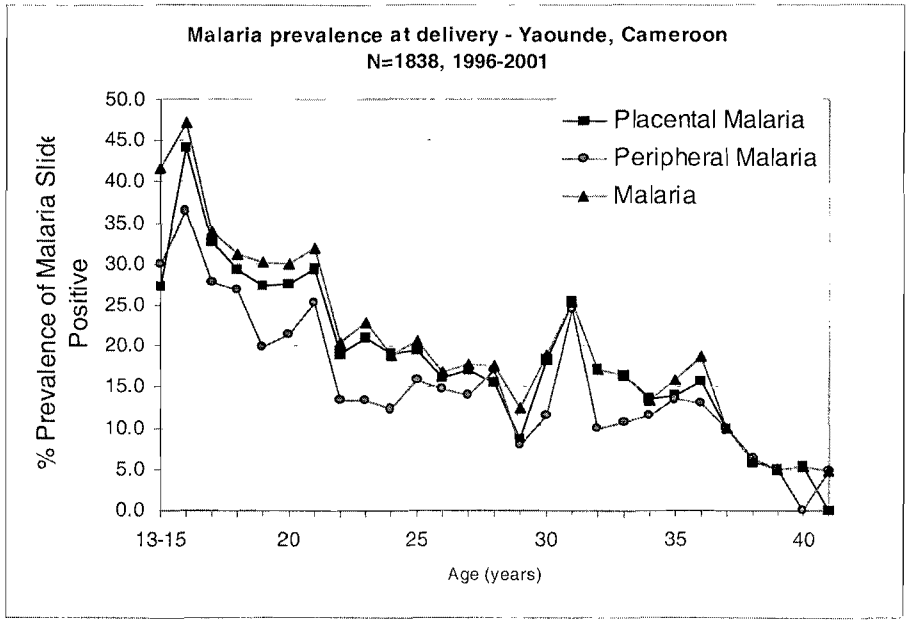

Figure 3: Distribution of *Plasmodium falciparum* malaria in 1,499 women residing in Yaounde, Cameroon. Individual plots represent the percentage of women with parasites detected by microscopy in the placenta (■) and the peripheral blood (○) and the total, i.e., present in the peripheral blood and/or the placenta ().

non-pregnant women living in Yaoundé (Mvondo, et al. 1992). No differences in Ab to CSP were found, but Ab to RESA were lower in pregnant women. Anti-RESA titers were also significantly lower in primigravidae compared multigravidae and inversely correlated with peripheral parasitemias. Thus, Ab to RESA might contribute to protection from infection in pregnant women (Mvondo, et al. 1992). However, in southern Cameroon Fievet *et al.* (1995) found similar Ab titers in pregnant and non-pregnant primigravidae to RESA and extracts of sexual and gametocyte stage parasites in (Fievet, et al. 1995). Subsequently, they reported Ab levels to RESA were actually higher in primigravidae during pregnancy than after delivery (Fievet, et al. 1997), suggesting they may not be protective. The difference in results could be due to varying rates of transmission and assay techniques.

Ab titers to CSP, liver-stage antigen 1 (LSA1), RESA, and the 19 kDa C-terminal sequence of merozoite-surface antigen 1 (MSP1-19) were determined for women in Yaoundé with and without placental malaria at delivery (Taylor, et al. 2004). Absence of Ab to MSP1-19, but not the other antigens, was associated with a significantly increased risk of placental malaria. Results from studies on anti-*var* Ab are discussed below.

Cell-Mediate Immune Responses to Malaria in Pregnant Women

Peripheral blood mononuclear cells (MNC) from Cameroonian primigravidae women living in Ebolowa had a reduced proliferative responses *in vitro* compared to non-pregnant woman when their MNC were cultured with extracts of schizonts and gametocytes (Fievet, et al. 1995). MNC collected at delivery also showed a decreased proliferative response following stimulation with MSP-1 and a schizont extract compared to MNC collected three months postpartum. Interestingly, when cells were cultured with two different lines of *P. falciparum,* the response to the strain that adheres to CSA in the placenta (see below) induced proliferation in a parity-dependent manner (Fievet, et al. 2002). MNC from pregnant and non-pregnant women secreted equivalent amounts of IL-4 and INF?, but the IL-2 to RESA was reduced (Fievet, et al. 1995). MNC obtained at delivery resulted in more INF? than IL-4 production (Achidi, *et al.* 2005). These data suggest that pregnant Cameroonian women can produce Th1-type responses to malaria.

Placental Malaria and Cytokine Production

Macrophages accumulate in the placenta in response to the presence of malarial parasites (Ordi, et al. 1998; Ordi, et al. 2001; Imamura, et al. 2002; Chaisavaneeyakorn, *et al.* 2002; Abrams, et al. 2003; Chaisavaneeyakorn, et al. 2005). Recently, maternal leukocytes in the intervillous space (IVS) of Cameroonian placentas were found to secrete large amounts of the ?-chemokines MCP-1, MIP-1α, MIP-1β, and IP-10 (Suguitan, et al. 2003). These chemokines are chemotactic for marcophages and most likely contribute to macrophage accumulation in the IVS. The presence of parasites in the placenta also alters the cytokine balance in the IVS. Both maternal leukocytes and fetal villous tissue secrete cytokines in response to malaria (Fievet, et al. 2001; Suguitan, et al. 2003). Maternal cells secrete TNFα, INFγ, and IL-10 in response to *P. falciparum*, whereas fetal tissues secrete IL-6, IL-1α, GM-CSF, TGF-β (Fievet, et al. 2001; Suguitan, et al. 2003) and IL-10 (Suguitan, et al. 2003).

Suguitan *et al.* compared cytokine concentrations in placentas of women who had preterm (PT) and full term (FT) deliveries (Suguitan, et al. 2003). No difference in TNFα, INFγ, IL-4 and IL-10 were found between malaria negative women with PT and FT deliveries, however, the concentrations of TNFα and IL-10 were significantly elevated in malaria-positive women with PTD compared to malaria-positive women with FTD. Parasitemias greater than 1.0%, maternal anemia, elevated levels of IL-10, and low TNFα:IL-10 ratios were significant risk factors for PTD. After adjusting for other covariates, however, only maternal anemia was a significant factor for PTD in Cameroonian women living in Yaoundé (Suguitan, et al. 2003).

Placental Malaria: Parasite sequestration and Antibodies that Inhibit Sequestration

Parasites sequestered in the IVS have a unique phenotype that allows them to bind to chondroitin sulfate A (CSA) expressed by trophoblasts (Fried and Duffy 1996). Studies in Cameroon demonstrated that the level of sulfation of placental CSA changes during gestation (Agbor-Enoh, et al. 2003), but sequestered parasites are able to bind to all forms expressed throughout gestation. Parasite sequestration has been studied by several groups in Cameroon. Maubert *et al.* (1998) demonstrated that parasitized erythrocytes from the peripheral and placental blood of Cameroonian women did not form rosettes, whereas parasites from non-pregnant individuals did. They also demonstrated that parasites isolated from the placentas of women in Yaoundé bind to trophoblasts *in vitro* via CSA (Maubert, et al. 2000). These parasites were later shown to express the variant (*var*) gene encoding the Duffy-binding-like domain (DBL) -γ (Khattab, et al. 2003).

Women in Yaoundé acquire Ab that block the binding of parasitized erythrocytes to CSA in a parity-dependent manner (O'Neil-Dunne, et al. 2001). These Ab are acquired as early as 12 weeks in multigravidae, whereas primigravidae do not develop inhibitory Ab until ~2 months later (O'Neil-Dunne, et al. 2001). The presence of Ab that inhibit sequestration are dependent on the endemicity of malaria, are less prevalent in women who take prophylaxis, and decline during the postpartum period (Staalsoe, et al. 2001). The predominant Ab response is IgG1 with some IgG3 (Megnekou, et al. 2005).

Malaria Control and Prevention

The severe effects of malaria during pregnancy can be prevented by prophylaxis. In 1995, chloroquine prophylaxis was reported to increase infant birth weigh and lower the percentage of LBW babies in Cameroon (Cot, et al. 1995). Data collected in the late 1990's showed that women who did not take chloroquine were at an increased risk of malaria (Zhou, et al. 2002). However, by the late 1990's drug resistance to chloroquine became extensive. Lemardely *et al.* (1997) found no difference in the prevalence of malaria in women in Yaoundé who took chloroquine and those who did not. The emergence of chloroquine resistance led Cameroon to adopt intermittent preventive treatment using sulfadoxine-pyrimethamine in early 2004. The efficiency of IPT in central African countries has not been evaluated.

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References

Abrams ET, H Brown, SW Chensue, GD Turner, E Tadesse, VM Lema, ME Molyneux, R Rochford, SR Meshnick and SJ Rogerson. 2003. Host response to malaria during pregnancy: placental monocyte recruitment is associated with elevated beta chemokine expression. J Immunol. **170:** 2759-2764.

Achidi E, A Kuoh, J Minang, B Ngum, B Achimbom, S Motaze, M Ahmadou and M Troye-Blomberg, M. 2005a. Malaria infection in pregnancy and its effects on haemoglobin levels in women from a malaria endemic area of Fako Division, South West Province, Cameroon. J Obstet Gynaecol. **25:** 235-240.

Achidi EA, JK Anchang, JT Minang, MJ Ahmadou and M Troye-Blomberg. 2005b. Studies on *Plasmodium falciparum* isotypic antibodies and numbers of IL-4 and IFN-gamma secreting cells in paired maternal cord blood from South West Cameroon. Int J Infect Dis. **9:** 159-169.

Agbor-Enoh ST, RN Achur, M Valiyaveettil, R Leke, DW Taylor and DC Gowda. 2003. Chondroitin sulfate proteoglycan expression and binding of *Plasmodium falciparum*-infected erythrocytes in the human placenta during pregnancy. Infect Immun. **71:** 2455-2461.

Carnevale P, G Le Goff, JC Toto and V Robert. 1992. *Anopheles nili* as the main vector of human malaria in villages of southern Cameroon. Med Vet Entomol. **6:** 135-138.

Chaisavaneeyakorn S, N Lucchi, C Abramowsky, C Othoro, SC Chaiyaroj, YP Shi, BL Nahlen, DS Peterson, JM Moore and V Udhayakumar. 2005. Immunohistological characterization of macrophage migration inhibitory factor expression in *Plasmodium falciparum*-infected placentas. Infect Immun. **73:** 3287-3293.

Chaisavaneeyakorn S, JM Moore, C Othoro, J Otieno, SC Chaiyaroj, YP Shi, BL Nahlen, AA Lal V and Udhayakumar. 2002. Immunity to placental malaria. IV. Placental malaria is associated with up-regulation of macrophage migration inhibitory factor in intervillous blood. J Infect Dis. **186:** 1371-1375.

Cot M, JY Le Hesran, P Miailhes, M Esveld, D Etya'ale, and G Breart. 1995. Increase of birth weight following chloroquine chemoprophylaxis during the first pregnancy: results of a randomized trial in Cameroon. Am J Trop Med Hyg. **53:** 581-585.

Fievet N, M Cot, C Chougnet, B Maubert, J Bickii, B Dubois, JY Le Hesran, Y Frobert, F Migot, F Romain and et al. 1995. Malaria and pregnancy in Cameroonian primigravidae: humoral and cellular immune responses to *Plasmodium falciparum* blood-stage antigens. Am J Trop Med Hyg. **53:** 612-617.

Fievet N, M Cot, P Ringwald, J Bickii, B Dubois, JY Le Hesran, F Migot and P Deloron. 1997. Immune response to *Plasmodium falciparum* antigens in Cameroonian primigravidae: evolution after delivery and during second pregnancy. Clin Exp Immunol. **107:** 462-467.

Fievet N, M Moussa, G Tami, B Maubert, M Cot, P Deloron and G Chaouat. 2001. *Plasmodium falciparum* induces a Th1/Th2 disequilibrium, favoring the Th1-type pathway, in the human placenta. J Infect Dis. **183:** 1530-1534.

Fievet N, G Tami, B Maubert, M Moussa, IK Shaw, M Cot, AA Holder, G Chaouat and P Deloron. 2002. Cellular immune response to *Plasmodium falciparum* after pregnancy is related to previous placental infection and parity. Malar J. **1:** 1-8.

Fondjo E., V Robert, G Le Goff, JC Toto and P Carnevale. 1992. [Urban malaria in Yaounde (Cameroon). 2. Entomologic study in 2 suburban districts]. Bull Soc Pathol Exot. **85:** 57-63.

Fried M. and PE Duffy. 1996. Adherence of *Plasmodium falciparum* to chondroitin sulfate A in the human placenta. Science. **272:** 1502-1504.

Imamura T, T Sugiyama, LE Cuevas, R Makunde and S Nakamura. 2002. Expression of tissue factor, the clotting initiator, on macrophages in *Plasmodium falciparum*-infected placentas. J Infect Dis. **186:** 436-440.

Khattab A, PG Kremsner and MQ Klinkert. 2003. Common surface-antigen var genes of limited diversity expressed by *Plasmodium falciparum* placental isolates separated by time and space. J Infect Dis. **187:** 477-483.

Leke RF, RR Djokam, R Mbu, RJ Leke, J Fogako, R Megnekou, S Metenou, G Sama, Y Zhou, T Cadigan, M Parra and DW Taylor. 1999. Detection of the *Plasmodium falciparum* antigen histidine-rich protein 2 in blood of pregnant women: implications for diagnosing placental malaria. J Clin Microbiol. **37:** 2992-2996.

Lemardeley P, J Raiga, R Chambon, JJ Keuzeta, V Foumane and J Chandenier. 1997. [Prevention and control of malaria in pregnant women in an urban setting (Yaounde, Cameroun)]. Sante. **7:** 239-245.

Manga L, V Robert, J Mess, M Desfontaine, and P Carnevale. 1992. Le paludisme urbain á Yaoundé, Cameroon. I. Étude entomologique dans deux quartiers cantraux. Mem Soc R Belg Entomol. **35:** 155-162.

Manga L, JC Toto and P Carnevale. 1995. Malaria vectors and transmission in an area deforested for a new international airport in southern Cameroon. Ann Soc Belg Med Trop. **75:** 43-49.

Maubert B, N Fievet, G Tami, C Boudin and P Deloron. 1998. *Plasmodium falciparum*-isolates from Cameroonian pregnant women do not rosette. Parasite. **5:** 281-283.

Maubert B, N Fievet, G Tami, C Boudin and P Deloron. 2000. Cytoadherence of *Plasmodium falciparum*-infected erythrocytes in the human placenta. Parasite Immunol. **22**: 191-199.

Megnekou R, T Staalsoe, DW Taylor, R Leke and L Hviid. 2005. Effects of pregnancy and intensity of *Plasmodium falciparum* transmission on immunoglobulin G subclass responses to variant surface antigens. Infect Immun. **73**: 4112-4118.

Meunier JY, I Safeukui, D Fontenille and c Boudin. 1999. [Malaria transmission in an area of future vaccination in equatorial forest of south Cameroon]. Bull Soc Pathol Exot. **92**: 309-312.

Mvondo JL, MA James and CC Campbell. 1992a. Malaria and pregnancy in Cameroonian women. Effect of pregnancy on *Plasmodium falciparum* parasitemia and the response to chloroquine. Trop Med Parasitol. **43**: 1-5.

Mvondo JL, MA James, AJ Sulzer and CC Campbell. 1992b. Malaria and pregnancy in Cameroonian women. Naturally acquired antibody responses to asexual blood-stage antigens and the circumsporozoite protein of *Plasmodium falciparum*. Trans R Soc Trop Med Hyg. 86: 486-490.

O'Neil-Dunn I, RN Achur, ST Agbor-Enoh, M Valiyaveettil, RS Naik, CF Ockenhouse, A Zhou, R Megnekou, R Leke, DW Taylor and DC Gowda. 2001. Gravidity-dependent production of antibodies that inhibit binding of *Plasmodium falciparum*-infected erythrocytes to placental chondroitin sulfate proteoglycan during pregnancy. Infect Immun. **69**: 7487-7492.

Ordi J, MR Ismail, PJ Ventura, E Kahigwa, R Hirt, A Cardesa, PL Alonso and C Menendez C. 1998. Massive chronic intervillositis of the placenta associated with malaria infection. Am J Surg Patho. **22**: 1006-1011.

Ordi J, C Menendez, MR Ismail, PJ Ventura, A Palacin, E Kahigwa, B Ferrer, A Cardesa and PL Alonso. 2001. Placental malaria is associated with cell-mediated inflammatory responses with selective absence of natural killer cells. J Infect Dis. **183**: 1100-1107.

Robert V, G Le Goff, JC Toto, L Mulder, E Fondjo, L Manga and P Carnevale. 1993. Anthropophilic mosquitoes and malaria transmission at Edea, Cameroon. Trop Med Parasitol. **44**: 14-18.

Staalsoe T, R Megnekou, N Fievet, CH Ricke, HD Zornig, R Leke, DW Taylor, P Deloron and L Hviid. 2001. Acquisition and decay of antibodies to pregnancy-associated variant antigens on the surface of *Plasmodium falciparum*-infected erythrocytes that protect against placental parasitemia. J Infect Dis. **184**: 618-626.

Suguitan AL, TJ Cadigan, TA Nguyen, A Zhou, RJ Leke, S Metenou, L Thuita, R Megnekou, J Fogako, RG Leke, and DW Taylor. 2003a. Malaria-associated cytokine changes in the placenta of women with pre-term deliveries in Yaounde, Cameroon. Am J Trop Med Hyg. **69**: 574-581.

Suguitan AL, RG Leke, G Fouda, A Zhou, L Thuita, S Metenou, J Fogako, R Megnekou and DW Taylor. W. 2003b. Changes in the levels of chemokines and cytokines in the placentas of women with *Plasmodium falciparum* malaria. J Infect Dis. **188**: 1074-1082.

Tako EA, A Zhou, J Lohoue, R Leke, DW Taylor and RF Leke. 2005. Risk factors for placental malaria and its effect on pregnancy outcome in Yaounde, Cameroon. Am J Trop Med Hyg. **72**: 236-242.

Taylor DW, A Zhou, LE Marsillio, LH Thuita, EB Leke, O Branch, DC Gowda, C Long and RF Leke. 2004. Antibodies that inhibit binding of *Plasmodium falciparum*-infected erythrocytes to chondroitin sulfate A and to the C terminus of merozoite surface protein 1 correlate with reduced placental malaria in Cameroonian women. Infect Immun. **72**: 1603-1607.

Walker-Abbey A, RR Djokam, A Eno, RJ Leke, VP Titanji, J Fogako, G Sama, LH Thuita, E Beardslee, G Snounou, A Zhou and DW Taylor. 2005. Malaria in pregnant Cameroonian women: the effect of age and gravidity on submicroscopic and mixed-species infections and multiple parasite genotypes. Am J Trop Med Hyg. **72**: 229-235.

Zhou A, R Megnekou, R Leke, J Fogako, S Metenou, B Trock, DW Taylor and RF Leke. 2002. Prevalence of *Plasmodium falciparum* infection in pregnant Cameroonian women. Am J Trop Med Hyg. **67**:, 566-570.